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Millipede phylogeny revisited in the light of the enigmatic order Siphoniulida

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Abstract

The discovery of six specimens of the enigmatic order Siphoniulida, including for the first time males, prompted a modern re-analysis of current phylogenetic schemes for the class Diplopoda derived from traditional morphological and developmental characters. The data matrix was constructed and analysed using PAUP. The resulting phylogenetic hypotheses corroborated the longest standing, traditional classification, but also demonstrated clearly that more characters must be included to reach a better resolution. Recent alternative phylogenetic hypotheses and classifications are discussed in the light of the current analysis. The validity of a putative helminthomorph synapomorphy, the location of male gonopods on the 7th body ring, is discussed. Scanning electron microscopy corroborated morphological characters already described for the Siphoniulida: modified anterior legs, an apparently legless 3rd body ring, pyriform head, antennae with clavate setae, and absence of ozopores. The highly modified gonopods of the Siphoniulida are described for the first time; only the anterior legs of the 7th ring are modified into gonopods, the posterior legs of this ring are developed as normal walking legs. The gnathochilarium differs from the Colobognatha and consists of well-developed stipites with palps, elongated lingual plates with palps and a narrow central sclerite, most likely the mentum. Structures on the epiproct may possibly be spinnerets. Despite the discovery of adult males, the Siphoniulida are still considered Helminthomorpha *incertae sedis*.

Key words: Diplopoda – Siphoniulida – systematics – Helminthomorpha – Mexico

Introduction

Existing classifications of many organism groups, traditionally based on morphological characters, are presently being re-evaluated by using molecular-based phylogenies (e.g. Shultz and Regier 1997). In some groups, morphology-based classifications had been subjected to testing using phylogenetics (e.g. Coddington and Levi 1991). However, for many invertebrate groups traditional classifications from pre-cladistic times are the only systematic framework currently available. Diplopod systematics received a 'head start', an early phylogenetic treatment in Enghoff's first cladistic analysis in 1984, before phylogenetic software packages were readily available. This seminal work resulted in a cladistic classification of the Diplopoda, with all supra-ordinal taxa defined as character-based monophyletic groups. The argumentation used in this first cladistic treatment of millipede phylogeny employed traditional, mostly morphological and developmental characters.

The enigmatic order Siphoniulida, previously reported from Sumatra and Guatemala, was placed as Helminthomorpha *incertae sedis* at the time of Enghoff's (1984) analysis, because the group was so poorly known (only three specimens of this order had ever been found). Recently, several siphoniulid specimens were sorted from Field Museum's extensive bulk sample collection. This exciting discovery prompted us to revisit millipede classification as a whole, especially, since adult siphoniulid males were discovered. Establishing Siphoniulida's place within the diplopod ordinal classification requires a review of current hypotheses of millipede ordinal relationships, especially since Regier and Shultz (2001), based on molecular data, proposed some strikingly different relationships from the traditional classification.

At present, the traditional, morphology-based phylogeny of the Diplopoda has not been converted into a data matrix and tested with a current phylogenetic software package (see Enghoff 2000). In an attempt to place the Siphoniulida, we generated a data matrix following the only existing morphological character list (Enghoff 1984), and scored, as closely as possible, the same characters for the Siphoniulida. It must be stressed that the character list and data matrix presented in Appendices A and B are not newly generated characters nor has the distribution of the character states across millipede taxa been comprehensively investigated for this study. Rather, they represent morphological and developmental characters that have a long standing in millipede systematics dating back to Pocock (1887), Verhoeff (1910), Attems (1926) and others and this treasure trove of potentially informative characters is currently distinctly under-analysed.

Material and methods

Specimens

The *Siphoniulus* specimens were sorted from litter samples housed in the Field Museum's bulk sample collection (FMHD numbers refer to the bulk sample database). Due to the scarcity of material, ultrasonic cleaning of the specimens was not attempted. Specimens were sputter-coated; the scanning electron microscope examinations were done with an Amray 1810. The first specimen was air-dried. Its anterior region experienced major distortions due to the drying process, with the head and first five body-rings being shrivelled almost beyond recognition. The body rings from the centre section and the caudal end remained undistorted, demonstrating the Julidan-like fusion of the sclerites of the middle and posterior body regions (see Figs 6 and 11). Two specimens were critical-point dried, one of which resulted in an undistorted anterior body region (see Figs 1 and 2). One head of another specimen was dissected and critical-point dried. The head was damaged during the process, but the gnathochilarium was intact and mounted. The scanning electron microscope images were recorded digitally and modified in Adobe Illustrator. The taxonomic and descriptive treatment of the newly discovered siphoniulid specimens is presented in Appendix C.

Phylogenetic analysis

To place the Siphoniulida into existing phylogenetic hypotheses, we generated a data matrix (see Appendix B), employing the traditional morphological characters, which were used by Enghoff (1984) and Enghoff et al. (1993). Several characters were constructed differently

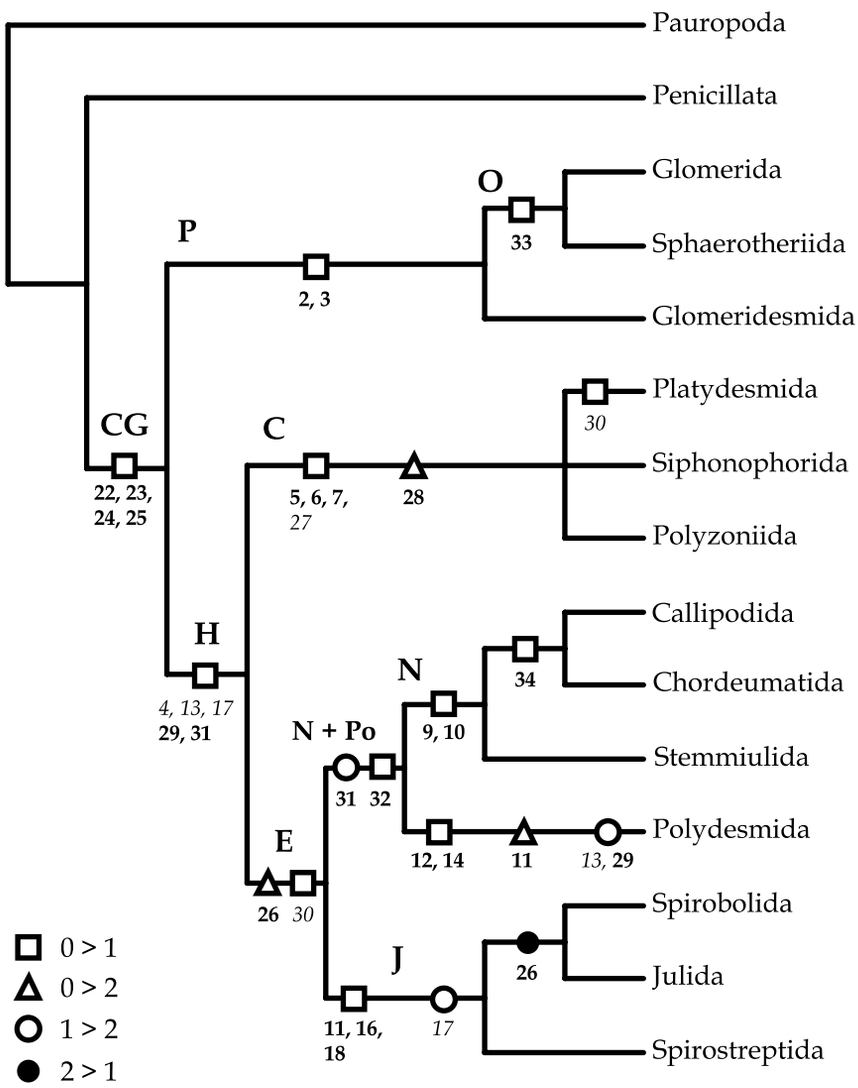


Fig. 1. Preferred cladogram of three, including all millipede orders except Siphoniulida, based on data matrix in Appendix B. Tree statistics: length 53 steps; CI 0.85 (excluding uninformative characters 0.83); RI 0.904; RC 0.77. Character numbers correspond to our numbers in Appendices A and B. The symbols denote unambiguous character state changes, with the state change indicated. Non-homoplastic character state changes bold; characters with a CI lower than 1 are indicated by italics. Abbreviations on tree branches: C: Colobognatha; CG: Chilognatha; E: Eugnatha; H: Helminthomorpha; J: Juliformia, N: Nematophora; Po: Polydesmida (Merocheta); O: Oniscomorpha

and redefined, diverting from Enghoff's indicated coding, as explained in the character discussion. Several of Enghoff's characters were reorganized by combining two of his binary characters into multistate series, if the attributes were logically dependent, e.g. characters 1, 4, 8, 11, 26–29 (see Wilkinson 1995, for rationale regarding composite and reductive character construction). Unknown character states were scored with '?'; the only truly non-applicable character state (in our character 5) was coded as a separate, fourth state in the outgroup (Pauropoda). The data matrix was analysed using PAUP* Version 4 (Swofford 1998) on a Power Mac G 4. The data matrix contains 34 characters, 11 multistate and 23 binary characters. Multistate characters were unordered; all characters were equally weighted. We employed the branch and bound algorithm. We ran two analyses on the data set: the first excluded the Siphoniulida; in the second analysis, the Siphoniulida were included. Since inclusion of Siphoniulida yielded seven most parsimonious trees and the resulting consensus tree showed low resolution, we reran the data matrix under successive weighting. Character optimization followed ACCTRAN. Character optimization was investigated using MacClade (Maddison and Maddison 1992).

Results

Methodology of the previous phylogenetic analysis

In 1984, Enghoff presented the first superordinal phylogenetic analysis of the Diplopoda using cladistic principles based mainly on morphological and developmental characters. Since this analysis predates the advent of computer-aided analyses, Enghoff listed 36 characters, indicating the presumed plesiomorphic and apomorphic character states, based clearly on outgroup comparison. The Pauropoda have been and still are considered the undisputed sister-taxon to the Diplopoda, based on several characters in the body plan (two sets of mouth parts, a collum segment and opening of the gonopore in the region of the 2nd leg pair), and serve as the outgroup in the current analysis. Enghoff's analysis used a ground-plan approach for ordinal and supra-ordinal terminals (see Yeates 1995 for a discussion about the exemplar versus ground-plan approach). In all cases, he carefully discussed the distribution of character states, including possible convergences, reversals and thus potential homoplasy. It was possible to construct the data matrix (Appendix B) and develop the character discussion (given later) from Enghoff's 1984 table. Certain 'characters' appear to have been reorganized from the original description, but most of the changes were necessitated by entering the data into a data matrix. In Enghoff's study, characters or states were listed as attributes supporting certain groupings, rather than listed as exclusive characters with all their states scored across terminals. Thus, in cases when we

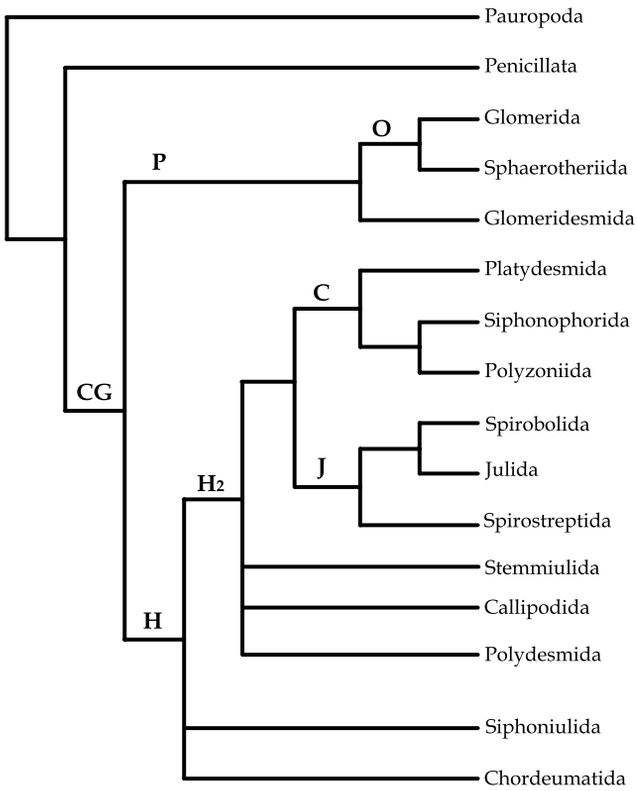


Fig. 2. Strict consensus tree of seven, including all millipede orders and Siphoniulida. Branches are labelled as in Fig. 1

apparently diverted from the original we reorganized two binary 'characters' into a single multistate character series, as explained in the 'Method' section. The only substantive change we made from Enghoff's original scheme is the reorganization of the helminthomorph gonopod characters (our character numbers 26–28), a possibility already raised by Enghoff et al. (1993: 211). In all other cases, we mirrored Enghoff's delineation of characters and the distributions of their states, even if several of them appear to deserve a closer study (e.g. character 31, ontogenetic development of the gonopods). In some cases, Enghoff briefly cited a publication (e.g. Hennings 1904, 1906), without discussing the character in detail, e.g. states of the Tömösvary organ (character 34). Such character systems should certainly be re-evaluated in future analyses.

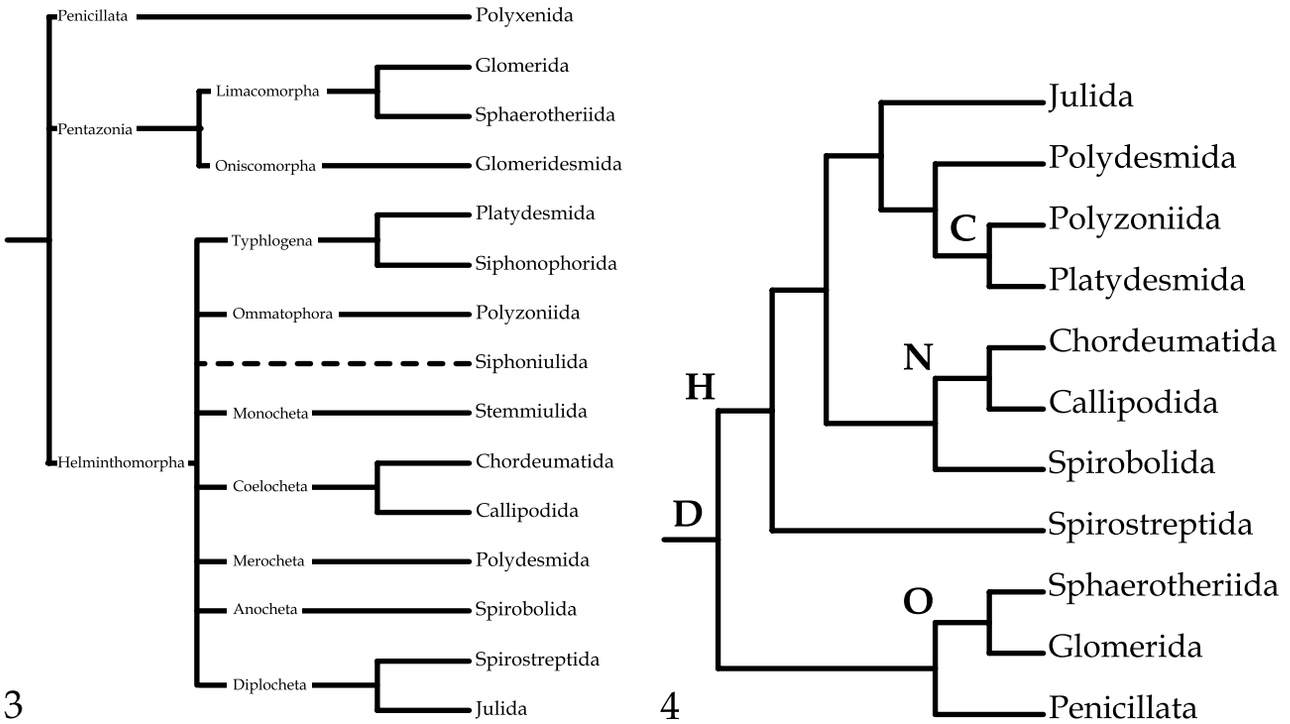
Results of previous phylogenetic analysis

Enghoff's results mirrored closely the traditional classifications of Attems (1926) and Verhoeff (1910), with the Helminthomorpha [H] comprising two clades: Colobognatha [C] (orders Platydesmida, Polyzoniida and Siphonophorida) and Eugnatha [E], consisting of the clades Nematophora [N] (orders Callipodida, Stemmiulida and Chordeumatida), Merocheta [Po] (order Polydesmida) and Juliformia [J] (orders Spirobolida, Spirostreptida and Julida); compare to our Figs 1 and 2 in this study. The relationships of the three eugnathan clades remained unresolved in Enghoff's analysis. Hoffman's (1980, see 3 Fig. 3) classification recognized the same orders, but the relationships among most helminthomorph orders were unresolved; he did not recognize the Eugnatha, Colobognatha, Nematophora or Juliformia. He argued against most supraordinal groupings, invoking the possibility of convergences in the case of the Colobognatha (Hoffman 1980: 45), or stressing the importance of maintaining homogeneity among groupings, arguing against a Nematophora clade (Hoffman 1980: 45). Enghoff et al. (1993: 116) discussed some additional characters, which slightly modified the previous tree, mainly by supporting sister-group relationships between the Merocheta (Polydesmida) and Juliformia, between the Glomerida and Sphaerotheriida and Chordeumatida and Callipodida (compare to Fig. 1).

Character discussion

The following discussion describes the characters and states we changed from the original scheme (see Appendix A). Each time we specify the difference in the character numbering. We altered Enghoff's character 1 and scored serrate setae as absent for the Pauropoda. Our literature search did not indicate the presence of serrated setae in Pauropoda and Ulf Scheller (in litt.) confirmed that he has not found any in Pauropoda to date. This modification also eliminates Enghoff's character 27, which identified the absence of serrated setae as a potential apomorphy of the Chilognatha. Characters 2 and 3 follow Enghoff (1984). Enghoff's 1984 analysis repeatedly used attributes of the repugnatory glands and gland openings. One problem, noted by Enghoff, is the uncertain homology of the repugnatory glands in the Glomerida versus the helminthomorph orders. We combined Enghoff's binary characters 4 and 26 into one multistate character (our number 4). By coding the glomerid middorsal glands and the helminthomorph paired lateral glands in an unordered multistate series, the test of homology will be performed through the analysis and no *a priori* decision is entered into the data matrix. We redefined Enghoff's characters 5 and 31. Three independent multistate characters, one for each leg pair, were constructed. Each leg pair, the 8th and 9th (7th ring) and the 10th (anterior legs of 8th ring) are treated as independent characters, each with their own states (for coding see Appendix A). Note that the Chordeumatida were scored as polymorphic for these three characters, following Shear's (2000) ordinal analysis. Enghoff's characters 6–11 were coded and scored as he suggested. Also, our characters 12–22, 24–26, 32 and 33 mirror Enghoff's, with an altered numerical order (Appendix A). We consolidated four of Enghoff's binary characters into multistate ones: his characters 12 and 32 are incorporated in our number 29 (segment number); his characters 13 and 17 are reflected in our number 11 (degree of fusion of the sternum to the pleurotergites). Coding Enghoff's character 34 (anterior gonopods without/with flagella) was not possible, since we redelineated the gonopod characters throughout. Enghoff (1984: 20) noted the fundamental homology problem influencing the coding and scoring of this character. Our characters 33 (enlarged 2nd tergite and anal shield in Glomerida and Sphaerotheriida) and 34 (structure of the Tömösvary organ, see Hennings 1904, 1906) were taken from Enghoff et al. (1993: 116).

Siphoniulid character states were scored based on the data presented herein (see Appendix C). The states for the following characters are presently unknown: characters 6 (number of 1st larval legs), 7 (potential protection of their eggs), 14 (synovial cavity), 15 (legs of 2nd larvae), 18 and 20 (spermatozoa), 24 (intersegmental tendons), 25 (particular muscles, see Enghoff 1984), 31 (development of gonopods),



Figs 3 and 4. Alternative phylogenies for the Diplopoda. (3) Diplopod phylogenetic relationships according to Hoffman's classification (1980). (4) Cladogram of diplopod orders by Regier and Shultz (2001)

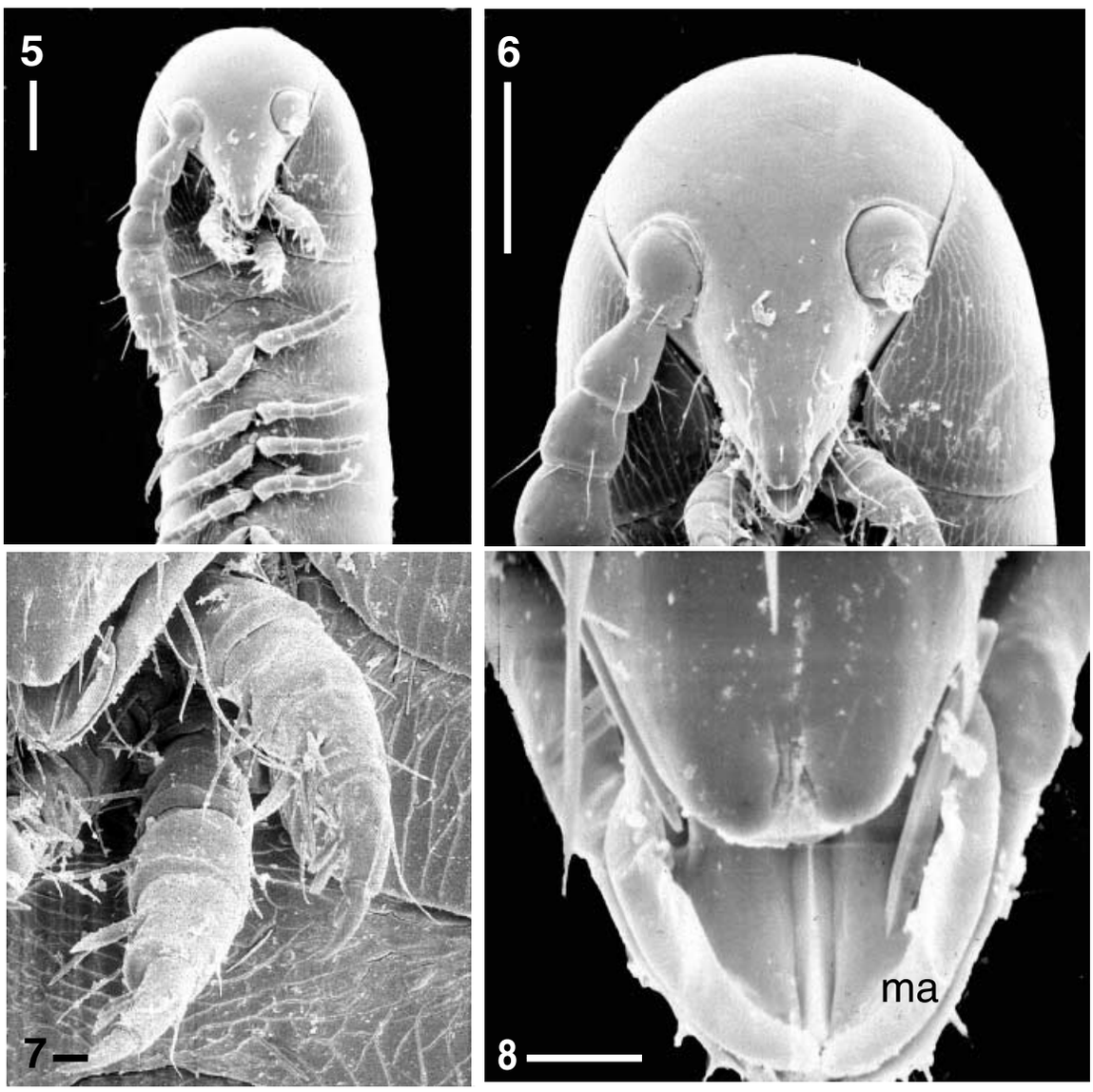
and 32 (position of male gonopore). Serrate setae (1, our character numbers in parentheses) and telopods (2) are absent and the sternite (3) is entire. Since the order lacks ozopores, we assume the absence of defence glands. Thus, several characters are not applicable to the Siphoniulida, position and form of defence glands (4, 8, 13) and the type of secretion (17) and are scored as absent. We assigned a distinct siphoniulid-state to the mouth parts (5). While they superficially resemble colobognathan mouth parts, siphoniulids possess gnathochilarial palps, which do not occur in colobognathan taxa. Clearly, millipede mouth parts deserve comparative study across taxa, which may result in additional characters and a more accurate definition of character states. Spinnerets (9) are present although their homology with those in other orders is uncertain. The mandibular cusps (10) appear to be absent. The sternites (11) were scored as being fused to the pleurites, with a visible suture; eyes (12) are absent. The collum (16) is large, covering the posterior edge of the head. Siphoniulida have diplosegments (19) and their antennae (21) display four cones; the cuticle (22) is calcified; and trichobothria (23) are scored as absent (see scanning electron micrographs below). Scoring characters 26–28 was straightforward, in Siphoniulida only the 8th leg is modified to form the gonopods. Siphoniulids have numerous segments (29) and the pleurotergites are fused (30).

Result of phylogenetic analysis

We conducted two PAUP runs, one excluding and one including Siphoniulida. The character matrix excluding Siphoniulida yielded three trees. In this data set, six of the 34 characters included are autapomorphies (characters 1, 12, 14, 19–21; our numbers). The three trees differ only in their resolution of the Colobognatha. In two, the relationships of the three colobognath orders are resolved into two alternate sister groups: Siphonophorida + Polyzoniida and Platydesmida + Polyzoniida. In the third tree (Fig. 1), which is identical with the strict consensus tree, the colobognathan orders form a polytomy, since the sister taxon relationship suggested by the discarded trees is supported only by the reversal of character 30 (pleurites free/fused). As Enghoff (1984: 20) noted, the fusion of pleurites to tergites in the Playdesmida is most likely an independent gain compared to the Eugnatha. The preferred tree (Fig. 1) agrees well with the traditional view of millipede systematics and Enghoff's analysis from 1984, with the only exception that in this analysis, the Polydesmida form the sister to the Nematophora, supported by unambiguous character state changes in characters 31 (gonopod development) and 32 (male gonopore position). All traditional supra-ordinal clades are recovered and well supported by this analysis.

Character optimization and clade support

Not surprisingly, all clades are supported largely by the same character state transformations (Fig. 1) Enghoff used in his 1984 analysis. Thus, coding them into a data matrix confirmed the traditional, morphology-based classification dating back to Attems and Verhoeff. The Chilognatha (CG) are supported by four character state changes (characters 22–25), which were also recognized in Enghoff's 1984 analysis, except that we reorganized Enghoff's character 27 (absence of serrate setae, see above), and the absence/presence of the paired lateral repugnatory gland (our number 4, E26). The latter supported the Chilognatha in Enghoff's analysis. As the characters are presently scored in our analysis, all defence

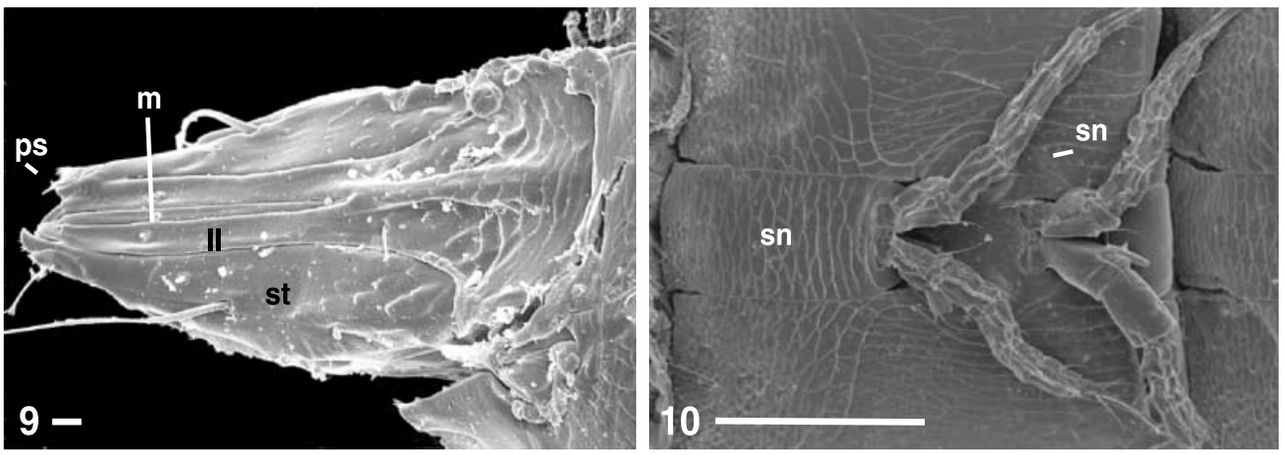


Figs 5–8. *Siphoniulus* aff. *neotropicus*. Head and frontal body section, ♂ specimen FMHD 83-813; critical-point dried; SEM images. (5) Head and front body section, left antenna removed. (6) Head enlarged, basal antennomeres of right antenna visible. (7) Legs 1 and 2, left side. (8) Apex of rostrum enlarged, mandible tips (*ma*) and inside of gnathochilarium visible. Scale bars – 5 and 6: 100 μm; 7 and 8: 10 μm

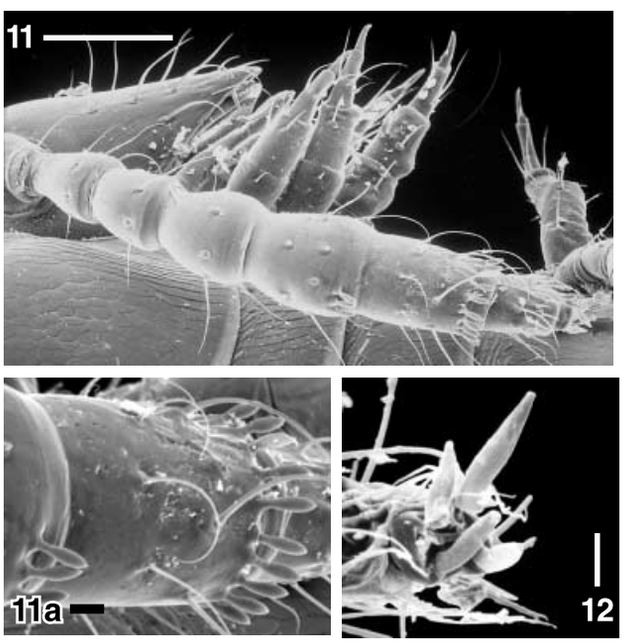
glands characters [4, 8 (ambiguous), 13, 17] support the Helminthomorpha [H], but this is certainly an inflation dependent upon the current character definition. However, defence gland characters will be defined in the future, the occurrence of paired lateral defence glands will most likely remain one of the major apomorphies of the Helminthomorpha. One of Enghoff's characters (adults generally with more than 21 body rings, our number 29) and the gonopod development (character 31) support the Helminthomorpha. Both characters will need re-evaluation.

One of the main helminthomorph characters, 'Legs of the 7th male segment transformed into copulatory structures (gonopods)' no longer supports this clade. This is certainly due to our re-definition of the gonopod characters (see our numbers 26–28). The presence of male gonopods on the 7th ring has been traditionally accepted as the major unifying character of the Helminthomorpha (e.g. see Pocock 1887: 294; = Proterandria, Verhoeff 1895: 17) and was used by Enghoff in his analysis (Enghoff 1984: 20, E31), treating both leg pairs of the 7th ring as one character unit. The concept of treating the legs of this ring as a character unit may have been influenced by the fact that legs in the Diplopoda are serial homologues, and may have been enforced by the existence of diplosegments, the fact that most body rings in the millipede body consists of two fused segments. However, the presence of unmodified walking legs in the anterior as well as posterior position of ring seven in different groups compellingly suggests exploring alternative treatments of the character(s), like coding the anterior and posterior legs and their modifications independently, as we have done here.

The Pentazonia (P) are supported by two unambiguous characters (characters 2 and 3) and its arrangement agrees with Enghoff's analysis. Due to our re-evaluation of the defence gland traits, the third character in Enghoff's analysis is not realized here. The Oniscomorpha (O) are supported, as suggested by Enghoff et al. (1993), by the enlarged 2nd



Figs 9 and 10. *Siphoniulus* aff. *neotropicus*. ♀ Specimen FMHD 83-818; SEM images. (9) Gnathochilarium, specimen damaged during critical point-drying; *st* stipes, *ps* palps of stipes, *m* mentum, *ll* lingual plates. (10) Anterior and posterior sternites (*sn*), mid-body section, specimen air-dried. Scale bars – 9: 10 μm; 10: 100 μm

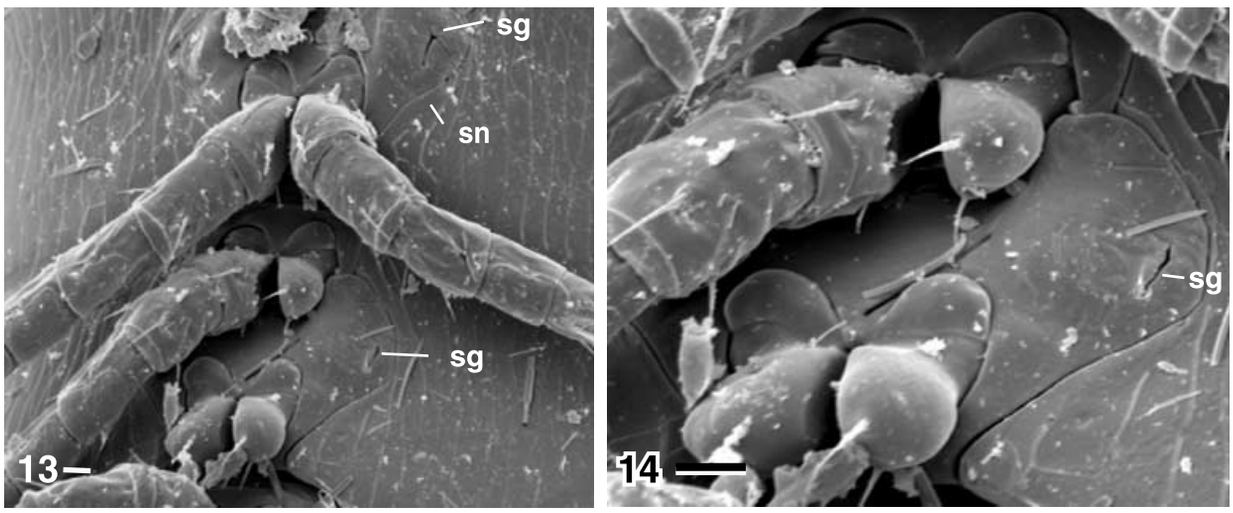


Figs 11 and 12. *Siphoniulus* aff. *neotropicus*. Antenna; SEM images. (11) Side view of frontal body section, antenna with apical clavate setae on antennomeres 5 and 6, ♂ specimen FMNH 83-813, critical-point dried. (11a) Antennomeres 5 and 6 enlarged, showing specialized setae. (12) Tip of antenna showing four long apical sensory cones, ♀ specimen FMHD 83-818, air-dried. Scale bars – 11: 100 μm; 11a and 12: 10 μm

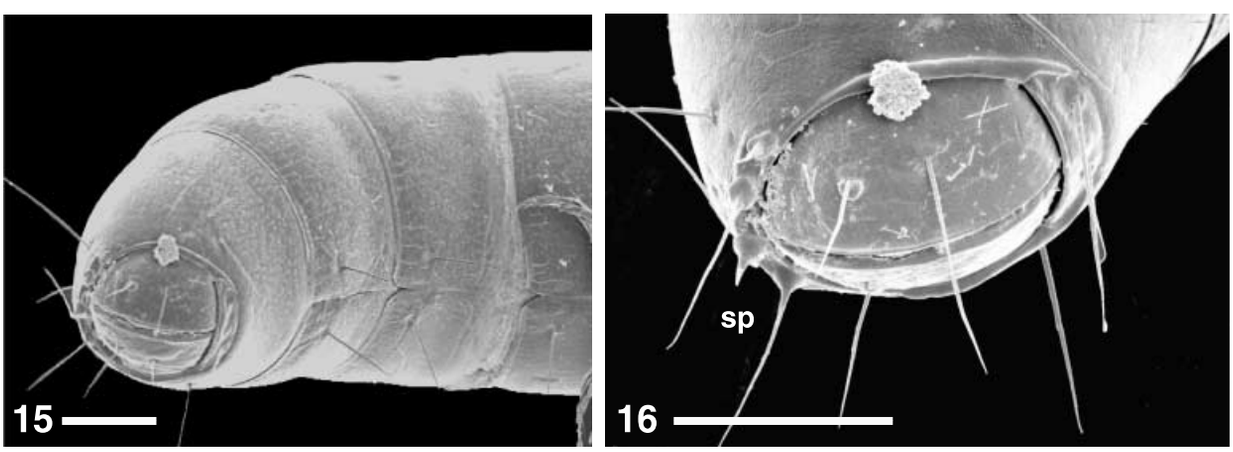
tergite and the anal shield (our character 33). In our analysis, the character optimizations for the Colobognatha (non-homoplasious characters 5–7 and 28) and Nematophora (characters 9 and 10) mirrors Enghoff's closely; the shape of the defence glands (our character 8, ambiguous) supports the Helminthomorpha, not the Colobognatha, as discussed by Enghoff (1984: 13). For the Eugnatha, only one non-homoplasious unambiguous character state change remains: the transformation of the 8th leg into the functional gonopod (character 26, with subsequent transformation to an accessory gonopod in the Spirobolida-Julida clade). The fusion of the pleurites to the tergites (character 30) occurs here and independently in the Platydesmida. The clade Nematophora + Polydesmida is supported by the male gonopore position (character 32, as Enghoff suggested in 1984) and the gonopod development mode (character 31). The Polydesmida are characterized by five unambiguous character state changes, as listed by Enghoff. The Juliformia, supported by four unambiguous character state transformations, also follow Enghoff's analysis.

Including the Siphoniulida in the analysis yielded seven most parsimonious trees, with a length of 58 steps (CI 0.79, RI 0.87, RC 0.68). Twenty-nine of the 34 characters were parsimony informative. Depicted in Fig. 2 is the strict consensus tree of these seven trees. In contrast to the results from the previous analysis excluding Siphoniulida, the strict consensus tree is not identical to one of the seven source trees and should not be interpreted as suggesting a modified classification. The consensus tree simply gives an overview of the elements common to all seven trees, each of which had higher resolution than the consensus tree. In all seven original trees the Chilognatha, Helminthomorpha, Pentazonia, Colobognatha and Juliformia are recovered and, except for the Helminthomorpha, supported mostly by the same characters as in the previous analysis. The helminthomorph characters are distributed over two nodes (marked H and H_2 in Fig. 2), with all defence gland character state changes concentrated on the more terminal node. The Eugnatha were not recovered in any tree.

In this analysis, the Colobognatha appear nested higher in the cladogram, a position that requires a highly doubtful reversal of character 26 (8th leg; from state 2 to state 0). This character optimization implies the reversal from a highly modified, functional gonopod into a standard walking leg. The Nematophora appear as a reduced group, containing Stemmiulida and Callipodida only, in five of the seven trees, supported in those five by character 10 (presence of molar cusps) alone, which is homoplastic under this optimization (because it also occurs in the Chordeumatida). The spinneret character state change (character 9, presence of spinnerets) is realized at node H and subsequently lost. The third nematophoran order, Chordeumatida, consistently appears



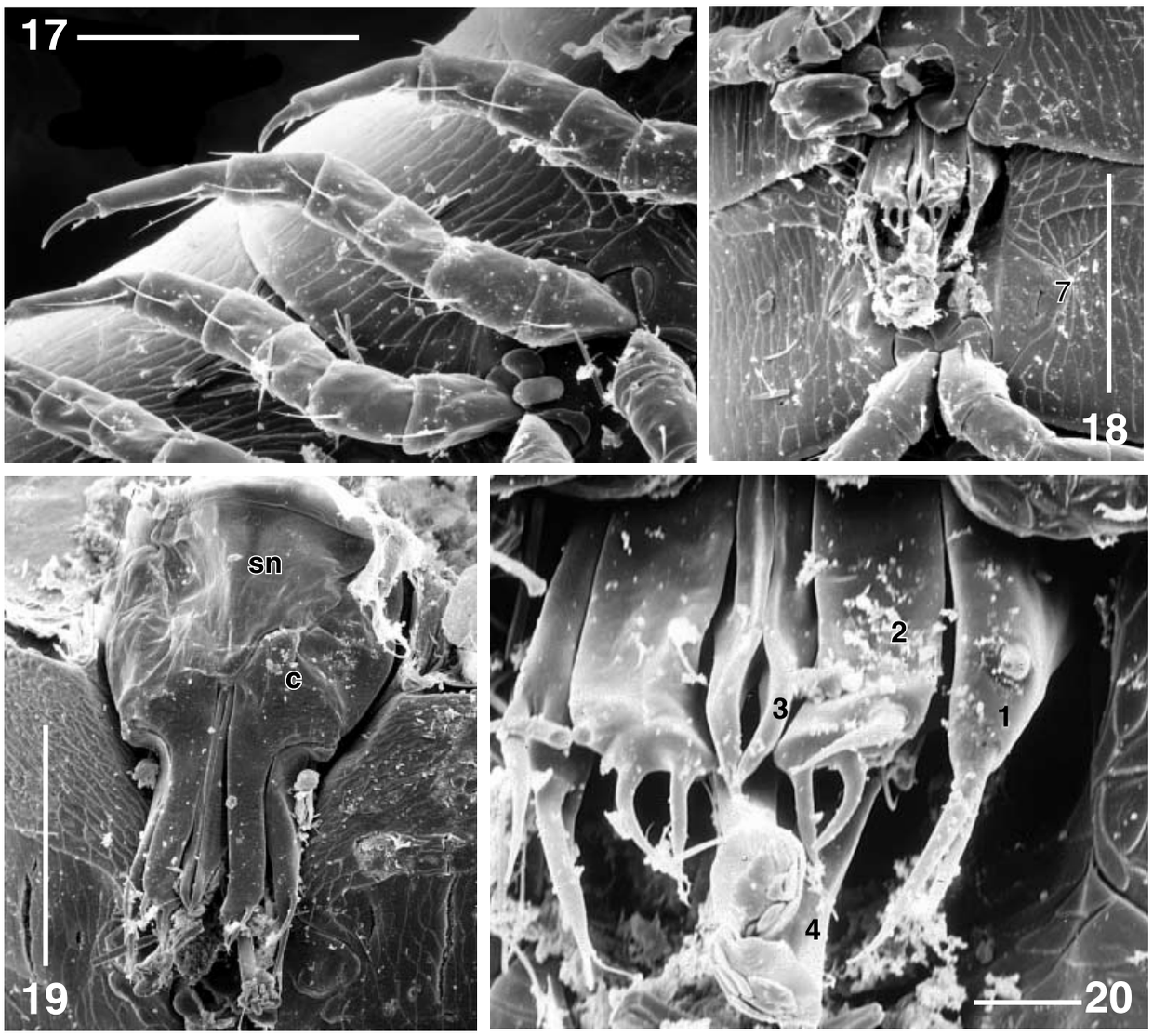
Figs 13 and 14. *Siphoniulus* aff. *neotropicus*, ♂ specimen FMHD 83-813, critical-point dried; SEM images. (13) Mid-body section, ventral view. *sn* sternum, *sg* stigma. (14) Leg base enlarged, stigma. Scale bars – 13 and 14: 10 μm



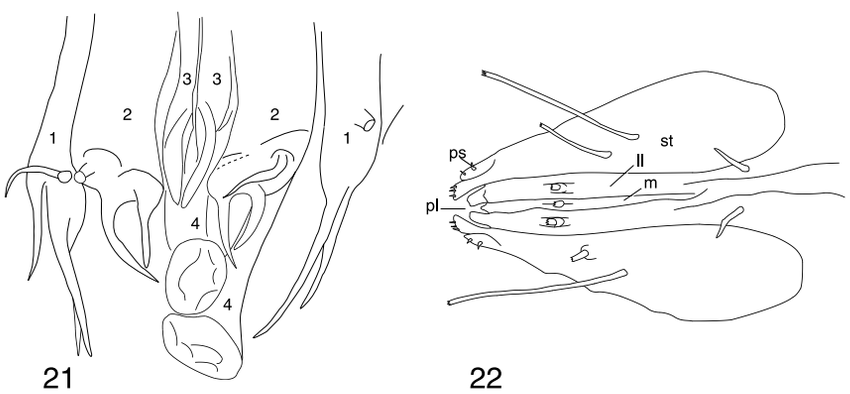
Figs 15 and 16. *Siphoniulus* aff. *neotropicus*, ♀ specimen FMHD 83-818, caudal end, air-dried; SEM images. (15) Caudal end with apodous segments showing pairs of ventral setae. (16) Caudal end enlarged, *sp* possible spinnerets. Scale bars – 15: 10 μm; 16: 100 μm

together with the Siphoniulida at the base of the Helminthomorpha, a position caused by the shared absence of defence glands and the presence of spinnerets. It must be noted that both character states are possibly independently derived; i.e. the lack of defence glands in Chordeumatida may be secondary and the spinnerets of the Siphoniulida may or may not be homologous to those in the currently still valid taxon Nematophora. In three of the seven trees, Siphoniulida and Chordeumatida form a sister-group, supported by the (homoplastic) presence of spinnerets. In four of the seven trees, the Polydesmida are grouped with the Colobognatha and Juliformia, in each of the remaining trees they occupy different positions. Despite the persistence, this grouping appears to be spurious, since it is supported by one reversal (character 9) and an ambiguous state change (character 15, 2nd larvae with seven leg pairs). The hypothesized loss of spinnerets (character 9) is simply a result of the current optimization of this character on these trees and is unlikely to reflect true phylogenetic relationship. As with most other character systems, spinnerets deserve a closer comparative look, especially since Adis et al. (2000) reported spinneret-like structures and spinning behaviour in a juvenile of a species of Polydesmida. Encouraged by Adis et al.'s description of polydesmidan spinnerets, we reran the data matrix with spinnerets (character 9) scored as present in the Polydesmida. The run, which included the Siphoniulida, resulted in 16 most parsimonious trees (58 steps, CI 0.793, RI 0.868, RC 0.689) and an identical consensus tree as depicted in Fig. 2.

The most curious result of our analysis is the persistence of the clade Juliformia + Colobognatha, supported by reversals in characters 31 (gonopod development) and 32 (male gonopore position). The former is poorly understood throughout the Diplopoda. Commonly (e.g. Enghoff 1984; Enghoff et al. 1993), a distinction between 'abrupt' and 'gradual' development is described, but to date, the different modes have not been comparatively described across millipede orders (Sierwald and Bond, unpublished). The postulated reversal of the position of the male gonopore is an unconvincing character state change and may be due to the



Figs 17–20. *Siphoniulus* aff. *neotropicus*. Legs and gonopods, two ♂ specimens: FMHD: 83-813, critical-point dried; SEM images. (17) Mid-section of body, ventral view, showing legs. (18) Male gonopods, *7* indicates number of body ring. (19) Male gonopods, second specimen of sample, anterior body section removed; *sn* sternite, *c* coxae. (20) Gonopods of specimen in '18', enlarged. Scale bars – 17–19: 100 μm; 20:10 μm



Figs 21 and 22. *Siphoniulus* aff. *neotropicus*. Drawings, composites from several scanning electron micrographs. (21) Male gonopods. (22) Gnathochilarium; *ll* lingual plates, *m* mentum, *pl* palps of lingual plates, *ps* palps of stipes, *st* stipes

overall lack of characters employed in millipede phylogenetic reconstruction. The lack of resolution of the data set including the Siphoniulida was not improved by using successive weighting of characters. The same seven trees resulted and the strict consensus tree is identical to the one in Fig. 2, an indication that the characters currently employed are unable to satisfactorily resolve millipede phylogeny (Kitching et al. 1998: 112).

Siphoniulids clearly possess one helminthomorph feature, undivided sternites each with a pair of walking legs and stigmata (Verhoeff 1928: 27, 30) as opposed to divided sternites as in the Pentazonia. However, the polarity of this character is unclear. The current position of Siphoniulida results from numerous characters that are unknown and the fact that it is supported mainly by homoplasious character state changes (numbers 11, 12 and 16), because the Siphoniulida share characters with both the Juliformia and Polydesmida. Of course, any one of these could be independently derived (e.g. the loss of eyes, or the sternite fusion) and if true the current coding of the states and their scoring would need to be altered accordingly.

Discussion

Despite the fact that males are now known for the order, the Siphoniulida are still Helminthomorpha *incertae sedis*. Hoffman (1980: 48) hoped that he would '… eventually have the pleasure of seeing the dénouement of this perplexing diplopodous riddle' referring to the relationship of Siphoniulida to other helminthomorph groups, once males were to be discovered. Pocock (1894) placed the immature female of *Siphoniulus albus* in the Colobognatha Brandt, 1834, which he considered as one of five suborders of the order Helminthomorpha. This placement was based on the pyriform head, since without a male, the position of the gonopods could not be established. Enghoff (1984: 24) assumed that the Siphoniulida are possibly '… a specialized subordinate taxon within some juliform or colobognathan order', and Hoffman (1980: 49) suggested placement close to either the Julida or Spirostreptida.

Our study does not add new characters or character systems, nor does it claim to use a large amount of existing data that have never been employed for supra-ordinal classification purposes. The phylogenetic analysis performed here, including the Siphoniulida, produced a consensus tree that bears some resemblance to Hoffman's 1980 classification in that the Eugnatha cannot be recovered (see Fig. 3). Regier and Shultz's (2001) tree, based on protein-coding gene (EF-1α) and protein amino acid sequences (Pol II), provides another hypothesis (Fig. 4). We compared our three trees from the first data set (excluding Siphoniulida) with Regier and Shultz's tree; in order to map the morphological and developmental characters on their tree, 10 to 11 additional steps are required. Regier and Shultz (2001: 483) stressed that their study focussed on recovering test clades rather than attempting to provide a new phylogeny. The currently available and conflicting hypotheses all appear to suffer from the same problem: the paucity of well documented and thoroughly examined morphological characters. Morphology-based millipede phylogenies will certainly change and improved over time, especially when character systems will have been explored in more detail. What is needed now are not only better substantiated homology-hypotheses for various character systems, e.g. sclerite homology within male gonopods, morphological characters of the female copulatory apparatus, but also more data on the distribution of character states across taxa. Most likely, some of these data already exist; the older literature focussing on comparative morphology (mainly Verhoeff and Attems, but also Hennings 1904, 1906, and numerous others) represents an unexploited treasure trove of potentially useful characters for a modern phylogenetic analysis. As one of the next steps in millipede phylogenetic research, an exemplar approach should be attempted employing species as terminals with a judicial and well-argued taxon selection rationale.

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Zusammenfassung

Die Phylogenie der Diplopoda revidiert im Lichte der rätselhaften Ordnung der Siphoniulida

Die Entdeckung von 6 Exemplaren der ungewöhnlichen Ordnung Siphoniulida, einschließlich der ersten Männchen, erforderte eine Neu-Analyse der derzeitig diskutierten, morphologisch begründeten Klassifikationen der Klasse Diplopoda. Die erstellte Datenmatrize wurde in PAUP analysiert. Die sich ergebenden phylogenetischen Hypothesen bestätigen in wesentlichen die früheren, traditionellen Klassifikationsschemata; allerdings zeigt diese Analyse auch, dass die Merkmalsbasis erweitert werden muss, um eine höhere Auflösung zu erreichen. Neuere, abweichende phylogenetische Klassifikationen und Kladogramme werden unter Berücksichtigung der jetzigen Analyse verglichen. Die Gültigkeit einer der vorgeschlagenen Apomorphien für das Taxon Helminthomorpha, die Position der Gonopden am 7. Körperring, wird diskutiert. Rasterelektronenmikroskopische Untersuchungen bestätigten die morphologischen Merkmale, welche bereits für die Siphoniulida angegeben wurden: modifizierte Vorderbeine, scheinbar beinloser dritter Körperring, kleiner, zugespitzter Kopf, Antennen mit abgeflachten Borstenhaaren, fehlende Saftlöcher. Die männlichen Gonopoden werden hier zum ersten Male beschrieben: nur das vordere Beinpaar des 7. Ringes bildet die Gonopoden, das hintere Beinpaar desselben Ringes ist als normales Laufbeinpaar ausgebildet. Die Gonopoden sind stark modifizierte Strukturen. Das Gnathochilarium weicht deutlich von dem der Colobognathen ab, es besteht aus tastertragenden Stipites, langgestreckten Lingula Lamellae mit Tastern und einem zentralen, schmalen Sklerit, wahrscheinlich dem Mentum. Der Epiproct trägt vier Strukturen, die wahrscheinlich als Spinngriffel zu interpretieren sind. Trotz der Entdeckung von Männchen müssen die Siphoniulida weiterhin als Helminthomorpha *incertae sedis* angesehen werden.

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Appendix A

Characters, their states and coding. Character numbers in brackets denote character numbers used by Enghoff (1984, p. 22, Table 2)

1. Serrate setae [E1 + E27]
   0 = absent
   1 = present, arranged in tufts
2. Telopods [E2]
   0 = absent
   1 = present
3. Sternite [E3]
   0 = entire
   1 = divided
4. Defence glands [E4 + E26]
   0 = absent
   1 = lateral
   2 = middorsal
5. Mouth parts [E6]
   0 = standard diplopodan
   1 = colobognathan type
   2 = siphoniulid type
   3 = non applicable
6. 1st larvae with [E7]
   0 = three leg pairs
   1 = four leg pairs
7. Egg protections [E8]
   0 = adults do not curl around eggs
   1 = adults curl around eggs
8. Defence glands [E9]
   0 = absent
   1 = subtubular
   2 = subspherical
9. Spinnerets [E10]
   0 = absent
   1 = present
10. Molar cusps [E11]
    0 = absent
    1 = present
11. Pleuro-sternites [E13 + E17]
    0 = not fused
    1 = fused, with suture
    2 = fused, without suture
12. Eyes [E14]
    0 = present
    1 = absent
13. Defence glands [E15]
    0 = absent
    1 = simple
    2 = bicompartmental
14. Synovial cavity [E16]
    0 = absent
    1 = present
15. 2nd larvae [E18]
    0 = without seven leg pairs
    1 = with seven leg pairs
16. Collum [E19]
    0 = small
    1 = large
17. Defence secretions [E20]
    0 = no defence secretions
    1 = without benzoquinones
    2 = with benzoquinones
18. Spermatozoa [E21]
    0 = without pseudoperforations
    1 = with pseudoperforations
19. Segments [E22]
    0 = single
    1 = diplo

20. Spermatozoa [E23]
    0 = flagellate
    1 = aflagellate
21. Antennae [E24]
    0 = without four apical cones
    1 = with four apical cones
22. Cuticle [E25]
    0 = non-calcified
    1 = calcified
23. Trichobothria on head [E28]
    0 = present
    1 = absent
24. Ventral intersegmental trunk tendons [E29]
    0 = present
    1 = absent
25. Certain muscles [E30]
    0 = present
    1 = absent
26. 8th leg in adult males [E5 + E31]
    0 = walking leg
    1 = accessory gonopod
    2 = functional gonopod
27. 9th leg in adult males [E5 + E31]
    0 = walking leg
    1 = accessory gonopod
    2 = functional gonopod
    3 = reduced
28. 10th leg in adult males [E5 + E31]
    0 = walking leg
    1 = accessory gonopod
    2 = functional gonopod
    3 = reduced
29. Number of body rings [E12 + E32]
    0 = up to 21
    1 = more than 21
    2 = subconstant, 18–29
30. Pleurites [E33]
    0 = free
    1 = fused to tergites
31. Gonopod development [E35]
    0 = gonopods absent
    1 = gradual
    2 = abrupt
32. Male gonopore position [E36]
    0 = behind 2nd coxa
    1 = through coxa of 2nd leg
33. 2nd tergite [Enghoff et al. 1993]
    0 = normal size
    1 = enlarged
34. Tömösvary organ [Enghoff et al. 1993]
    0 = normal structure or absent
    1 = special structure

Appendix B

Datamatrix of 34 characters and 15 ingroup and one outgroup (Pauropoda) taxon

Pauropoda	00003?0000000000000000000000000000
Penicillata	10000000000000000011100000000000000
Glomerida	01120000000000000011111110000000010
Glomeridesmida	01100000000000000011111110000000000
Sphaerotheriida	01100000000000000011111110000000010
Platydesmida	00011111000010001011111111012111000
Siphonophorida	0001111?000010001011111111012101000
Polyzoniida	00011111000010001011111111012101001?
Siphoniulida	00002??010110??10?1?111??20011??00
Stemmiulida	00010002110010001011111112301121 00
Chordeumatida	00000000110000000011111111AAB112101
Callipodida	00010002110010001011111111200112101
Polydesmida	00010002002121101011111111200212100
Spirobolida	00010002001010112111111111120111000
Spirostreptida	00010002001010112111111111230111000
Julida	00010002001010112111111111120111000

Polymorphisms: 1 and 2 = A; 0 and 1 and 2 = B.

Appendix C

Taxonomic descriptions

Pocock (1894: 340) described the first specimen in his newly proposed family Siphoniulidae and placed it in what was then the suborder Colobognatha of the order Helminthomorpha (Colobognatha is now considered a subterclass of the Infraclass Helminthomorpha, Subclass Chilognatha; Enghoff 1984; Enghoff et al. 1993: 116). Cook (1895) was the first to propose ordinal-group status, erecting the suborder 'Siphoniuloidea' (today the '… oidea' suffix is reserved for the superfamilial level). Despite Cook's (1895: 6) statement that the '… absence of legs from the third and fourth segments [see note below under *S. albus*] indicates want of affinity with the Colobognatha, while the form of the head and antennae will doubtless exclude it from the Diplocheta' (comprising the Spirostreptida and Julida), he listed the 'Siphoniuloidea' in the Diplocheta. Hoffman and Lohmander (1964: 111) included the order Siphoniulida in their listing for the Diplopoda without commentary, the first usage at this taxonomic rank. Hoffman (1979) noted that males were required to enable systematic placement. Here, we report two males, a female, and several juvenile specimens from Veracruz and Chiapas, Mexico, in the collection of the Field Museum, Chicago, and provisionally assign them to the geographically proximate species, *Siphoniulus neotropicus* Hoffman. The locality in Palénque, Chiapas, is around 260 km west of the type locality in Petén Dept., Guatemala, the locality at Catemaco, Veracruz, is 600 km northwest of the type locality. For the first time, specimens were studied using scanning electron microscopy and morphological details are added to the existing description. The fragility of the specimens and the relatively short legs are noteworthy: siphoniulids superficially resemble nematodes. We surmise that they may be deep humus dwellers that are missed by collectors focusing on larger, subsurface forms. Berlese extraction may be the best method of obtaining siphoniulids and one should probably include substantial humus as well as loose litter.

Order Siphoniulida Pocock 1894, Family Siphoniulidae Pocock 1894

Diagnosis: Small (up to ca. 7.5 mm length), eyeless helminthomorph Chilognatha with conic-pyriform head and slender antennae with seven antennomeres; body cylindrical and polished, almost hairless; ozopores absent; rings without median dorsal sutures, pleurites not evident, 3rd ring legless. Gnathochilarium with distinct lingual plates, stipites and mentum; gonopods developed from first leg pair of 7th ring only.

Component: *Siphoniulus* Pocock 1894.

Distribution: Indonesia, Sumatra; Mexico; Guatemala.

Siphoniulus Pocock 1894

Siphoniulus Pocock 1894: 340.
Siphoniulus, Jeekel 1971: 44; Hoffman 1979: 536; Hoffman 1980: 48, 189;
Hoffman et al. 1996: 28; Hoffman 1999: 180.

Type species: *Siphoniulus albus* Pocock 1894, by monotypy.
Diagnosis: Same as the characters for the family.
Note: Pocock's description of the anterior legs is unclear; he notes '… the 3rd and 4th segments *appear* [emphasis ours] to be apodous, while the 1st, 2nd and 5th are furnished with a single pair of legs each.' Unfortunately, the anterior portion of the type specimen is missing (see below) and the characters thus cannot be established. Pocock likely mistook the large gap at the 3rd ring as apodous segments 3 and 4. In all our specimens, ring 5 is furnished with two leg pairs.
Species: Two.
Distribution: Same as that of family.

***Siphoniulus albus* Pocock 1894**

Siphoniulus albus Pocock 1894: 341, pl. 19, Figs 13, 13a. Female (?) holotype from Sumatra; ZMUA; examined.

Note: The holotype, the only known specimen of this species, consists of two pieces. The caudal piece, with 16 leg-bearing and six legless rings, is cream-white; the other, with nine leg-bearing rings, is brown. Apodous rings at the caudal end each carry a pair of stiff ventral setae. The epiproct is furnished with four stiff, long setae, each with an enlarged base (spinnerets?). The setation on the hypoproct and paraprocts is identical to that of *S. neotropicus* (see Fig. 16). Pocock (1894: 341) reported '*Pores* conspicuous'; however, no ozopores were visible under a dissecting microscope (100×) when the first author examined the specimen. A large section of the anterior body, including the head, is missing, and no diagnostic characters for the species are apparent. Judging from the colour difference, both pieces may not have come from the same individual (see colour description below for the Field Museum's specimens).

***Siphoniulus neotropicus* Hoffman 1979**

Siphoniulus neotropicus Hoffman 1979: 536–539, Figs 1–6. Female or juvenile holotype and paratype from Guatemala; MNHG; examined.

Siphoniulus neotropicus, Hoffman 1999: 180.

Diagnosis: American siphoniulid with up to 45 rings, white to tan or brown, 5th and 6th antennomeres with three to four and nine to 12 apical clavate setae, respectively; both specimens with apodous rings at caudal ends. Distinction from *S. albus* uncertain; lack of specimens of this species, especially males, precludes nomenclatorial changes at present.

Note: The holo- and paratypes (Hoffman 1979: 537) of *Siphoniulus neotropicus* are cream-white. The holotype (head lost) consists of two pieces, a mid-body piece with 11 rings, and a tail piece with 31 rings with two pairs of legs each and four apodous rings at the caudal end. The first three apodous rings each carry one pair of stiff ventral setae, the pre-anal ring, which is twice as long, as the remaining rings does not have ventral setae. The setation on the epiproct, paraprocts and hypoproct is identical to that of our Mexican specimens (Fig. 16). The paratype is complete, consisting of 34 segments with six legless segments at the caudal end, of which five carry pairs of ventral setae. The 7th ring of this specimen has two normally developed walking legs, thus is certainly not an adult male.

***Siphoniulus* aff. *neotropicus* Hoffman 1979, Figs 1–18**

Measurements
The Field Museum's three complete specimens have the following measurements and number of body rings: 14 (including eight legless), 1.8 mm (FMHD 83-838, juvenile); 36 (including six legless); 4.7 mm (FMHD 83-830, ♀?); and 45 (including five legless); 7.5 mm (FMHD 83-818). The other specimens were recovered in pieces and could not be measured.

Description
Coloration. Preserved individuals are uniform in coloration, the colours range from light to almost dark brown. Head (Figs 1, 2, 4): deflexed in ventroposteriad position (Fig. 5), conic-pyriform, drawn out into rostrum, latter broad at base, tapering distally with blunt tip (Fig. 6); no labral teeth (Fig. 8), but with distinct median emargination or shallow groove; ocelli and frontal macrosetae absent; no median frontal suture; three pairs of short setae on rostrum, Tömösvary organ absent. Mouth parts (Figs 8, 9, 22): Gnathochilarium (Fig. 22) composed of well-developed stipites, lingual plates long and narrow, separating the stipites, evidently basally fused, also possibly fused to intermentum–hypostome complex. Stipites with two palps each, lingual plates with one palp each; narrow long sclerite, possibly the mentum, between the lingual plates. Two long macrosetae on each stipes, one seta each on the lingual plates and the mentum (Figs 9 and 22). Transverse basal sclerite as described by Hoffman (1979: 537) not visible on SEM-prepared specimen. Slender, curved, distal sections of mandible tips visible in mouth (Fig. 5). Basal parts of mandibles not visible in side view of head, apparently not extending outward beyond the gnathochilarium.
Antennae (Figs 11 and 12). Positioned laterally along rostrum; seven antennomeres, distinctly constricted between 1st and 2nd; 5th and 6th antennomeres rounded and swollen, 6th antennomere longest (Fig. 11); 5th and 6th antennomeres with apical series of three to four and nine to 12 submarginal clavate (club-shaped) setae, respectively, setae located laterally (Fig. 7a); all antennomeres with few long setae, 6th with two rings of long setae; antennal cones long, completely separated on all sides (Fig. 12). Collum (Fig. 6): dorsally as long as 2nd and 3rd tergites combined; anterior margin covering posterior margin of head; lateral margins curved and extended towards the bases of 1st legs.
Body (Figs 13–16). Long, resembling a nematode. Surface of rings smooth and polished, with few setae ventrally (none dorsally), without median dorsal suture or groove; pleurites not evident, sterna fused to pleuroterga with distinct suture lines (Figs 10, 13 and 14). Pleurite regions of anterior body with striations (Fig. 11). Anterior sternite of each ring narrow, with straight edges, posterior sternites widened in the middle. Slit-like stigmata visible in flared sections of sterna (Figs 13 and 14). Sterna in anterior region may not be completely fused to pleuroterga, since in the air-dried specimen the anterior section of the body contracted strongly, leading to severe distortions. Pair of bean-shaped structures at the base of each walking leg (Fig. 14) in each sternite, possibly part of the coxa. Rings 2 and 3 narrower than following rings (Fig. 4); ring 2

apparently with two pairs of legs, ring 3 appears legless (Figs 4, 7 and 11), ring 4 with one leg pair; ring 5 to legless rings at caudal end with two pairs of legs each (Figs 13, 14 and 17). Body tapering towards caudal end. All specimens with legless rings at the caudal end (Fig. 15); these with one pair of long ventral setae apiece, setae insert submarginally at apical end of each apodous ring; tips of setae diverging, about as long as diameter of body and decreasing in length posteriorly. Pre-anal ring tapering, twice as long dorsally as the penultimate ring. Epiproct not produced, carrying four setae with bulbous bases resembling spinnerets (Fig. 16); paraprocts smooth with two large setae each; hypoproct short and narrow, with two lateral setae.

Legs (Figs 7, 11, 13 and 17). Walking legs 6-jointed and slender, podomeres short, only tarsus elongated, legs medially in contact, tarsal claw long with basal accessory unguis (Fig. 17). Coxal glands apparently absent. Each podomere with one pair of ventrolateral setae, tarsus with one pair of ventral setae (Fig. 17). Legs 1 and 2 modified in all specimens (including juvenile), short and thick, with five podomeres (Fig. 7): 1st podomere not clearly visible, 2nd podomere with distal swollen rim forming a protruding bulge, 3rd and 4th podomeres short, thick and conical, tapering distally, 4th podomere with one large, flat, spatula-shaped seta mesiad. 6th podomere (tarsus) as long as 4th but comparatively slender, carrying two large, flat, spatula-shaped seta mesiad. Tarsal claw large, with juxtaposed shorter (accessory) claw as in the remaining walking legs. Anterior leg pair of 7th body ring modified into gonopods in males, almost completely covered by posterior legs of 6th ring, posterior legs of 7th ring are unmodified walking legs.

Male gonopods (Figs 18–21) arising from anterior leg pair of 7th ring, partially retracted into the body. Gonopod sternum (*sn*) broadly subtriangular in anterior view (Fig. 19); coxae (*c*) swollen, rounded, attached to sternum posteriorly. Distal part of each gonopod consists of four evident divisions. The lateralmost (1) narrow, bowed slightly outward arising posteriorly on coxa, terminating in three acute processes; next mesal division (2) broader, slightly shorter, arising more anteriorly, continuous with body of coxa and ending in three curved, acute processes. Between these are a closely appressed pair of laterally flattened laminae (3), without terminal processes, which appear to arise from the coxa between the two more lateral pairs. Posterior to these three paired divisions lie two long rod-like structures (4) with expanded tips. These structures have been confirmed by SEM studies for both examined males. It is not possible at this time to determine homologies for the gonopod divisions.

List of examined material

Mexio: Chiapas, Ococingo Rd., 71 km S Palénque, Rte 175, 700 m, 5 July 1983, S. & J. Peck, rainforest litter from ravine, two specimens (FMNH, FMHD# 83-818). Bonampak Rd., 100 km SE Palénque, 230 m, 24 July 1983, S. & J. Peck, rainforest litter, one (♀?) (FMNH, FMHD# 83-830); and 8 July 1983, S. & J. Peck, Berlese of rotten sapote fruit, two ♂ (one broken in pieces), additional pieces of more than one specimen (FMNH, FMHD# 83-813). Veracruz, 33 km NE Catemaco, 160 m, Los Tuxtlas Biol. Station, 1 August 1983, Berlese from tree base litter, S. & J. Peck, one juvenile (FMNH, FMHD 83-838). Holotype of *S. albus*: Indonesia: Sumatra: Manindjau [close to Sasak on the east coast], M. Weber, 1888; head missing (ZMUA). Holotype and paratype of *S. neotropicus*: Guatemala: Dept. Petén, Tikal, 20 m, beyond public campground on airfield, 28 December 1975, A. de Chambrier leg. Winckler extraction (MHNG).

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CRYPTIC SPECIATION IN THE *ANADENOBOLUS EXCISUS* MILLIPEDE SPECIES COMPLEX ON THE ISLAND OF JAMAICA

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Abstract.—*Anadenobolus excisus* is a large species of millipede endemic to the Caribbean Island of Jamaica. Initial detailed morphological studies showed little or no discrete variation across this species' distribution in somatic or, in particular, genitalic morphology. However, a molecular survey based on ~1000 base pairs of the mitochondrial (mtDNA) 16S rRNA gene that examines 242 individuals sampled from 54 localities reveals three highly divergent mtDNA lineages. A lack of discrete morphological differentiation suggests that genetic and morphological divergence may be decoupled, a pattern inconsistent with a number of evolutionary models. In contrast to minimal morphological divergence, size variation among mtDNA lineages suggests that character displacement has occurred and that these lineages are cohesive in sympatry. We conclude that *A. excisus* is actually a complex of three cryptic species and that morphological approaches to delineating millipede species may sometimes underestimate evolutionary diversity.

Key words.—Character displacement, Diplopoda, mitochondrial DNA, phylogeography, phylogenetic species, Rhinocricidae.

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''A thorough understanding of the biological properties of species is necessary not only for the evolutionist, but for every biologist'' (Mayr 1963, p. 12). Although written some 40 years ago, Ernst Mayr's dictum is no less valid today. The characterization of ''species'' is particularly important because species definitions can bias phylogenetic, comparative, and diversification studies, particularly if the units of analysis are not equivalent (Shaw 1998). Given the fundamental nature of this issue, it is not surprising that the ''species problem'' remains the subject of contentious debates within the biological sciences (Goldstein et al. 2000).

The advent and more recent accessibility of modern molecular techniques have immoderately changed how evolutionary biologists explore and, ultimately, define species. Rather than simplifying the problem, insights gained through a molecular perspective have instead added another layer of complexity to the issue. We now have the capability to conceptualize and examine species boundaries in the reductive terms of gene genealogies (Baum and Donoghue 1995; Baum and Shaw 1995). This approach has the capacity to delimit populations and species at a very fine scale yielding numerous accounts of cryptic species across many disparate organismal groups (e.g., pseudoscorpions: Wilcox et al. 1997; cave spiders: Hedin 1997a, b; mice: Riddle et al. 2000; marine worms: Schulze et al. 2000; mosses: Shaw 2000; trapdoor spiders: Bond et al. 2001). The presence of ''molecular'' species that are morphological indistinguishable or contravene patterns predicted by morphology (e.g., Baric and Sturmbauer 1999) suggests that defining species on the basis of morphology alone may be misleading.

Likewise, there are a number of reasons why species defined on the basis of molecules could also be misleading (i.e., gene tree species tree incongruence, for summary, see Maddison 1996). Mitochondrial genes, for example, might reflect a simple disruption of gene flow more rapidly than nuclear genes (for summary, see Avise 2000). Crandall et al. (2000) suggest that at least for the purposes of conservation both molecular and ecologically significant divergence should be requirements for species recognition (following Templeton 1989, 1998a). Lack of gene flow either in the past or in contemporary populations is not a sufficient criterion for species recognition. That is, molecular divergence in the absence of adaptive divergence implies only that populations are structured and therefore should be treated as a single population (see fig. 1 in Crandall et al. 2000).

Nevertheless, the treatment of geographically subdivided, but not ecologically divergent populations as ''a single population'' raises an important question about the speciation process, quite independently of its implications for conservation. Does geographic subdivision of populations only predicate ecological change or are the constraints of gene flow one of the primary overriding factors in the speciation process (sensu Bond et al. 2001)? In this paper we address the latter part of this question: What role does population subdivision play in speciation and, in more pragmatic terms species recognition, particularly when selective regime is apparently constant?

Our study species is the millipede *Anadenobolus excisus* (Karsch) (Diplopoda: Spirobolida, Rhinocricidae), the largest nominal millipede species on the island of Jamaica, where it is both endemic and nearly ubiquitous. It is associated predominantly with limestone formations where it seeks shelter and forages. *Anadenobolus excisus* varies considerably in size across the island, but the taxon is otherwise morphologically conservative. Although there are other species of *Anadenobolus* on Jamaica, other islands in the Greater and Lesser Antilles, and in Central America, *A. excisus* is clearly a distinct ''species'' on the basis of genitalic morphology, the set of features most commonly used to distinguish millipede species. A second species, *Anadenobolus holomelanus* (Pocock), has been described from the central, Mandeville, area of Jamaica, but is considered by Hoffman (1999) to be a subspecies of *A. excisus*.

The millipede *Anadenobolus excisus* is an ideal candidate for the study of the effects of population subdivision (Cruzan and Templeton 2000) because of its putative low vagility (Verhoeff 1928, p. 1781), high level of philopatry, and relatively long life span (Verhoeff 1942; Krabbe 1982). Furthermore, *A. excisus* has a restricted, insular distribution, with minimal variation in climate and habitat and appears to be an obligate calciphile. We might expect that for groups with extremely limited dispersal capabilities, geographic subdivision due to population isolation would be prevalent and that they may be particularly prone to population fragmentation. In stark contrast to the morphological evidence, we present molecular data (mtDNA 16S rRNA gene) that suggests a much more complex pattern of underlying millipede diversification. Based on an analysis of millipede size, we find that character displacement has occurred, indicating that three molecularly defined lineages, probably resulting from long-term geographical isolation, maintain lineage cohesion in sympatry. The observed genetic structure demonstrates the prevailing influence gene flow constraints have played within the confines of a relatively homogenous insular system.

Materials and Methods

Sampling

One to 22 individuals were sampled from 54 localities across Jamaica (Appendix 1). Every effort was made to collect at least five individuals per locality. However, due to difficult terrain (deep, loose limestone rubble) and the paucity of specimens at some sites, this goal was not always achieved. Larger sample sizes at select localities represent our attempts to accurately assess haplotype diversity and to maximize the numbers of haplotypes recovered for each clade at localities where major clades were sympatric. Each specimen was given a unique voucher number (FMJB001–FMJB311) and deposited in The Field Museum of Natural History (Chicago, IL) Insect Division collection. For specimens included in the molecular aspect of this study (ingroup and outgroup taxa), a label denoting 16S rRNA haplotype designation, corresponding to those listed in Appendix 1, was added to each vial.

Collection of DNA Sequences

Genomic DNA was extracted from 10–15 mg of tissue using the Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, CA). The polymerase chain reaction (PCR) was used to initially amplify (in a few select specimens) a 3′ region of the 16S rRNA mitochondrial gene using the universal primers 12Sai and 16Sbr (Hillis et al. 1997). Subsequent PCR reactions were carried out using millipede specific primers designed at positions internal to 12Sai and 16Sbr (see primer map, Appendix 2). Standard PCR reactions were conducted in 50 μl volumes for 25 cycles, each consisting of a 30 sec denaturation at 95°C, 30 sec annealing at 50–55°C, and 45 sec extension at 72°C, with an initial denaturation step of 95°C for 2.5 min and a final extension step of 72°C for 3 min.

Gel purified PCR products were sequenced with an ABI PRISM 377 automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase. Because these sequences lacked complex insertions and deletions, alignment was straightforward and could be accomplished manually. The computer programs CLUSTALW (Higgins et al. 1996) and MacClade version 4.0 (Maddison and Maddison 2000) were used to assemble the multiple sequences into a useable format for phylogenetic analysis.

Phylogenetic Inference and Analysis of Sequence Characteristics

Parsimony analyses were performed using PAUP* version 4.0b8 (Swofford 2000) with the aid of MacClade version 4.0 (Maddison and Maddison 2000). Gaps were treated as a fifth character state. Heuristic searches consisted of 500 random addition sequence replicates with a maximum of fifty trees held per replicate and rearranged using TBR branch swapping. The resulting 20,150 trees were filtered in PAUP* to remove non-binary trees if more fully resolved, compatible trees existed (Coddington and Scharff 1996). Relative branch support was evaluated using nonparametric bootstrap analysis (Felsenstein 1985a) carried out with PAUP* on a Sun E3500 Enterprise Server (Sun Microsystems, Inc., Santa Clara, CA). Bootstrap values are based on 1000 replicates with 100 trees saved at each replicate and rearranged using TBR branch swapping. Uncorrected pairwise proportional divergence values and chi square tests of homogeneity of base frequencies were computed in PAUP*. Four rhinocricid outgroup taxa collected on Jamaica (two *Eurhinocricus* species and two *Anadenobolus* species) were used to estimate the location of the phylogeny's root.

For maximum-likelihood analyses fifty haplotypes were randomly chosen (to reduce the size of the data set) from the set of 144 by randomizing the order of taxa in the data matrix using MacClade and then deleting the last 94 haplotypes from the data set. Using the complete data set, the computer program Modeltest version 3.0 (Posada and Crandall 1998) was used to determine the appropriate model of DNA substitution (by likelihood ratio test). We used the computer program MrBayes (Huelsenbeck 2000) to infer tree topology using the maximum-likelihood model indicated by Modeltest. We ran four simultaneous Markov Chain Monte Carlo (MCMC) chains for 251,000 generations saving the current tree to file every 10 generations (Huelsenbeck and Hall 2000). Trees prior to –Ln likelihood stabilization (''burn in'') were discarded and clade posterior probabilities determined by computing a 50% majority rule consensus of the trees remaining after burn in using PAUP*. Model parameter value estimations (base frequencies, ti/tv ratio, and Γ) were evaluated simultaneously in MrBayes during the course of the analysis.

All pairwise divergence values (uncorrected *p*) were computed by PAUP*. We used the computer program DNAsp version 3 (Rozas and Rozas 1999) to test that all mutations are selectively neutral using the *D*-test statistic (Tajima 1989). Transition–transversion ratios were calculated in MacClade.

TABLE 1. *Anadenobolus excisus* clade pairwise uncorrected proportional divergence values based on mtDNA 16S rRNA sequences. Average divergence is in bold, ranges of divergence are listed directly below in parentheses. Values along diagonal represent within-clade divergence.

	Outgroup	Clade I	Clade II	Clade III
Outgroup	**0.163** (0.036–0.208)	**0.175** (0.160–0.191)	**0.177** (0.157–0.206)	**0.180** (0.148–0.205)
Clade I	—	**0.020** (0.001–0.036)	**0.125** (0.112–0.143)	**0.124** (0.112–0.138)
Clade II	—	—	**0.033** (0.001–0.058)	**0.137** (0.123–0.152)
Clade III	—	—	—	**0.047** (0.001–0.104)

Analysis of Continuous Character Evolution: Millipede Size

We thought it necessary to implement two complementary approaches to analyzing change in millipede size: a phylogenetic approach and a traditional statistical approach. First, because we are dealing with clades, related individuals, and lineages, we need to account for the confounding role that these relationships might play in our analyses. Thus, the underlying assumption of parametric statistical tests' independence, may not necessarily be valid for these data (see Felsenstein 1985b). Conversely, because we are dealing with populations, clades at the interface between populations and species, variation in millipede size may not be phylogenetic but instead may represent localized, population-level, selection pressures. This analytical problem is particularly amplified in subclades that consist of allopatric and sympatric populations. Under this alternate paradigm traditional parametric statistical tests are appropriate.

Our estimate of millipede specimen size is based on average horizontal width. Width measurements ($n = 231$ specimens) were made using digital calipers, accurate to 0.01 mm, at three positions: directly behind the collum (1st segment), at approximately segment 15 (counting back from the anterior), and at approximately segment 10 (counting forward from the posterior end of the animal). We only measured specimens that were gravid females, or females with fully formed copulatory devices, and mature males, those with fully developed gonopods. The three widths were averaged for each specimen.

The average size and standard error for each clade were used as terminal values in a phylogenetic analysis of continuous character evolution. Values for multiple specimens with the same haplotype were averaged across all specimens to represent that haplotype when computing clade averages. Terminal branch lengths of the consolidated phylogeny are the average distance of all terminals that belong to the terminal clades measured to the base of their respective clade. The computer program ANCML (Ancestral States Using Maximum Likelihood) version 1.0 (Ludwig and Schluter 1997) was used to reconstruct ancestral character state values, and a 95% confidence limit for those values, according to a Brownian motion process (Felsenstein 1985b, Schluter et al. 1997). We chose a maximum-likelihood based method of reconstructing ancestral characters states over a parsimony based method because maximum-likelihood approaches allow for a quantification of uncertainty, allow for higher rates of character change, and more appropriately mimics evolution by selection (Schluter et al. 1997).

As a second phylogenetic approach to investigating change in millipede size we use Felsenstein's (1985b) independent contrasts in a fashion similar to that employed by Radtkey et al. (1997). The computer program Compare, version 4.3 (Martins 1999), was used to calculate independent contrasts for nodes experiencing a change in sympatry versus allopatry status. We calculated these values incorporating branch-length information and using a ''punctuated'' model (all branch lengths equal) and then compared sympatric versus allopatric values using a nonpaired Student's *t*-test (Radtkey et al. 1997). Independent contrast values for females, under the punctuated model, were log transformed to correct for unequal variances.

In standard statistical analyses, those not corrected for phylogeny, individual specimen values were used (i.e., we did not average across haplotypes represented by more than one specimen, see above). The dataset was partitioned into five class variables based first on individual membership in one of the three major clades and then further broken down on the basis of whether or not the specimen belonged to a population comprising individuals from one or two clades (allopatric or sympatric). Because we probably did not sample individuals of both haplotype clades at localities where they likely co-occur we considered the following localities sympatric because of their proximity to sympatric populations: all Manchester localities not already strictly classified as sympatric (man), trwII, wst I, trwIV, jam I, and jamII. All statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC)

RESULTS

Sequence Characteristics

The results presented in this study are based on ~1030 bp of 16S mtDNA surveyed for 242 individuals. Sequence alignment was straight-forward and required 34 single, seven double, three triple, and one four-base-pair insertion/deletions (indels). Many indels, almost half, were restricted to direct outgroup/ingroup differences. Of these 242 sequences 144 unique ingroup haplotypes were recovered (GenBank accession numbers AF501371–AF501514). The uncorrected base frequency composition across all ingroup haplotypes is homogenous ($\chi^2 = 96.96$, df = 429, $P = 1.00$) with a nucleotide

base composition that is A–T rich (A: 36%, C: 25%, G: 9%, T: 30%). The divergence values (uncorrected *p*) across all haplotypes ranges from 0.1 to 15.2% with a mean divergence of 9.6%. Table 1 summarizes the pairwise range and average divergence values with respect to all major clades (recovered in the parsimony analysis) and outgroups. We are unable to reject the null hypothesis of neutrality for these data ($D = 0.13$, $P > 0.10$).

Phylogenetic Inference

For the aligned 16S mtDNA data set 486 sites are variable, 397 of which are parsimony informative. The transition to transversion ratio computed in MacClade across all trees is approximately 6:1. A heuristic search in PAUP* identified over 20,000 most parsimonious (MP) trees (1265 steps, CI = 0.52, RI = 0.95). After filtering for compatible binary trees, the set of MP trees was reduced to 10,045. Figure 1A is the strict consensus of the 10,045 trees from the PAUP* analysis. All three of the major clades have very strong bootstrap support (>95%). However, the node that unites Clades II and III as sister taxa, exclusive of Clade I, lacks support.

Modeltest indicated that the most suitable maximum-likelihood (ML) model for these data is the HKY + Γ (Hasegawa et al. 1985). A Bonferroni correction for multiple tests does not change this outcome. Stabilization, burn in, of -Ln likelihood values occurred after 18,000 generations, therefore 1800 trees were discarded. Clade posterior probabilities are based on a 50% majority rule consensus of the remaining 23,210 trees and indicate very high support for all major nodes (Fig. 1B). Model parameter values reported here were computed in PAUP* using the GTR + Γ model for the tree with the highest −ln (Fig. 3B; −ln = 4287.90553; estimated base frequencies A: 0.37, C: 0.25, G: 0.09, T: 0.29; ti/tv ratio = 8.19; Γ = 0.269027). Although based on a limited subsample of the dataset this analysis recovers the three major clades obtained in the parsimony analysis. The parsimony and likelihood analysis differ only slightly in the internal resolution of Clade II.

The phylogenetic analysis of the 16S mtDNA sequence data reveals three very strongly supported lineages. Figure 2 summarizes our phylogenetic hypothesis for the *Anadenobolus excisus* complex and the geographic distribution of the major clades and their respective haplotypes. Clade I is restricted to the northeastern/eastern end of the island with most of its haplotypes distributed throughout the John Crow mountain range. Clade II comprises haplotypes found throughout the central part of the island, with a distribution extending both to the south and the northwest. Clade III haplotypes are distributed in the north central part of the island, with a pocket of populations along the western-most tip. Clade II and Clade III haplotypes co-occur along a relatively narrow zone of overlap on the central part of the island.

Each of the three major clades has been further partitioned into subclades (Fig. 1A) for subsequent analyses. Subclades I-1 and III-3, not recovered in the parsimony analysis (indicated by gray bars across the polytomy in Fig. 1A), are resolved in the maximum-likelihood analysis with strong support (subclade I-1 only), and in analyses (J. E. Bond, unpubl. data) that employ the TCS parsimony algorithm (Templeton 1998b; Templeton et al. 1992; both subclades).

Analysis of Continuous Character Evolution: Millipede Size

A total of 231 specimens from each of the three major Jamaican *Anadenobolus excisus* clades were measured. Because millipedes are generally sexually size dimorphic males and females were treated separately in all analyses (males: $\bar{x} = 7.84$; females: $\bar{x} = 8.71$; $t = 4.83$, $P < 0.0001$). Table 2 summarizes the descriptive statistics for millipede width for each subclade (as defined in Fig. 1A).

Figure 3 summarizes the reconstruction of male and female millipede body size using maximum likelihood. Males and females of subclades II-2 and II-1, both predominantly sympatric with Clade III, are smaller with respect to the Clade III ancestral node (Fig. 3). The opposite pattern of size change is observed for females in Clade III, in which its sympatric members are larger on average than the ancestral values for Clades II and III (e.g., subclade III-3, whose members all co-occur with individuals of Clade II). Size change in sympatry is not as evident for subclades III-2 and III-1. However, the allopatric subclade III-4 (sister to the sympatric subclade III-3) is much smaller than all other Clade III subclades. The pattern for Clade III males is not as clear as that for females given the considerable size variation in adult males. However, male size in the allopatric subclade, III-4, is much smaller than those in the sympatric subclade III-3. Finally, it is important to note how different the pattern of size variation is for Clade I, all of whose members are allopatric and intermediate in size.

The phylogenetic trends observed for millipede size (Fig. 3) are further supported by the independent contrasts. Independent contrasts for the five nodes that undergo change from allopatric to sympatric, or vice versa, were compared under the null hypothesis that there is no size difference (Radtkey et al. 1997). For females we reject the null hypothesis for both the branch length corrected ($t = -2.96$, $P = 0.03$) and punctuated model ($t = -6.83$, $P = 0.001$). For male body size we can only reject the null hypothesis for the punctuated model ($t = -3.74$, $P = 0.02$). The *P*-value for the *t*-test that takes into account branch length was marginally not significant ($P = 0.06$).

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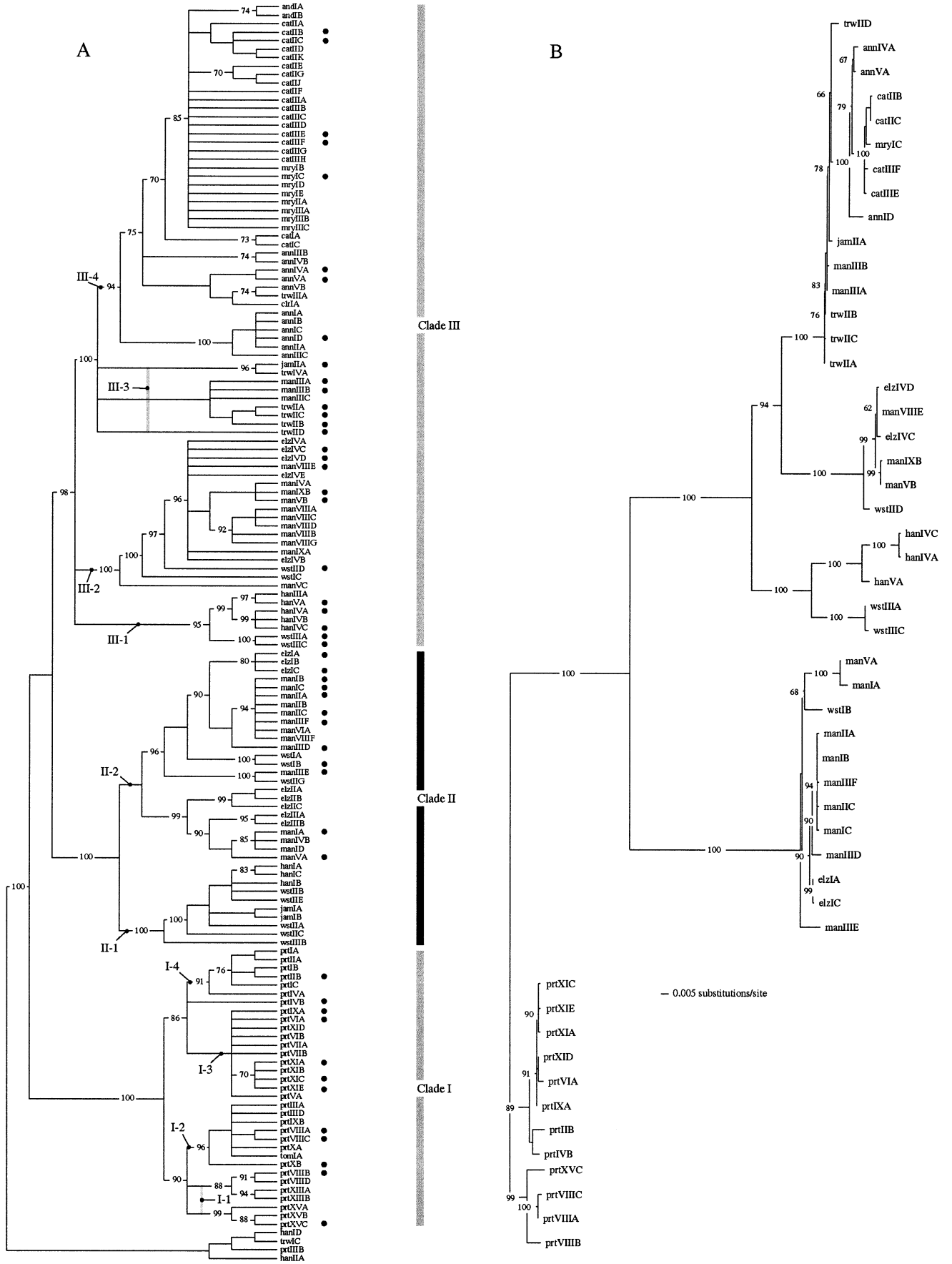
FIG. 1. Results of phylogenetic analyses of *Anadenobolus excisus* mtDNA haplotypes. Haplotype designation refers to localities and haplotypes defined in Appendix 1. (A) Strict consensus of 10,045 trees showing relationships of 144 mtDNA haplotypes based on an analysis using maximum parsimony. Numbers placed directly on nodes are bootstrap values, Roman Numerals offset from nodes indicate ''subclade'' numbers, and solid circles denote haplotypes included in analysis using maximum likelihood. Gray bars placed directly on the tree denote subclades that were resolved using methods other than standard parsimony (see Methods section for detailed explanation). This tree is rooted on the basis of outgroup comparison (outgroup haplotypes = hanIID, trwIC, prtIIIB, hanIIA). (B) Unrooted fifty percent majority rule consensus of 23,310 trees obtained using Bayesian inference and the HKY85 model of DNA substitution for 50 mtDNA haplotypes chosen at random. Numbers at nodes are posterior clade probabilities.


A
B
Clade III
Clade II
Clade I
III-4
III-3
III-2
III-1
II-2
II-1
I-4
I-3
I-2
I-1
andIA
andIB
catIIA
catIIB
catIIC
catIID
catIIK
catIIE
catIIG
catIIJ
catIIF
catIIIA
catIIIB
catIIIC
catIIID
catIIIE
catIIIF
catIIIG
catIIIH
mryIB
mryIC
mryID
mryIE
mryIIA
mryIIIA
mryIIIB
mryIIIC
catIA
catIC
annIIIB
annIVB
annIVA
annVA
annVB
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annIC
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annIIA
annIIIC
jamIIA
trwIVA
manIIIA
manIIIB
manIIIC
trwIIA
trwIIC
trwIIB
trwIID
elzIVA
elzIVC
elzIVD
manVIIIE
elzIVE
manIVA
manIXB
manVB
manVIIIA
manVIIIC
manVIIID
manVIIIB
manVIIIG
manIXA
elzIVB
wstIID
wstIC
manVC
hanIIA
hanVA
hanIVA
hanIVB
hanIVC
wstIIIA
wstIIIC
elzIA
elzIB
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wstIIIB
prtIA
prtIIA
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prtIIB
prtIC
prtIVA
prtIVB
prtIXA
prtVIA
prtXID
prtVIB
prtVIIA
prtVIIB
prtXIA
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prtXIC
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prtVA
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prtIXB
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prtVIIID
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prtXVA
prtXVB
prtXVC
hanID
trwIC
prtIIIB
hanIIA
trwIID
annIVA
annVA
catIIB
catIIC
mryIC
catIIIF
catIIIE
annID
jamIIA
manIIIB
manIIIA
trwIIB
trwIIC
trwIIA
elzIVD
manVIIIE
elzIVC
manIXB
manVB
wstIID
hanIVC
hanIVA
hanVA
wstIIIA
wstIIIC
manVA
manIA
wstIB
manIIA
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manIIIE
prtXIC
prtXIE
prtXIA
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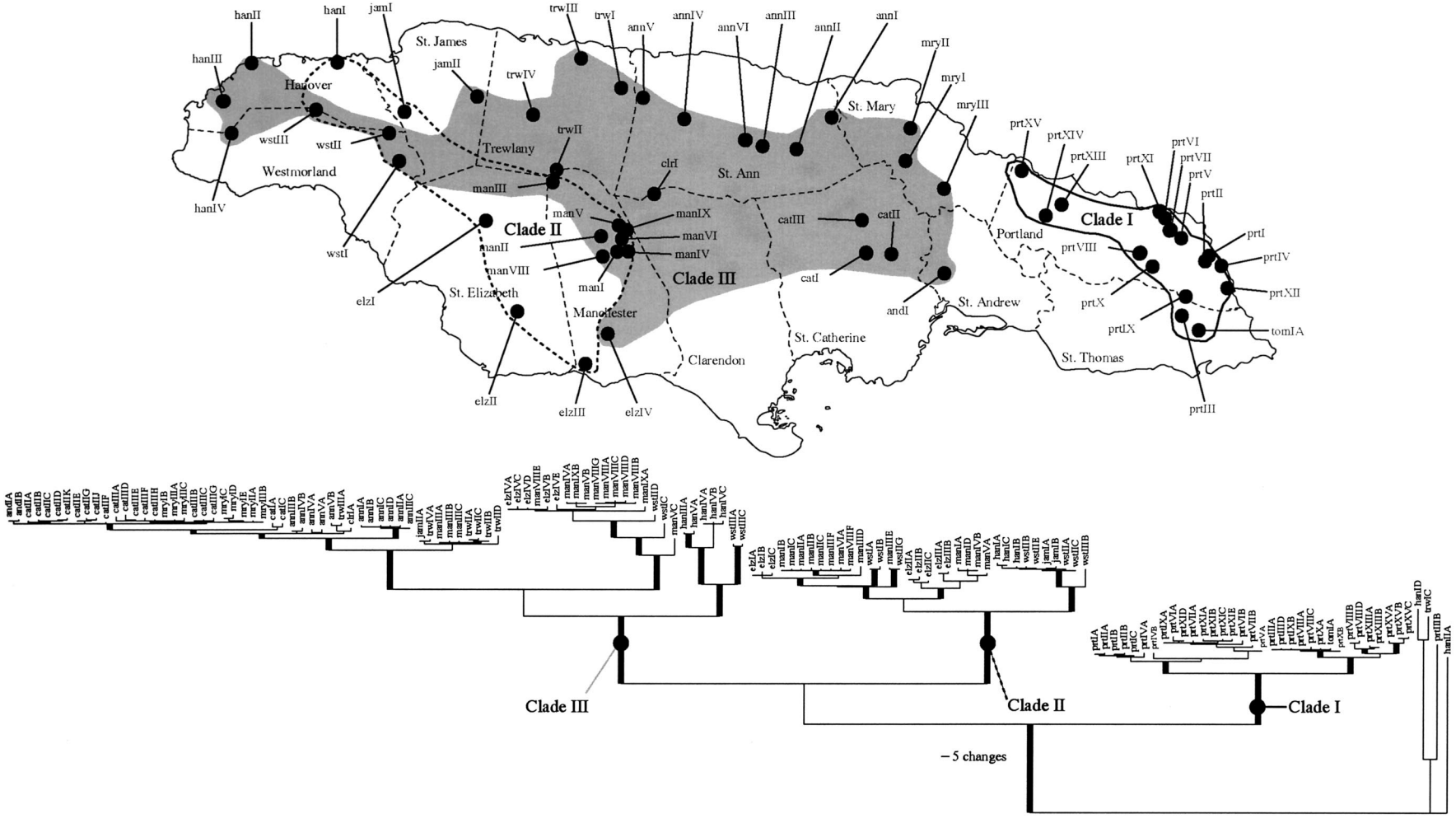

FIG. 2. One of the most parsimonious trees (branches with bootstrap support by > 70% thickened, branch lengths computed on the basis of nucleotide substitutions using parsimony), and a map of Jamaica with collecting localities indicated, illustrating the distribution and generalized range of each of the major mtDNA haplotype clades.

TABLE 2. Summary descriptive statistics of *Anadenobolus excisus* body size (females and males) for each of the major clades (Sym/Allo indicates values for each clade when broken down on the basis of sympatry/allopatry status, respectively. Tot refers to total clade values).

Clade	*n*	Mean	Range	SE	SD
I_{Allo}	41	8.61	6.65–11.89	0.89	1.92
	12	8.11	6.51–10.01	0.34	1.17
II_{Tot}	18	7.41	6.29–8.72	0.19	0.84
	36	6.89	4.98–8.44	0.12	0.74
II_{Sym}	13	7.36	6.44–8.63	0.23	0.82
	24	7.02	5.55–8.39	0.14	0.67
II_{Allo}	5	7.52	6.29–8.72	0.44	0.99
	12	6.61	4.98–8.44	0.24	0.83
III_{Tot}	67	9.12	7.04–12.76	0.17	1.36
	57	8.37	5.78–11.20	0.19	1.43
III_{Sym}	22	10.58	8.86–12.76	0.23	1.08
	19	9.58	6.63–11.97	0.32	1.39
III_{Allo}	45	8.41	7.04–11.19	0.12	0.80
	38	7.77	5.78–11.20	0.16	1.01

Figure 4 summarizes the size distributions for all clades, ANOVA outcomes comparing clade sizes, and results of the pairwise comparisons of mean body width for each clade. These results show that there are significant differences in size and consequently these differences are consistent with a pattern that predicts change in size for sympatric populations. In sympatry Clade II males and females differ significantly in size from Clade III, a distinction that cannot be made for Clade III individuals in allopatry. However, it is important to note that Clade III sympatric individuals are significantly different in size (larger) than Clade III allopatric individuals. The only exception to the predictive pattern of size change in allopatry versus sympatry is for Clade II, which may reflect our inability to differentiate all Clade II populations as definitionally sympatric. As a consequence the sample sizes for allopatric Clade II were very small (Table 2). Finally, for females in sympatry we observed no overlap in size between Clade II and Clade III individuals.

DISCUSSION

The Molecular Evidence for Incipient Speciation

Based on the hypothesized mtDNA gene tree *Anadenobolus excisus* likely represents a sibling species complex that consists of three deeply separated lineages. These lineages are diagnosable (unique nonhomoplasious characters define these lineages), well supported (by high bootstrap and posterior Probabilities), and highly divergent. Clade I comprises a basal lineage that is largely restricted to the John Crow Mountains on the island's easternmost end (Fig. 2). Clades II and III, located to the west, are sympatric from the center of the island westward. The sympatry observed for these two haplotype arrays can probably be attributed to secondary contact following divergence in allopatry (Category II: deep gene tree: major lineages sympatric; Avise 2000).

Within and between clade divergence (uncorrected *p*, Table 1) is very high (11–15%) and is an order of magnitude higher than reports for comparable population and species level studies in other animal groups (for summary, see Vogler et al. 1993). Similar divergence values (12.6%) have been recently reported for a fossorial, trapdoor spider species that inhabits sand dunes along the California coast and consequently exhibits similar life history characteristics (e.g., limited dispersal capabilities) that make its populations prone to geographic isolation (Bond et al. 2001).

A pattern of such deep divergence is indicative of long, nontrivial temporal isolation. As a rough approximation we apply Brower's (1994) 2.3% rate of mtDNA divergence per million years for arthropods to estimate divergence times of the major mtDNA lineages within the *Anadenobolus excisus* complex. We use combinations of the range and mean pairwise uncorrected p values from Table 1 as broad estimates of divergence. Average range of divergence time between all three major clades is approximately 5–6 million years before present (mybp) with ranges that vary from ~4.87–6.60 mybp. Divergence during the late Miocene/early Pliocene is consistent with what has been reported (summarized by Schubart et al. 1998) for other Jamaican endemics (frogs: Hedges 1989; lizards: Hedges and Burnell 1990; terrestrial crabs: Schubart et al. 1998) and postdates the final emergence of Jamaica in the early to mid-Miocene. An early-Pliocene divergence also may explain the east/west disjunction between Clade I and Clades II and III as a function of the uplift of the Blue Mountains (Buskirk 1985), which would have isolated Clade I in the east from the other two lineages.

The Morphological Evidence

Millipede species are identified and described almost exclusively on the basis of male genital morphology; thus speciation and divergence of male genital morphology are considered to be tightly coupled. Prior detailed studies of *Anadenobolus excisus* gonopod morphology using scanning electron microscopy (J. E. Bond and P. Sierwald, unpubl. ms. in review) revealed no qualitative gonopod differences across much of its distribution. Consistent with these results morphometric analyses (J. E. Bond and P. Sierwald, unpubl. ms.) of anterior gonopod shape do not reveal any discrete association of gonopod shape with any of the mtDNA lineages or particular geographic region. Based on male genital morphology additional species within the *Anadenobolus excisus* species complex would not be recognized. However, the pattern of change in millipede size that we discuss below is interesting because it is inconsistent with the observation that the *A. excisus* complex comprises only one species. Size is relatively homogenous across the species complex except where two major mtDNA lineages co-occur (II and III).

Does Millipede Size Matter?

Overall body size is generally considered to be a characteristic that has implications for many aspects of millipede life history (Enghoff 1992). Feeding, burrowing, and habitat choice are all features that are constrained by body size. The results of our analyses of *Anadenobolus excisus* body size indicate that significant size differences occur when major mtDNA lineages are sympatric (Fig. 5). Because body size differences can be attributed to nodes in the haplotype phylogeny where a shift from allopatry to sympatry occurs, it is appropriate to consider a causal mechanism like ecological character displacement to account for this size disparity in

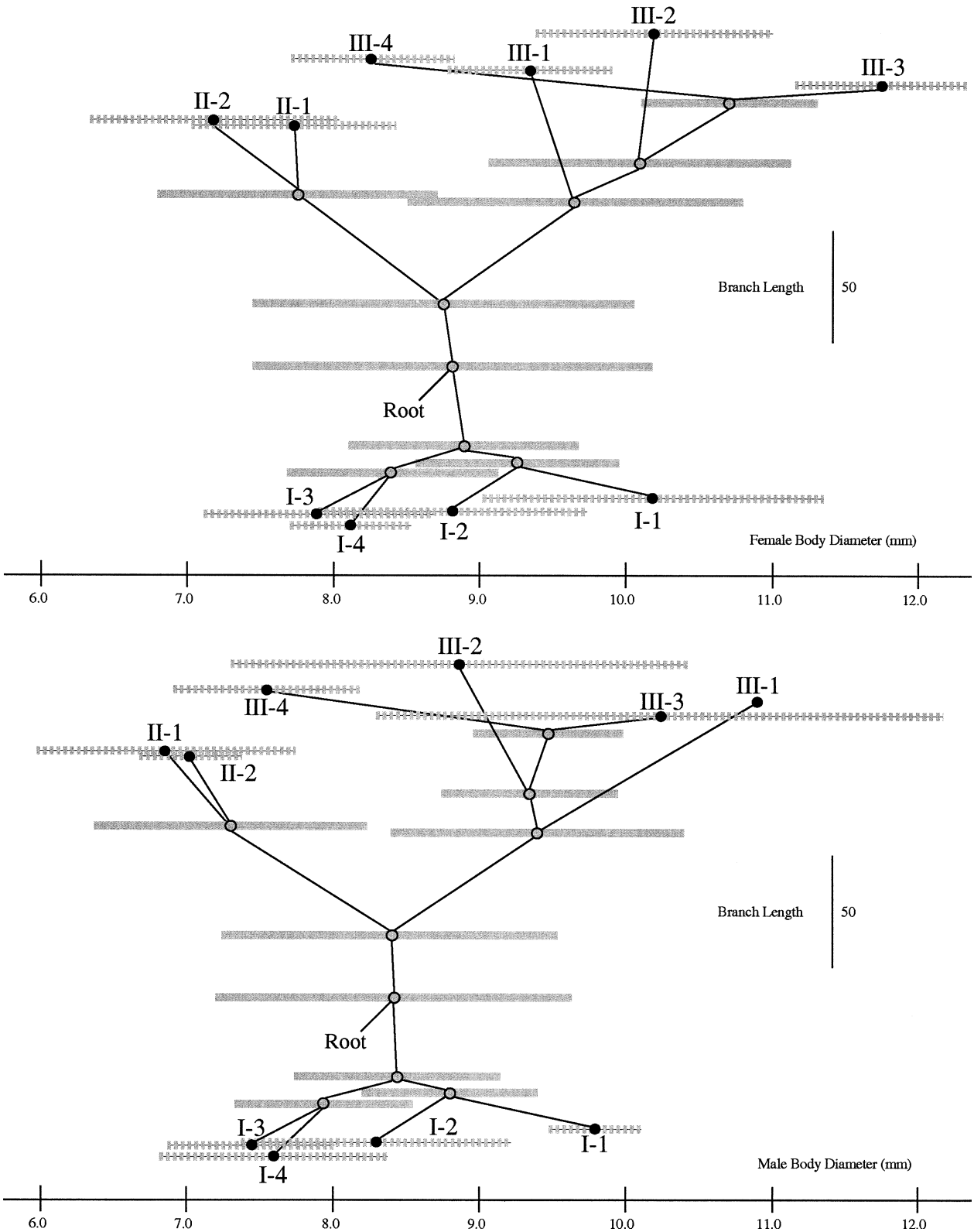

FIG. 3. Ancestor reconstruction of female and male body size using a maximum likelihood model of Brownian Motion. At the tips, extant clade averages are represented by filled circles with ± 1 standard error represented by dashed lines. Ancestral values (hollow circles) are plotted along the X-axis (body size in millimeters) at their corresponding position in the phylogeny based on branch length (the Y-axis), gray solid lines denote 95% confidence limits of the ancestral values. Clade numbers (roman numerals) correspond to those clades defined in Figure 1A.

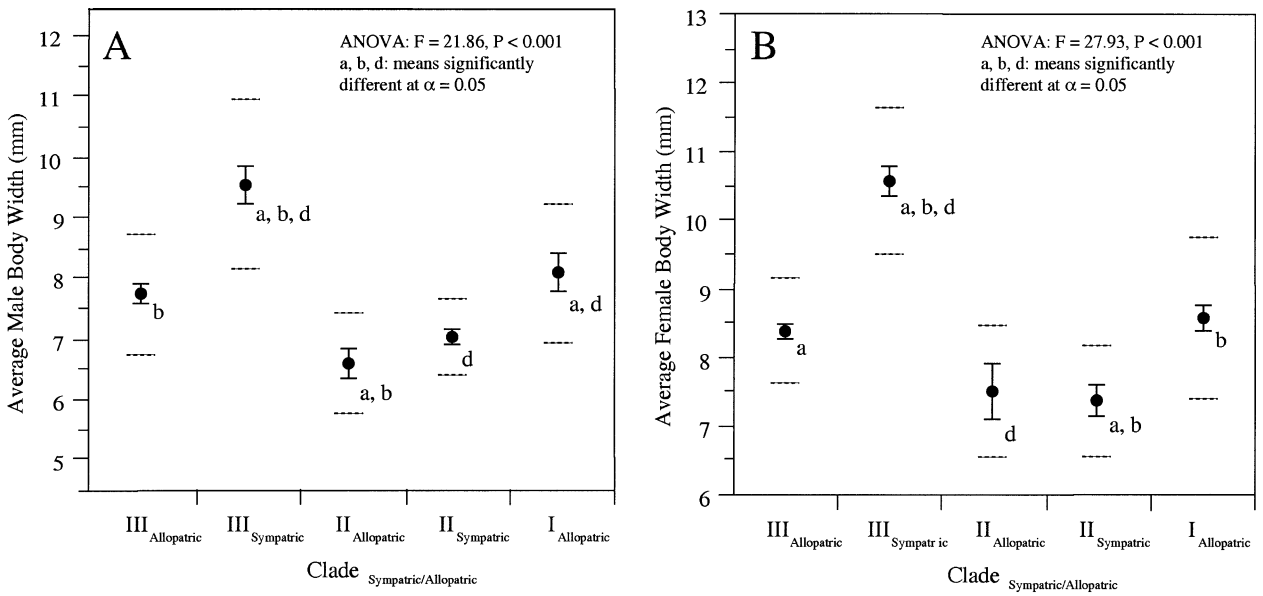

FIG. 4. Summary of parametric statistical analysis (ANOVA) of *Anadenobolus excisus* body size. (A) Average male body size. (B) Average female body size.

sympatry. However, the results presented in this aspect of the study should be viewed as somewhat preliminary because our initial collecting efforts were not focused on addressing character displacement.

The model of ecological character displacement predicts that when intermediate—sized species are sympatric, their sizes will evolve in opposite directions until competition is relaxed and stable coexistence is possible (Losos 1992; Schluter 2000). Six standard criteria must be met to demonstrate that ecological character displacement has occurred (Schluter and McPhail 1992, summarized in detail by Losos 2000 and Schluter 2000): (1) phenotypic differences must have a genetic basis; (2) the pattern of size difference cannot be attributed to chance; (3) differences in size must be related to differences in resource use; (4) resources are limited; (5) resources are the same in allopatry and sympatry; and (6) the differences evolved in sympatry. Although a number of authors have attributed differences in body size of coexisting millipede species to the influence of competition in sympatry (for summary, see Enghoff 1992), the majority of these studies lack empirical support (e.g., Enghoff 1983) for true character displacement because they do not formally address the six criteria described above. Enghoff (1983, 1992) acknowledges this shortcoming and points out that comparisons of taxa that have both allopatric and sympatric populations would be a more informative way of addressing potential character displacement in millipedes.

Character displacement may be an appropriate explanation for the disparity in *Anadenobolus excisus* size where major mtDNA lineages are sympatric. We address at least half of the six criteria for establishing ecological character displacement in the *A. excisus* species complex (criteria 2, 5, and 6). Both phylogenetic and standard parametric statistical analyses indicate the pattern of size disparity in the *A. excisus* species complex to be nonrandom (criterion 2); where Clades II and III overlap they differ in size. Resources, habitat, limestone substrate, and vegetation appear to be the same in allopatry and sympatry (criterion 5; J. Bond, pers. obs.). Habitat preference in *A. excisus* is very specific across its distribution and without exception is a key character when searching for these millipedes (Bond, unpubl. data). Based on phylogenetic analysis (Losos 2000), the most parsimonious explanation is

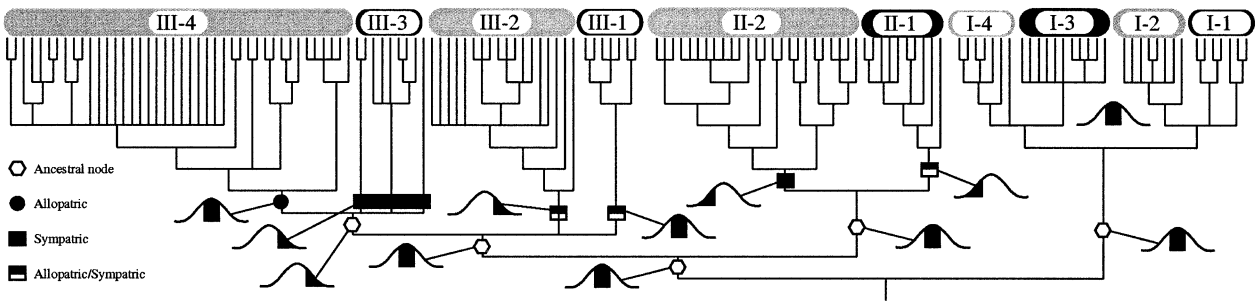

FIG. 5. Overall summary of change in millipede body size plotted onto the haplotype phylogeny. Clade numbers, denoted by roman numerals, correspond to those clades defined in Figure 1A. Graphical representation of a normal distribution is used to depict changes in body size at each node: intermediate body size = center of distribution filled, large body size = right side of distribution filled, and small body size = left side of distribution filled.

that *A. excisus* size differences evolved in sympatry (criterion 6). Our hypothesis of haplotype/clade relationships indicates that in five instances shifts to sympatry, or back to allopatry, and changes in millipede size are concordant. Although it would be surprising if size did not have an overwhelming genetic component (criterion 1), the genetic basis of size does need to be evaluated formally. Of the noninteractive parameters that Enghoff (1992) considers to strongly affect millipede size (ancestry, age, sex, food, latitude, altitude, and habitat), we suspect that ancestry and food are the only two that could come into play within the context of this system. We have demonstrated that character displacement has probably occurred, but we have not distinguished what kind (e.g., ecological or reproductive). Within a broader context the results of this study are interesting because of the paucity of character displacement examples in invertebrates. The majority, thoroughly documented, and best known recent studies of ecological character displacement comprise vertebrate systems (e.g., *Anolis* lizards: Losos 1990; *Cnemidophorus* lizards: Radtkey et al. 1997; *Plethodon* salamanders: Adams and Rohlf 2000).

Individuals from all three clades (Figs. 3 and 4) are indistinguishable in allopatry but in sympatry clades II and III can be easily distinguished on the basis of size and apparently coexist as distinct lineages. If Clade II and III individuals did hybridize, very different molecular and morphological patterns would be expected. First, given the relatively old age of these sympatric lineages (see branch lengths, Fig. 2) we might expect to see a distinct, or separate, mitochondrial lineage in the overlap zone (i.e., a fourth mtDNA lineage), particularly if hybridization is unidirectional. Second, we would expect to observe intermediate body sizes in sympatry. In the absence of mating constraints (mating is bidirectional) between lineages intermediate sized millipedes would have equal probabilities of having a haplotype belonging to either clade.

Conclusions

Based on a combined analysis of the molecular and morphological data we strongly advocate the recognition of three *nominal* species in the *Anadenobolus excisus* species complex. Although formal taxonomic considerations will be addressed elsewhere (J. E. Bond and P. Sierwald, unpubl. ms.), the data do not support Hoffman's (1999) recommendation that *A. holomelanus* be considered a subspecies of *A. excisus*. Rather *A. holomelanus* is probably a valid nominal taxon representing Clade III (based on the size of the *A. holomelanus* holotype). This study demonstrates the relative importance and the overriding nature that the constraints of gene flow may play in millipede species diversification. Although little is known about the ecology and dispersal patterns of millipedes, it is clear from these data, as well as studies of millipede distributions (Verhoeff 1928) and other insular species swarms (e.g., Enghoff 1982, 1983, 1992), that millipede vagility is probably very low. In the *A. excisus* complex speciation was likely a vicariant event with ecological and morphological evolution playing only a secondary rather than a punctual role (Bond et al. 2001, Peterson et al. 1999).

In this example it is clear there would be some danger in failing to recognize the importance of geographically subdivided populations as distinct evolutionarily significant units (ESUs, e.g., units of conservation, species, etc.). This study demonstrates that putative neutral molecular change and geographic subdivision of populations across a rather homogenous selective regime reflects underlying divergence that is sufficient enough to be maintained in sympatry. Had we failed to sample sympatric populations or secondary contact had yet to occur, we might have incorrectly treated these three lineages as one species. That is not to say that we do not agree, at least in part, with concern that the ever-reductive resolution of molecular approaches could very well lead to the ''inappropriate diagnosis of ESUs within functionally equivalent populations'' (Crandall et al. 2000, p. 290). Although the ramification of oversplitting based on molecules is the unwieldy proliferation of names and conservation units, the failure to recognize ''real'' molecular based ESUs increases the risk of grossly underestimating true evolutionary diversity and is in our opinion, a less desirable alternative.

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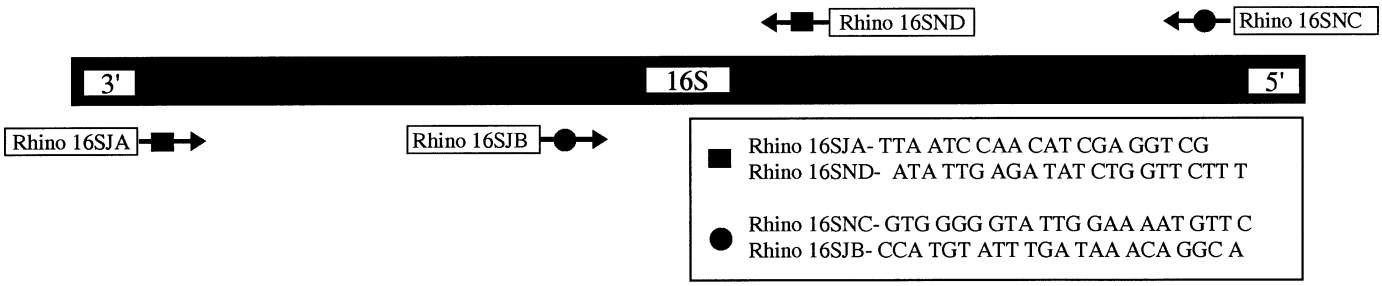
APPENDIX 1

List of Jamaican parishes, locality acronyms, geographic location, number of individuals sampled, and haplotype composition for all of the localities sampled as part of this study [haplotypes correspond to GenBank Accession nos. AF501371–AF501514]. Total sample sizes, *N*, for localities that comprise both Clade II and Clade III individuals are given in parentheses.

Parish	Locality	Latitude/longitude	*N*	Haplotype composition (*N*)
Clade I				
Portland	prtI	N 18°05′30.7″/W 76°19′49.4″	4	prtIA (1), prtIB(2) prtIC* (1)
Portland	prtII	N 18°04′52.6″/W 76°20′1.4″	4	prtIIA (1), prtIIB (1), prtIC (2)
Portland	prtIV	N 18°04′27.9″/W 76°17′45.3″	2	prtIVA prt(1)IVB (1)
Portland	prtV	N 18°07′34.4″/W 76°26′19.1″	3	prtVA (1), prtIC (2)
Portland	prtVI	N 18°09′48.9″/W 76°25′04.7″	2	prtVIA (1), prtVIB (1)
Portland	prtVII	N 18°08′30.5″/W 76°24′08.3″	2	prtVIIA (1), prtVIIB (1)
Portland	prtVIII	N 18°05′42.9″/W 76°27′45.9″	6	prtVIIIA (1), prtVIIIB (2), prtVIIIC (2), prtVIIID (1)
Portland	prtIX	N 18°00′48.3″/W 76°22′43.5″	3	prtIXA (1), prtIXB (1), prtIIIA (1)
Portland	prtX	N 18°03′33.2″/W 76°24′52.7″	4	prtXA (2), prtXB (1), prtIIIA (1)
Portland	prtXI	N 18°10′19.3″/W 76°25′23.1″	6	prtXIA (1), prtXIB (2), prtXIC–E (1)
Portland	prtXII	N 18°01′43.9″/W 76°17′28.1″	2	prtIC (2)
Portland	prtXIII	N 18°08′43.2″/W 76°34′56.4″	2	prtXIIIA (1), prtXIIIB* (2)
Portland	prtXIV	N 18°08′12.0″/W 76°37′28.8″	3	prtXIIIB (3)
Portland	prtXV	N 18°14′15.0″/W 76°42′06.6″	5	prtXVA (3), prtXVB (1), prtXVC (1)
St. Thomas	prtIII	N 17°58′40.1″/W 76°22′49.5″	5	prtIIIA* (4), prtIIID (1)
St. Thomas	tomI	N 17°56′58.2″/W 76°20′57.6″	2	prtIIIA (1), tomIA (1)
Clade II				
St. Elizabeth	elzI	N 18°09′16.3″/W 77°45′25.3″	6	elzIA (4), elzIB (1), elzIC (1)
St. Elizabeth	elzII	N 17°58′58.8″/W 77°41′38.9″	3	elzIIA–C (1)
St. Elizabeth	elzIII	N 17°52′54.3″/W 77°33′23.4″	5	elzIIIA (1), elzIIIB (4)
Manchester	manI	N 18°05′42.8″/W 77°29′32.9″	5	manIA (1), manIB* (2), manIC (1), manID (1)
Manchester	manII	N 18°07′16.6″/W 77°31′40.8″	6	manIB (3), manIIA–C (1)
Manchester	manIII	N 18°13′19.7″/W 77°37′24.8″	3 (9)	manIIID–F (1)
Manchester	manIV	N 18°05′34.7″/W 77°28′48.3″	2 (3)	manIVB* (2)
Manchester	manV	N 18°08′34.3″/W 77°29′37.2″	3 (7)	manVA (1), manIVB (1), manIB (1)
Manchester	manVI	N 18°06′10.8″/W 77°29′18.6″	1	manVIA
Manchester	manVIII	N 18°04′59.4″/W 77°30′52.2″	5 (22)	manVIIIF (1), manIB (4)
Manchester	manIX	N 18°08′01.2″/W 77°27′34.2″	1 (7)	manIB (1)
Hanover	hanI	N 18°26′48.2″/W 78°03′02.9″	5	hanIA–C (1), wstIIE (2)
St. James	jamI	N 18°18′50.6″/W 77°51′58.3″	5	jamIA (2), jamIB (3)
Westmorland	wstI	N 18°15′42.0″/W 77°55′46.1″	2 (3)	wstIA (1), wstIB (1)
Westmorland	wstII	N 18°18′18.1″/W 78°01′03.2″	5 (7)	wstIIA–C (1), wstIIE* (1), wstIIG (1)
Westmorland	wstIII	N 18°21′26.4″/W 78°05′39.7″	2 (4)	wstIIIB (2)
Clade III				
St. Andrew	andI	N 18°03′32.2″/W 76°51′11.6″	2	andIA (1), andIB* (1)
St. Ann	annI	N 18°21′50.9″/W 77°05′33.6″	6	annIA (1), annIB* (3), annIC (1), annID (1)
St. Ann	annII	N 18°17′15.6″/W 77°08′44.3″	4	annIIA (1), annIB (3)
St. Ann	annIII	N 18°17′38.2″/W 77°12′29.3″	2	annIIIB* (1), annIIIC* (1)
St. Ann	annIV	N 18°20′33.1″/W 77°22′00.6″	3	annIVA (2), annIVB (1)
St. Ann	annV	N 18°22′43.3″/W 77°27′00.0″	2	annVA (1), annBV (1)
St. Ann	annVI	N 18°18′23.9″/W 77°14′37.3″	3	annIIIB (2), annIIIC (1)
St. Catherine	catI	N 18°05′36.5″/W 76°57′25.1″	3	catIA (1), catIC (1), andIB (1)
St. Catherine	catII	N 18°05′54.5″/W 77°00′15.7″	11	catIIA–C(1), catIID (3), catIIE–G (1), catIIJ (1), catIIK (1)
St. Catherine	catIII	N 18°09′18.0″/W 76°59′37.8″	8	catIIIA–H (1)
Clarendon	clrI	N 18°12′07.0″/W 77°25′16.6″	2	clrIA (2)
St. Elizabeth	elzIV	N 17°56′17.4″/W 77°30′49.1″	9	elzIVA (4), elzIVB (1), elzIVC (1), elzIVD (2), elzIVE (1)
Hanover	hanIII	N 18°26′47.9″/W 78°13′09.8″	2	hanIIIA (2)
Hanover	hanIV	N 18°18′54.0″/W 78°15′37.5″	4	hanIVA (2), han IVB (1), hanIVC (1)
Hanover	hanV	N 18°22′36.1″/W 78°16′28.5″	1	hanVA (1)
St. James	jamII	N 18°22′19.2″/W 77°45′10.8″	1	jamIIA (1)
Manchester	manIII	N 18°13′19.7″/W 77°37′24.8″	6 (9)	manIIIA (2), manIIIB (1), manIIIC (3)
Manchester	manIV	N 18°05′34.7″/W 77°28′48.3″	1 (3)	manIVA (1)
Manchester	manV	N 18°08′34.3″/W 77°29′37.2″	4 (7)	manVB (3), manVC* (1)
Manchester	manVIII	N 18°04′59.4″/W 77°30′52.2″	17 (22)	manVIIIA* (1) manVIIIB (2) manVIIIC–E (1), manVIIIG (11)
Manchester	manIX	N 18°08′01.2″/W 77°27′34.2″	6 (7)	manIXA (1), manIXB (1), manVC (1), manVIIIA (1), manVIIIG (2)
St. Mary	mryI	N 18°16′08.8″/W 76°55′49.1″	5	mryIB* (1) mryIC (1), mryID (2), mryIE (1)
St. Mary	mryII	N 18°17′36.0″/W 76°55′14.4″	5	mryIIA (1), annIB (4)
St. Mary	mryIII	N 18°11′39.0″/W 76°51′16.8″	9	mryIIIA (1), mryIIIB (2), mryIIIC (3), mryIB (3)
Trewlany	trwII	N 18°14′47.9″/W 77°36′53.7″	5	trwIIA (2), trwIIB–D (1)
Trewlany	trwIII	N 18°27′46.9″/W 77°37′24.6″	1	trwIIA (1)
Trewlany	trwIV	N 18°19′16.2″/W 77°38′01.2″	1	trwIVA (1)
Westmorland	wstI	N 18°15′42.0″/W 77°55′46.1″	1 (3)	wstIC (1)
Westmorland	wstII	N 18°18′18.1″/W 78°01′03.2″	2 (7)	wstIID (2)
Westmorland	wstIII	N 18°21′26.4″/W 78°05′39.7″	2 (4)	wstIIIA (1), wstIIIC (1)

* Denotes haplotype found at more than one locality.



APPENDIX 2. *Anadenobolus excisus* primer combinations used to amplify a 1200 base-pair region of 16S mtDNA.