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THE ROYAL SOCIETY

Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences

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The internal phylogeny of the 'myriapod' class Chilopoda is evaluated for 12 species belonging to the five extant centipede orders, using 18S rDNA complete gene sequence and 28S rDNA partial gene sequence data. Equally and differentially weighted parsimony, neighbour-joining and maximum-likelihood were used for phylogenetic reconstruction, and bootstrapping and branch support analyses were performed to evaluate tree topology stability. The results show that the Chilopoda constitute a monophyletic group that is divided into two lines, Notostigmophora (= Scutigeromorpha) and Pleurostigmophora, as found in previous morphological analyses. The Notostigmophora are markedly modified for their epigenic mode of life. The first offshoot of the Pleurostigmophora are the Lithobiomorpha, followed by the Craterostigmomorpha and by the Epimorpha s. str. (= Scolopendromorpha + Geophilomorpha), although strong support for the monophyly of the Epimorpha s. lat. (= Craterostigmomorpha + Epimorpha s. str.) is only found in the differentially weighted parsimony analysis.

Keywords: Arthropoda; Chilopoda; molecular; phylogeny; ribosomal; evolution

1. INTRODUCTION

The Chilopoda, or centipedes, is a class of terrestrial Myriapods comprising approximately 3000 extant species distributed over all continents, from sea level to high altitudes. Centipedes are nocturnal predators that live under stones, under the bark of tree trunks, in moss, in leaf litter and in caves. They are predominantly soft-bodied myriapods, mostly measuring 1–10 cm long, and bearing 15–191 pairs of legs (Minelli 1993). For a review of their biology see Lewis (1981) and Minelli (1993).

(a) *Background to centipede biology and taxonomy*

A total of five orders of centipedes are currently recognized within the extant Chilopoda: Scutigeromorpha, Lithobiomorpha, Craterostigmomorpha, Scolopendromorpha and Geophilomorpha. One extinct order, Devonobiomorpha, is also accepted (but, see Borucki 1996). Scutigeromorpha (i.e. the synanthropic cosmopolitan house centipede *Scutigera coleoptrata*), have pseudofaceted eyes resembling the compound eyes of insects, 15 pairs of very elongated legs, and dorsal respiratory openings (= Notostigmophora). The haemolymph contains a respiratory pigment. In the remaining orders, the body is more elongated and more or less flattened, with lateral spiracles (= Pleurostigmophora), and the haemolymph does not contain respiratory pigments. The categories Notostigmophora and Pleurostigmophora have been considered to be subclasses of the Chilopoda by some authors.

There are also 15 pairs of legs in Lithobiomorpha and Craterostigmomorpha, a poorly known group comprising two species from New Zealand and Tasmania. The Geophilomorpha comprises 11 families of worm-like bodied centipedes, bearing 29–191 pairs of short legs. The largest centipedes (reaching 25 cm long) belong to the Scolopendromorpha, and bear 21–23 pairs of legs. The bite of some Scolopendromorpha can inflict serious injury to humans.

Scutigeromorpha and Lithobiomorpha have a type of development known as anamorphic, in which segment number increases during postembryonic life (= 'Anamorpha'). In contrast, Scolopendromorpha and Geophilomorpha have an epimorphic development, in which the definitive number of body segments appears upon hatching (= Epimorpha). The condition of Craterostigmomorpha is unclear because they are not strictly anamorphic, as they achieve the final number of legs after only one moult, and have been suggested to be the sister group of the Epimorpha (Shinohara 1970; Dohle 1980, 1985, 1990; Shear & Bonamo 1988; Borucki 1996; Prunescu 1996).

The oldest fossil centipedes, attributable to the extant order Scutigeromorpha, have been found in the Late Silurian and Early Devonian (Almond 1985; Jeram *et al.*

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1990). The fossil order Devonobiomorpha has recently been described from the Middle Devonian Gilboa (New York, USA) (Shear & Bonamo 1988, 1990). Well-preserved whole scutigeromorphs appear in siderite nodules from Mazon Creek (Illinois, USA), from the Late Carboniferous (Mundel 1979); other centipedes present in this fossil bed are a scolopendromorph, and a possible geophilomorph. Therefore, there appears to be no Mesozoic fossil record for centipedes. Extant genera are well represented in Tertiary amber beds (Hoffman 1969), and in onyx deposits (Chamberlin 1949).

(b) *The problems in centipede systematics*

The relationships between the chilopod orders have been discussed by several authors, and two hypotheses have been considered. Whether the primitive chilopod is a scutigeromorph-like animal (with heteronomous segmentation and 15 body segments) or a geophilomorph-like one (with homonomous segmentation and many body segments) has been widely debated. The first hypothesis deals with the 'elongation' theory which implies an increase of the number of body segments during evolution, whereas the second one deals with the 'tachygenesis' theory which implies that the number of body segments has decreased during evolution. In addition, the plesiomorphic or apomorphic status of the epimorphism seems to be another crucial issue in centipede evolution.

Another important question refers to whether the order Lithobiomorpha should be grouped with the order Scutigeromorpha to form the subclass 'Anamorpha', or with Scolopendromorpha and Geophilomorpha to form the subclass Pleurostigmophora. A third question deals with the phylogenetic position of the enigmatic Craterostigmomorpha.

Historical reviews of the different classical chilopod phylogenetic hypotheses were presented by Lewis (1981) and Dohle (1985). More recently, some approaches based on morphological characters have tried to evaluate centipede evolution based on cladistic principles (Prunescu 1965, 1969*b*, 1996; Shinohara 1970; Dohle 1985; Shear & Bonamo 1988, 1990; Borucki 1996). Such analyses support the existence of two main evolutionary lines in the Chilopoda, the Notostigmophora (=Scutigeromorpha) and the Pleurostigmophora, Scutigeromorpha being the first offshoot in chilopod phylogeny. The phylogenetic trees presented by those authors are summarized in figure 1, and the main characters used are summarized in table 1.

The monophyly of the Chilopoda has never been in doubt, and a few synapomorphies for all chilopod groups are reported (Dohle 1980, 1985; Kraus & Kraus 1994; Borucki 1996). With the exception of the Craterostigmomorpha (which is monogeneric), the monophyly of the remaining four orders seems to be widely accepted. However, no clear synapomorphies exist for the monophyly of the Lithobiomorpha (see Dohle 1985; Shear & Bonamo 1988; Borucki 1996). The aims of this work are: (i) to establish phylogenetic patterns for the centipede orders, using molecular data sets from the 18S rDNA and 28S rDNA genes, testing out the classical morphological classifications with the topologies obtained using molecular data; and (ii) to produce an evolutionary

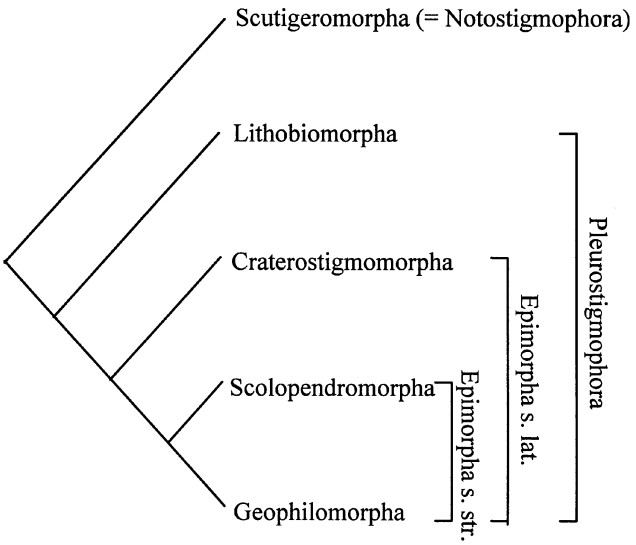

Figure 1. Cladogram of the orders of the Chilopoda based on cladistic morphological data analyses (Dohle 1985; Shear & Bonamo 1988; Borucki 1996).

Table 1. *Main systematic characters for the Chilopoda (based on the character coding of Dohle (1985), Shear & Bonamo (1988), Borucki (1996))*

Order Scutigeromorpha
Dorsal respiratory openings; haemolymph with a respiratory pigment; domed head; pseudofaceted eyes; fusion of the maxillae 2 coxae; absence of coxal–anal organs; absence of brood care; anamorphic development; heteronomous segmentation; presence of Tömosvary organs; 15 pairs of legs; segments 7–9 covered by a single tergite; multiannulate antennal segments.

Order Lithobiomorpha
Lateral paired spiracles; flattened head; maxilliped coxae with partial fusion; absence of brood care; anamorphic development; heteronomous segmentation; presence of Tömosvary organs; 15 pairs of legs; female gonopods with spines and a terminal article with a broad claw; presence of unpaired median testes in males.

Order Craterostigmomorpha
Lateral paired spiracles; flattened head; brood care; haemianamorphic development; heteronomous segmentation; presence of Tömosvary organs; 15 pairs of legs; divided long tergites; presence of anogenital capsule; segment 16 a complete cylinder.

Order Scolopendromorpha
Lateral paired spiracles; flattened head; brood care; epimorphic development; heteronomous segmentation; tibia and tarsus of the maxilliped podomeres incomplete; absence of Tömosvary organs; 21–23 pairs of legs; fusion of the maxilliped tergite to that of the next posterior segment.

Order Geophilomorpha
Lateral paired spiracles; flattened head; brood care; epimorphic development; homonomous segmentation; absence of Tömosvary organs; 29–191 pairs of legs; antennal annuli not variable (fixed at 14).

hypothesis for the evolution of the developmental patterns in the Chilopoda.

2. METHODS AND MATERIALS

(a) *Biological samples*

A total of nine centipede species belonging to the five extant centipede orders (two Scutigeromorpha, one Lithobiomorpha, one Craterostigmomorpha, three Scolopendromorpha, and two Geophilomorpha) were collected and reared in the laboratory, frozen at −80 °C or preserved in absolute ethanol for the molecular study. In addition we used two unpublished sequences from G. S. Min and co-workers (unpublished data) (one Lithobiomorpha and one Geophilomorpha), and a partial sequence from Friedrich & Tautz (1995) (a Lithobiomorpha).

In the taxonomic sampling an attempt was made to analyse the maximum morphological diversity within each clade, whenever possible, which is assumed to correspond to the maximum genetic diversity. A checklist of the sampled species and their systematic position is given in table 2. Detailed information on collecting localities can be provided upon request to the authors.

(b) *Sample preparation for the molecular study*

Genomic DNA samples were obtained from fresh, frozen or ethanol-preserved tissue, homogenized in a solution of guanidinium isothiocyanate following a modified protocol for RNA extraction (Chirgwin *et al.* 1979). The 18S rDNA was PCR amplified in two or three overlapping fragments of about 950, 900 and 850–1200 bp each, using primer pairs 1F-5R, 3F-18Sbi and 5F-9R, respectively. Primers used in amplification and sequencing are described by Giribet and co-workers (1996), except for forward primer 18Sa2.0 (5′-ATG GTT GCA AAG CTG AAA C-3′) and reverse primer 18Sbi (5′-GAG TCT CGT TCG TTA TCG GA-3′). The 28S rDNA fragment, of about 400 bp, was amplified by using primer pair 28Sa (5′-GAC CCG TCT TGA AAC ACG GA-3′) and 28Sb (5′-TCG GAA GGA ACC AGC TAC TA-3′). Amplification was done in a 100 µL volume reaction, with 0.6 units of DynaZyme™ polymerase, 100 µM of dNTPs and 0.5 µM of each primer. The PCR programme consisted of an initial denaturing step of 5 min at 95 °C and 35 amplification cycles (94 °C for 45 s, 49 °C for 45 s, 72 °C for 1 min).

Samples were purified with GenecleanR II kit (BIO 101 Inc.) and directly sequenced by using an automated ABI Prism 377 DNA sequencer. Cycle sequencing with AmpliTaq DNA Polymerase, FS using dye-labelled terminators (ABI PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit) is based on the Sanger method, and was performed in a Perkin-Elmer GeneAmp PCR system 9600. Amplification was done in a 20 µl volume reaction: 8 µl of Terminator Ready Reaction Mix, 10–30 ng ml^{-1} of PCR product, 5 pmol of primer and dH20 to 20 µl. The cycle sequencing programme consisted of an initial step of 94 °C for 3 min, 25 sequencing cycles (94 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min), and a rapid thermal ramp to 4 °C and hold. The Dye-labelled PCR products were ethanol precipitated with 0.1 volumes of 3 M sodium acetate, pH 5.2 and 2 volumes of 95% ethanol; 10 min on ice and 20 min centrifuging at 12 500 rpm. The pellet was cleaned with 50 µl of 70% ethanol and dried in a speed-vac at 60 °C for 5 min. All the new sequences obtained have been deposited in GenBank (see accession codes and taxonomic categories in table 2).

Table 2. *Chilopoda taxa and molecular data used with GenBank accession codes*

	18S rDNA	28S rDNA (D3 region)
Order Scutigeromorpha		
F. Scutigeridae		
Scutigera coleoptrata	AF000772[a]	AF000779[a]
Thereuopoda clunifera	AF000787[a], AF000788[a]	
Order Lithobiomorpha		
F. Lithobiidae		
Lithobius variegatus	AF000773[a]	AF000780[a]
Lithobius forficatus	X90653, X90654	X90656
F. Ethopolyidae		
Bothropolys asperatus[b]		
Order Craterostigmomorpha		
F. Craterostigmidae		
Craterostigmus tasmanianus	AF000774[a]	AF000781[a]
Order Scolopendromorpha		
F. Scolopendridae		
Scolopendra cingulata	U29493	AF000782[a]
F. Cryptopidae		
Cryptops trisulcatus	AF000775[a]	AF000783[a]
Theatops erythrocephala	AF000776[a]	AF000784[a]
Order Geophilomorpha		
F. Geophilidae		
Clinopodes cfr. *poseidonis*	AF000777[a]	AF000785[a]
F. Himantariidae		
Pseudohimantarium mediterraneum	AF000778[a]	AF000786[a]
F. Mecistocephalidae		
Nodocephalus doii[b]		

[a] New sequences from this study.
[b] Unpublished 18S sequences from G. S. Min.

(c) *Phylogenetic analysis*

DNA sequences were aligned by hand using the GDE editor, and helped by the 18S rRNA secondary structure prediction from the spider *Eurypelma californica* (Hendriks *et al.* 1988*b*). Some extremely divergent regions that could not be aligned unambiguously were excluded from the data set. Of these zones, one is region 41 (named after the secondary structure prediction of Hendriks *et al.* (1988*a*,*b*)), that has an insertion of about 350 bp in two of the geophilomorph sequences (*Pseudohimantharium mediterraneum* and *Clinopodes* cfr. *poseidonis*), making it impossible to align them. Only the data that was impossible to analyse phylogenetically was excluded.

There were two different alignments which were generated and analysed independently. The first alignment, including several sequences of non-centipede arthropods (Symphyla, Pauropoda, Diplopoda, Protura, Diplura, Collembola, Archaeognatha, Zygentoma, Chelicerata and Pycnogonida) was used to test the monophyly of the Chilopoda as well as to determine the most basal Chilopoda order. The second alignment containing only the 12 centipede sequences was generated include the maximum number of positions. This alignment including the 18S and 28S data sets had 2043 positions (288 variable; 112 parsimony-informative). It is accessible by anonymous ftp to the site porthos.bio.ub.es/incoming/pub/18sphylogeny (in GDE format). The trees were rooted from the

Table 3. *Step matrix used in the differentially weighted parsimony analysis*

	A	C	G	T
A	—	10	1	5
C	7	—	10	1
G	1	10	—	10
T	3	1	10	—

Scutigeromorpha, as this group was found to be the sister group of the remaining centipedes from the analysis of the previous alignment.

Parsimony analyses of the 18S rDNA and of the combined 18S+28S rDNA data sets were done using Nona v. 1.5.1 (Goloboff 1993) and a test version 4.0d57 of PAUP, written by David L. Swofford. For the equally weighted parsimony analyses we used Nona and PAUP. With Nona we performed an exact search (hold*;mult*100 and mswap*9), while a 'branch and bound' search was performed in PAUP with the ACCTRAN character optimization option, and multistate taxa interpreted as uncertainty. Only informative sites were used for the analysis, and thus steps and CI are expressed only considering the informative positions. Gaps were treated both as missing data and as a fifth character state. Nonparametric bootstrapping (1000 replicates) (Felsenstein 1985) and branch support or decay indexing (Bremer 1988, 1994; Donoghue *et al.* 1992) were done to evaluate the robustness of branches in the phylogenetic estimates (see a review of these methods in Swofford *et al.* (1996)).

MacClade 3.06 (Maddison & Maddison 1992, 1996) was used for exploring trees and to study character evolution on them. The frequency of unambiguous changes between states over the maximum parsimonious trees (MPTs) was estimated with the 'state changes & stasis' option in MacClade, and a transformation type was generated with the 'chart to type' option, using the function

$$K_{ij} = -\ln(X_{ij}/X_i),$$

where K_{ij} is the cost of going from state i to state j, X_{ij} is the number of $i \rightarrow j$ changes shown on the chart, and X_i is the number of changes from i to any state on the tree. This function is similar to those proposed by Wheeler (1990), and is monotone decreasing. This means that the higher the observed frequency, the lower the cost, the weights being scaled from 1 (the most frequent cost) to 10 (the least frequent kind of change) (table 3). An asymmetrical step matrix implementing these weights was analysed.

A neighbour-joining (NJ) analysis and a maximum-likelihood (ML) analysis were performed because it is assumed by some authors that both may be less sensitive to the long-branch attraction problem. The NJ analysis was done by using a substitution rate matrix (parameters estimates via ML with an assumed nucleotide frequency estimated from data), as implemented in the test version 4.0d57 of PAUP. A Kimura two-parameter model was also investigated.

The ML analysis was performed by estimating the optimal transition–transversion ratio using PAUP to be 2.22353. Among-site rate variation was estimated using a discrete approximation to the gamma distribution (shape parameter 0.760315, with four rate categories (Yang 1996)). Gaps were treated as missing data. Employing the HKY85 model (Hasegawa *et al.* 1985), which allows for two substitution types and unequal base frequencies, and estimating the proportion of invariant sites (0.623650) using ML, a single tree of log-likelihood (4944) was produced.

3. RESULTS

Phylogenetic analysis of the alignment including different arthropod groups yielded a monophyletic Chilopoda more closely related to Diplopoda (as obtained in previous molecular analyses: Wheeler *et al.* 1993; Friedrich & Tautz 1995; Wheeler 1995) than to any other arthropod group, although the topology is extremely sensitive to the parameters used and to taxa removal. The Scutigeromorpha are the most basal of the centipede lineage with bootstrap values of 81 for the represented Pleurostigmophora (results not shown).

The 18S rDNA sequences of two Scutigeromorpha, three Lithobiomorpha, one Craterostigmomorpha, three Scolopendromorpha and three Geophilomorpha species yielded an alignment of 1749 positions (94 parsimony-informative). The transition–transversion ratio for the entire 18S rDNA molecule is 1.70:1, similar to the ratio found by Smith and co-workers (1995) for echinoids, even though Vawter & Brown (1993) found no consistent transition–transversion bias in 18S rDNA genes. We also obtained a fragment of about 350 bp of the 28S rDNA D3 region from one Scutigeromorpha, two Lithobiomorpha, one Craterostigmomorpha, two Geophilomorpha and three Scolopendromorpha species.

The parsimony analysis of the combined 18S+28S rDNA data set of the 12 centipede species, rooting the trees from the Scutigeromorpha, resulted in a single cladogram of 228 steps (CI=0.627, RI=0.599) when gaps were treated as missing data (figure 2), and in an identical cladogram of 242 steps (CI=0.632, RI=0.588) when gaps were treated as a fifth character state. There were two cladograms differing from the previous trees only in the internal topology of the Scolopendromorpha which were obtained for the 18S data set alone, when gaps were treated as missing data (185 steps, CI=0.648, RI=0.630). The frequency distribution of tree lengths for the combined data set (gaps=missing data), which are up to 16 steps longer than the MPTs (table 4) were obtained through a 'branch and bound' search in PAUP.

The monophyly of the Scolopendromorpha, Geophilomorpha, Scutigeromorpha and *Lithobius* is obtained, showing decay index values between 2 and >17; and bootstrap values between 75 and 100 (in the combined analysis). The monophyly of the Epimorpha s. lat. (=Craterostigmomorpha+Epimorpha s. str.) depicts a decay index value of 2 and a bootstrap value below 60. The monophyly of the three representatives of the Lithobiomorpha was not indicated by the parsimony analyses, but it was found in the distance and maximum-likelihood analyses. The long branch depicted by *Bothropolys* may be the cause of this difference. Finally, the monophyly of the Epimorpha s. str. has a bootstrap value of 71 in the 18S data set alone, even though it is not well supported in the combined analysis. The differentially weighted parsimony analysis using the estimated asymmetrical step matrix (table 3) of the combined data set yielded a single cladogram of 1008 steps identical to the tree obtained in the equally weighted parsimony analysis.

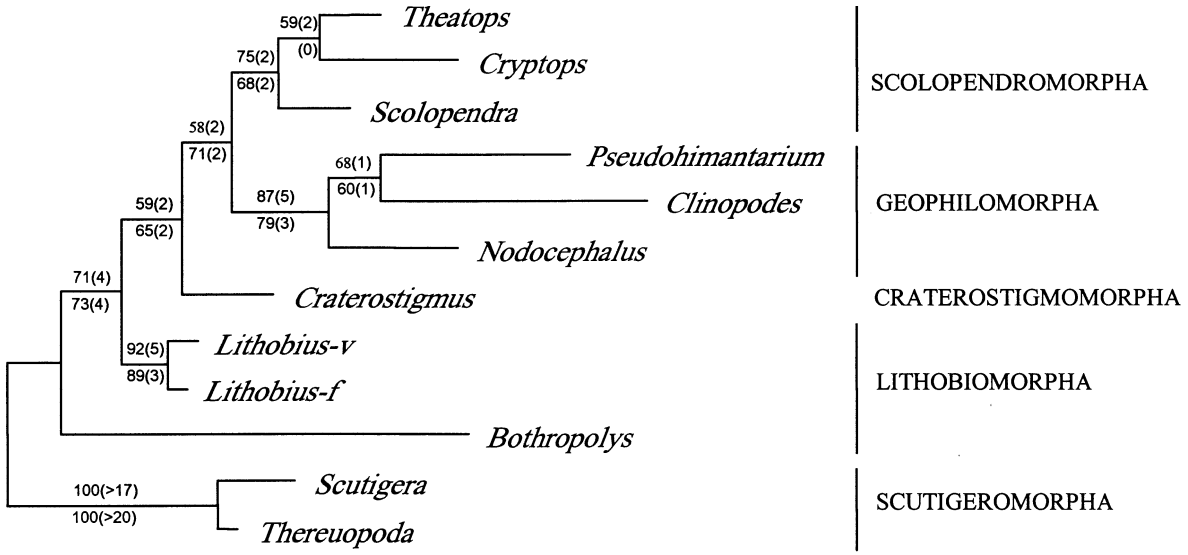

Figure 2. The single cladogram obtained from the equally weighted parsimony analysis of the combined 18S + 28S data set (gaps treated as missing data (228 steps, CI = 0.627, RI = 0.599) or as a fifth character state (242 steps, CI = 0.632, RI = 0.588)). Above each branch the bootstrap value and branch support index (in parentheses) for the combined analysis of 18S + 28S data sets are shown (gaps = missing data). The same symbols under the branch refer to the 18S data set alone. An additional tree differing in the internal topology of the Scolopendromorpha was found when analysing the 18S data set alone (gaps = missing data) (*Cryptops* (*Scolopendra* + *Theatops*)).

Table 4. *Frequency distribution of tree lengths for the combined data set which are up to 16 steps longer than the MPT in the equally weighted parsimony analysis (gaps = missing data)*

length	number of trees
228	1
229	1
230	9
231	13
232	18
233	38
234	53
235	75
236	144
237	201
238	346
239	485
240	712
241	1057
242	1364
243	1859
244	2577

The NJ (figure 3) and the ML trees apart from the monophyly of Scolopendromorpha, Geophilomorpha, Epimorpha s. str., and Epimorpha s. lat., also show the Lithobiomorpha as a monophyletic group (supported by a bootstrap value of 71 in the NJ analysis). They only differ in the internal topology of the Scolopendromorpha, and they differ from the MP trees in the position of *Bothropolys*.

4. DISCUSSION

The 18S data set seems to be a good indicator of internal centipede phylogeny, as it is able to recover their monophyly as well as the monophyly of some clearly morphologically defined groups. Nevertheless, the number of parsimony-informative positions used in the present analyses is low (about 5% of the total alignment), whereas for other arthropod groups it is higher (i.e. 10.5% for the arachnid order Opiliones (Giribet 1997)).

The 28S rDNA data set used in our analyses is not complete because the DNA samples of two of the species used (*Nodocephalus doii* and *Bothropolys asperatus*) were not obtained by the authors, and only the 18S rDNA sequences were provided by Min and co-workers. Furthermore, the species *Thereupoda clunifera* (Scutigeromorpha) could not be amplified by using the primer pair 28Sa and 28Sb. Also the 28S sequence of the two Geophilomorpha (*Pseudohimantharium* and *Clinopodes*) is extremely divergent (with an insertion of about 300 bp) and some regions could not be aligned with the remaining sequences. Nevertheless, we decided to include the 28S data set in the final analyses because it still contains some phylogenetic signal that increases resolution in some branches such as the Scolopendromorpha.

(a) *Molecular data agree with the accepted morphological hypothesis*

The results obtained here are completely consistent with the morphological centipede trees proposed by several authors (Prunescu 1965, 1996; Shinohara 1970; Dohle 1985; Shear & Bonamo 1988, 1990; Borucki 1996), indicating the presence of two main evolutionary lines: Notostigmophora (= Scutigeromorpha) and Pleurostigmophora (the remaining chilopod orders). Thus, the 'Anamorpha' as a taxonomic concept may be fully rejected, and anamorphic development can be considered as plesiomorphic, with the epimorphism being regarded as a synapomorphy of the Scolopendromorpha and the Geophilomorpha.

The split of the Chilopoda into Notostigmophora and Pleurostigmophora is consistent with their mode of life.

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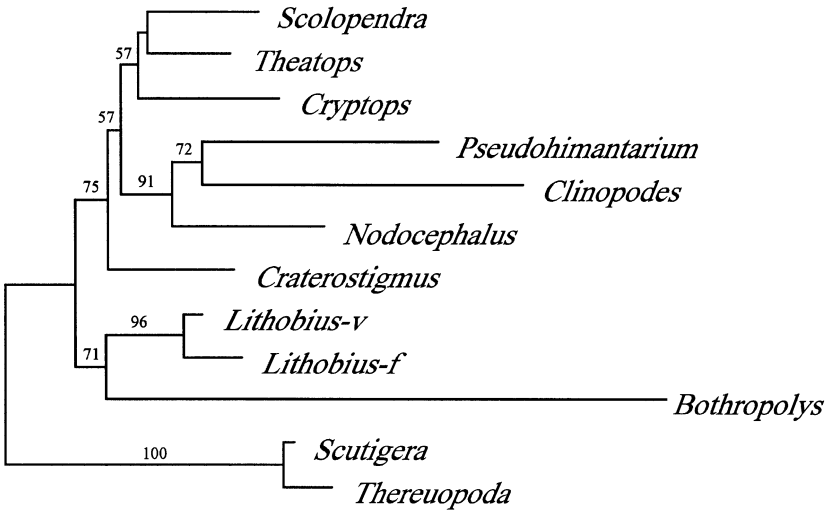

Figure 3. NJ tree of the combined 18S+28S data matrix. Numbers above branches represent bootstrap values. A ML analysis (using PAUP 4d57; initial parameters estimated from the NJ tree: TS–TV=2.22, proportions of invariable sites 0.623, approximately to gamma distribution (four categories)=0.76) produced a tree of −log-likelihood 4944, which differed from the NJ tree only in relationship with Scolopendromorpha (*Scolopendra* (*Theatops*+*Cryptops*)). The most parsimonious and NJ trees were calculated to be −log 4949 and 4945 respectively. According to the K/H test (Kishino & Hasegawa 1989) neither the MP nor the NJ were significantly worse than the ML tree ($p=0.31$ and 0. 39, respectively).

The Notostigmophora are adapted to an epigeous way of life, hunting in the open air, on rocky walls, in houses and on open beaches, and thus have well-developed pseudofaceted eyes and adaptations in the respiratory system. Prunescu (1996) recently commented that the Notostigmophora preserved most of the plesiomorphic features of the primitive chilopods, but also adapted their metabolism and some of their organs to this different environment. In contrast, the Pleurostigmophora reflects a stepwise approach towards subterraneous habitats (Borucki 1996), with adaptations such as body flattening and elongation, reduction of eyes, and a very different respiratory system.

(b) *The Lithobiomorpha are problematic*

The emergence of the groups within the Pleurostigmophora is also consistent with the morphological studies yielding the following structure: (Lithobiomorpha (Craterostigmomorpha (Scolopendromorpha + Geophilomorpha))), with the only difference that in our parsimony analyses, the Lithobiomorpha represent a paraphyletic group, as *Bothropolys* do not cluster with the two *Lithobius* species. However, we have to consider that the genera *Lithobius* and *Bothropolys* belong to different families, although they are morphologically very similar. Such a result could be explained by the fact that the *Bothropolys* 18S rDNA sequence (G. S. Min, unpublished data) is extremely divergent and has numerous substitutions as well as indel events. This suggests the possibility of an artefactual paraphyly of the Lithobiomorpha, that contrasts with morphological data and with the results obtained from the NJ and ML analyses. *Bothropolys* is the longest branch in the tree, and it is followed in length by the branches of the Scutigeromorpha, which in turn seems to indicate an inconsistency of the 'long-branch attraction effect' kind (Felsenstein 1978; Hendy & Penny 1989).

In a recent phylogenetic study based on the male genital system of the Chilopoda, Prunescu (1996) regarded the Lithobiomorpha as a paraphyletic group. However, it is the family Henicopidae (not sampled in our study) which was paraphyletic in the study of Prunescu who did not include the Ethopolyidae. Referring to the status of the Lithobiomorpha, only a few morphological synapomorphies have been proposed for the monophyly of the group, and as commented by Dohle (1985), the Lithobiomorpha do not have many common characters that could be designated as synapomorphic. He commented that 'probably the unpaired tubular testis in connection with the pair of large seminal vesicles in the male, and the female gonopods, with a basal article with spines and a terminal article with a broad claw, are synapomorphies'. But he only mapped the first character in his phylogenetic tree. Lately, Shear & Bonamo (1988) listed the same two synapomorphies for the Lithobiomorpha: the presence of unpaired median testes, and the presence of female gonopods with macrosetae. Finally, the presence of unpaired testes is the only synapomorphy listed by Borucki (1996), although it is considered as the plesiomorphic state within the Pleurostigmophora.

Whether monophyletic or not, the Lithobiomorpha represent the first offshoot within the Pleurostigmophora, and they retain several plesiomorphic chilopod characters, as has also been proposed by many morphologists on the basis of the 15 leg-bearing segment body plan and the anamorphic development.

(c) *The position of the enigmatic* Craterostigmus, *and the 'Epimorpha' clade*

The Craterostigmomorpha, or the species *Craterostigmus tasmanianus*, has been considered as an enigmatic group, because it presents 15 pairs of walking legs in adult individuals (as in the Scutigeromorpha and Lithobiomorpha), but the development is not strictly anamorphic, because the first stage has 12 pairs of legs and the final number of legs is achieved after only one moult. Nevertheless, the distribution of the spiracles is more similar to that of the Scolopendromorpha than to any other group. Based on the presence of a brood-care behaviour in *Craterostigmus*, Manton (1965) suggested that it lies in the Epimorpha, whereas many authors place it as the sister group of the Epimorpha (Shinohara 1970; Dohle 1980, 1985, 1990; Shear & Bonamo 1988; Borucki 1996; Prunescu 1996), or as the sister group of the Scolopendromorpha (Prunescu 1969*a*) or as a group of Lithobiomorpha. Our results agree with the proposal that *Craterostigmus* is the sister group of the Epimorpha s. str.

The Epimorpha s. str. is another of the clades obtained here that share, among other characters, an epimorphic

development (although the presence of a variable number of segments in some species of the Geophilomorpha has created suspicions about a postembryonic addition of new segments, but this has not been confirmed to date).

5. CONCLUSIONS

The phylogenetic pattern of the Chilopoda obtained using molecular data of the ribosomal genes is consistent with the data obtained from morphological studies. Both sources of data indicate that the epimorphic development is apomorphic, while the anamorphic development is the plesiomorphic state within the centipedes. The monophyly of the three Scolopendromorpha, the three Geophilomorpha, the two species of the genus *Lithobius*, and the two Scutigeromorpha are the best-resolved areas of our cladograms, while some internal branches are not well supported in the equally weighted parsimony analyses. These problems should be resolved by adding more taxa to the data matrix, such as some Lithobiomorpha of the family Henicopidae, which would contribute to shortening the branches on the trees (see Hillis 1996; Purvis & Quicke 1997), and thus hopefully avoid the putative 'inconsistency problem' caused by *Bothropolys*.

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