Cladistics 16, 204–231 (2000)
doi:10.1006/clad.1999.0128, available online at http://www.idealibrary.com on IDEAL®

A Review of Arthropod Phylogeny: New Data Based on Ribosomal DNA Sequences and Direct Character Optimization

Gonzalo Giribet,*[,1] and Carles Ribera†

**Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024 and †Departament de Biologia Animal, Universitat de Barcelona, Avinguda Diagonal 645, 08071 Barcelona, Spain*

Accepted December 15, 1999

Ribosomal gene sequence data are used to explore phylogenetic relationships among higher arthropod groups. Sequences of 139 taxa (23 outgroup and 116 ingroup taxa) representing all extant arthropod "classes" except Remipedia and Cephalocarida are analyzed using direct character optimization exploring six parameter sets. Parameter choice appears to be crucial to phylogenetic inference. The high level of sequence heterogeneity in the 18S rRNA gene (sequence length from 1350 to 2700 bp) makes placement of certain taxa with "unusual" sequences difficult and underscores the necessity of combining ribosomal gene data with other sources of information. Monophyly of Pycnogonida, Chelicerata, Chilopoda, Chilognatha, Malacostraca, Branchiopoda (excluding *Daphnia*), and Ectognatha are among the higher groups that are supported in most of the analyses. The positions of the Pauropoda, Symphyla, Protura, Collembola, Diplura, Onychophora, Tardigrada, and *Daphnia* are unstable throughout the parameter space examined.

[1]Present address: Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138. E-mail: ggiribet@oeb.harvard.edu.

INTRODUCTION

Arthropods may well constitute the most speciose animal group ever to have existed. According to Nielsen (1995:158), "the insects alone are now believed to comprise more than a million living species, while the other arthropods number more than 100,000." They were also numerous in the fossil record, constituting probably the most diverse group of animals as early as the Cambrian (Fortey *et al.*, 1996, 1997).

Arthropods are especially well known morphologically. Their understanding at the molecular level is ever more important, given the prominence of *Drosophila*. The internal relationships among the four main arthropod groups, however, are still being furiously debated (e.g., paleontologists vs neontologists vs molecular biologists; see Edgecombe, 1998a; Fortey and Thomas, 1998; Melic *et al.*, 1999).

Classical morphological studies have considered arthropods monophyletic (Lankester, 1904; Snodgrass, 1938), diphyletic (Tiegs and Manton, 1958), or polyphy-

0748-3007/00 $35.00

letic[2] (Anderson, 1973; Manton, 1973). The protestations of Fryer (1996, 1998) aside, the monophyly of arthropods is well established. The monophyly of Mandibulata, all the arthropods with mandibles (crustaceans, myriapods, and hexapods), is supported by neontological and molecular data (i.e., Snodgrass, 1938, 1950, 1951; Weygoldt, 1979; Boudreaux, 1987; Kukalová-Peck, 1992; Wägele, 1993; Wheeler *et al.*, 1993; Wheeler, 1995, 1998a,b; Giribet and Ribera, 1998). On the other hand, many paleontologists consider the Crustacea to be the sister group of the Chelicerata (and certain fossil groups such as Trilobita) in the Schizoramia (i.e., Cisne, 1974; Briggs and Fortey, 1989; Schram and Emerson, 1991; Briggs *et al.*, 1992; Budd, 1993, 1996; Wills *et al.*, 1994, 1995, 1998; Waggoner, 1996; Emerson and Schram, 1998). Both hypotheses are summarized in Fig. 1. One feature common to almost all morphological hypotheses of arthropod relationships is the monophyly of "Atelocerata" or "Tracheata," a taxon comprising myriapods and hexapods (Snodgrass, 1938, 1950, 1951; Briggs and Fortey, 1989; Schram and Emerson, 1991; Bergström, 1992; Briggs *et al.*, 1992; Wägele, 1993; Wheeler *et al.*, 1993; Kraus and Kraus, 1994, 1996; Wills *et al.*, 1994, 1995, 1998; Fortey *et al.*, 1997; Emerson and Schram, 1998; Kraus, 1998; Wheeler, 1998a, b; but see the total evidence analysis of Zrzavý *et al.*, 1998). The molecular studies based on ribosomal gene sequence data, however, disagree, proposing a sister-group relationship between crustaceans and hexapods (Turbeville *et al.*, 1991; Friedrich and Tautz, 1995; Giribet *et al.*, 1996; Giribet and Ribera, 1998).

Several studies have attempted to solve the internal phylogenetic pattern of the arthropod groups using different molecular markers including 18S rRNA (Turbeville *et al.*, 1991; Wheeler *et al.*, 1993; Friedrich and Tautz, 1995; Giribet *et al.*, 1996; Giribet and Ribera, 1998; Spears and Abele, 1998; Wheeler, 1998a,b; Zrzavý *et al.*, 1998a), 28S rRNA (Friedrich and Tautz, 1995; Wheeler, 1998a,b; Zrzavý *et al.*, 1998a), 5.8S rRNA (Zrzavý *et al.*, 1998a), 12S rRNA (Ballard *et al.*, 1992; Wägele and Stanjek, 1995; Zrzavý *et al.*, 1998a), 16S rRNA (Zrzavý *et al.*, 1998a), ubiquitin (Wheeler *et al.*, 1993; Wheeler, 1998b; Zrzavý *et al.*, 1998a), histone H3 (Colgan *et al.*, 1998), U2 snRNA (Colgan *et al.*, 1998), elongation factor 1-α (Regier and Shultz, 1997, 1998), the largest subunit of RNA polymerase II (Regier and Shultz, 1997), and mitochondrial gene order (Boore *et al.*, 1995, 1998). Some of the approaches are especially interesting in that they combine several sources of molecular data plus morphological characters (i.e., Wheeler *et al.*, 1993; Wheeler, 1998a,b; Zrzavý *et al.*, 1998a), but in general these studies depicted a poor taxon sampling, with the exception of the study of Wheeler (1998a).

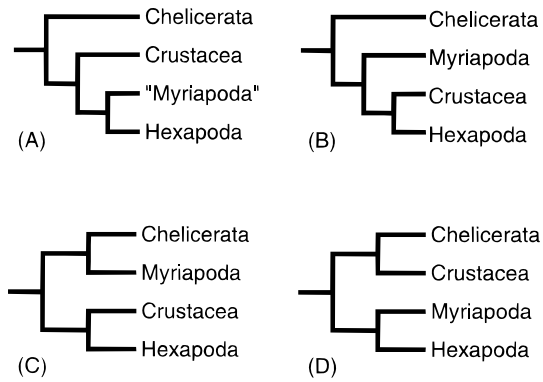

FIG. 1. Main hypotheses of internal relationships for the Arthropoda. (A) Mandibulata and Atelocerata (Snodgrass, 1938; Weygoldt, 1979; Wägele, 1993; Wheeler *et al.*, 1993; Wheeler, 1995, 1998a,b); (B) Mandibulata and Pancrustacea (Zrzavý *et al.*, 1998a; Giribet *et al.*, 1996; Giribet and Ribera, 1998); (C) Pancrustacea and (Myriapoda + Chelicerata) (Turbeville *et al.*, 1991; Friedrich and Tautz, 1995; Giribet *et al.*, 1996); (D) Schizoramia (Cisne, 1974; Briggs *et al.*, 1992; Budd, 1993).

This analysis attempts to approach the study of the internal arthropod relationships by using complete 18S rRNA and partial 28S rRNA (D3 region) sequences of 139 terminal taxa, including a wide sampling within all major arthropod groups. Compared to previous molecular analyses of arthropods based on nuclear ribosomal sequence data, the present analysis incorporates a much broader taxon sampling. Of previous studies, only Wheeler (1998a) is comparable in terms of molecular taxon sampling, but the present study also provides the first complete 18S rRNA sequences from important arthropod groups such as symphylans, pauropods, proturans, and diplurans. For the first time all major groups of myriapods are well represented. This analysis also incorporates molecular data from the remaining groups of Ecdysozoa, which had not been used previously as outgroups in phylogenetic studies

[2]Here we use the term "polyphyletic" as found in the arthropod literature, but the relationships proposed by these authors did not consider any sister-group relationships for the arthropod groups to other non-arthropod phyla (except the Onychophora).

of internal arthropod relationships, but are important to test arthropod monophyly (see Giribet, 1999). In addition to the improved taxon sampling, this study attempts to evaluate the impact of data exploration on the choice of a particular parameter set, as previously emphasized by Wheeler (1995, 1998b).

MATERIALS AND METHODS

Taxon Sampling

Outgroups. The choice of outgroup taxa for the study of internal arthropod relationships is a contentious issue. Two competing hypotheses, "Articulata" and "Ecdysozoa," have been proposed (see a review in Giribet, 1999). The first hypothesis places arthropods with mollusks and annelids due to their segmented body plan (i.e., Nielsen *et al.*, 1996). The second hypothesis places arthropods within a clade of animals that molt their cuticles (Aguinaldo *et al.*, 1997). Due to the current evidence, arthropod trees were rooted with other molting taxa such as Nematoda, Nematomorpha, Kinorhyncha, Priapulida, Onychophora, and Tardigrada (see also Giribet and Ribera, 1998; Zrzavý *et al.*, 1998b; Giribet and Wheeler, 1999b; Giribet *et al.*, 2000). Outgroup taxa are thus represented by sequences of 23 taxa belonging to six animal phyla.

Ingroup. Arthropod sequences have been carefully chosen to represent almost every arthropod order for which there were (1) complete 18S rRNA sequence data or (2) in few cases, partial 18S rRNA sequences (with more than 1000 bp) of taxa for which the D3 region of the 28S rRNA gene fragment was available. In total, 66 extant arthropod orders were represented, as follows:

• Chelicerata: Twenty-one complete 18S rRNA sequences and 19 sequences of the D3 region from the 28S rRNA gene fragment. These sequences include 4 pycnogonids, 2 xiphosurans, and 15 arachnids representing all the arachnid orders except palpigrades.

• Myriapoda: Sixteen 18S rRNA sequences and 14 sequences from the D3 region of the 28S rRNA gene. The sequences include 8 centipedes, 5 millipedes, 2 symphylans, and 1 pauropod.

• Crustacea: Twenty-eight complete 18S rRNA sequences and 3 sequences from the D3 region of the 28S rRNA gene. These sequences include representatives of the Branchiopoda, Maxillopoda, and Malacostraca.

• Hexapoda: Forty-one 18S rRNA sequences and 25 sequences from the D3 region of the 28S rRNA gene. We have focused on generating sequences of the entognathous hexapods (Protura, Diplura, and Collembola) and basal ectognaths (Zygentoma and Archaeognatha). All extant basal hexapod orders are represented, and many orders of Ectognatha are also included in the analysis.

Sequences

Forty-four complete 18S rRNA sequences (2 onychophorans, 1 tardigrade, 19 chelicerates, 15 myriapods, and 7 hexapods) and 36 sequences of the D3 region of the 28S rRNA loci were generated by the authors. All the new sequences were deposited in GenBank (see accession codes in Table 1).

Genomic DNA samples were obtained from fresh, frozen, or ethanol-preserved tissues, homogenized in a solution of guanidinium isothiocyanate following a modified protocol for RNA extraction from Chirgwin *et al.* (1979). The 18S rRNA gene was PCR-amplified in two or three overlapping fragments of about 950, 900, and 850 bp each, using primer pairs 1F–5R, 3F–18Sbi, and 5F–9R, respectively. Primers used in amplification and sequencing were described in Giribet *et al.* (1996, 1999c). The 28S rRNA fragment, of about 400 bp, was amplified using primer pair 28Sa and 28Sb (Whiting *et al.*, 1997). Amplification was carried out in a 50- to 100-μl volume reaction, with 0.6 units of DynaZyme polymerase, 100 μM dNTPs and 0.5 μM each primer. The PCR program consisted of a first 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) in a Perkin–Elmer 480 thermal cycler.

Some samples were purified and ligated into pUC 18 *Sma*-I/BAP dephosphorylated vector using the SureClone Ligation Kit (Pharmacia P-L Biochemicals) as described in Giribet *et al.* (1996, 1999c). Sequencing was performed by the dideoxy termination method (Sanger *et al.*, 1977) using T7 DNA polymerase (^{T7}Sequencing Kit from Pharmacia Biotech).

Other samples were purified with Geneclean II kit (BIO 101, Inc.) and directly sequenced using an automated ABI Prism 373 or 377 DNA sequencer. Cycle-sequencing with AmpliTaq DNA polymerase FS using

TABLE 1
Terminal Taxa and Loci Used

		18S	28S
Phylum Kinorhyncha (1 sp.)	*Pycnophyes kielensis*	X	
Phylum Priapulida (1 sp.)	*Priapulus caudatus*	X	
Phylum Nematomorpha (3 sp.)	*Gordius aquaticus*	X	
	Gordius albopunctatus	X	
	Chordotes morgani	X	
Phylum Nematoda (12 sp.)			
Class Adenophorea			
Order Araeolaimida	*Plectus* sp.	X	X
Order Desmodorida	*Desmodora ovigera*	X	
Order Chromadorida	*Metachromadora* sp.	X	
Order Enoplida	*Enoplus brevis*	X	
Order Dorylaimida	*Longidurus elongatus*	X	
Order Mermithida	*Mermis nigrescens*	X	
Order Trichocephalida	*Trichinella spiralis*	X	
Class Secernentea			
Order Tylenchida	*Globodera pallida*	X	
Order Aphelenchida	*Bursaphelenchus* sp.	X	
Order Strongylida	*Haemonchus placei*	X	
Order Ascaridida	*Anisakis* sp.	X	
Order Spirurida	*Brugia malayi*	X	
Phylum Onychophora (3 sp.)			
F. Peripatopsidae	*Peripatopsis capensis*	AF119087	X
	Euperipatoides leukartii	X	
F. Peripatidae	*Epiperipatus biolleyi*	X	
Phylum Tardigrada (3 sp.)			
Class Eutardigrada			
Order Parochela	*Hypsibius* sp.	X	
	Macrobiotus hufelandi	X81442	
Order Apochela	*Milnesium tardigradum*	X	
Phylum Arthropoda			
Chelicerata			
Class Pycnogonida			
F. Ammotheidae	*Achelia echinata*	AF005438	AF005459
F. Pallenidae	*Callipallene* sp.	AF005439	AF005460
F. Endeidae	*Endeis laevis*	AF005441	AF005462
F. Colossendeidae	*Colossendeis* sp.	AF005440	AF005461
Class Merostomata			
Order Xiphosura	*Limulus polyphemus*	U91490	U91492
	Carcinoscorpius rotundicaudatus	U91491	U91493
Class Arachnida			
Order Scorpiones	*Belisarius xambeui*	AF005442	AF124954
Order Ricinulei	*Pseudocellus pearsei*	U91489	AF124956
Order Solifugae	*Gluvia dorsalis*	AF007103	AF124957
	Eusimonia wunderlichi	U29492	AF124958
Order Schizomida	*Stenochrus portoricensis*	AF005444	
Order Uropygi	*Mastigoproctus giganteus*	AF005446	AF062990
Order Amblypygi	*Paraphrynus* sp.	AF005445	AF124959
Order Araneae	*Liphistius bicoloripes*	AF007104	AF124960
	Nesticus celullanus	AF005447	AF124961
Order Pseudoscorpiones	*Roncus* cfr. *pugnax*	AF005443	AF124962
Order Acari	*Boophilus annulatus*	X	
	Ixodes ricinus	X	
Order Opiliones	*Parasiro coiffaiti*	U36999	U91495
	Centetostoma dubium	U37002	U91499
	Equitius doriae	U37003	U91503

TABLE 1—*Continued*

		18S	28S
Myriapoda			
Class Chilopoda			
Order Scutigeromorpha	*Scutigera coleoptrata*	AF000772	AF000779
	Thereuopoda clunifera	AF173239	AF173270
Order Lithobiomorpha	*Lithobius variegatus*	AF000773	AF000780
Order Craterostigmomorpha	*Craterostigmus tasmanianus*	AF000774	AF000781
Order Scolopendromorpha	*Scolopendra cingulata*	U29493	AF000782
	Cryptops trisulcatus	AF000775	AF000783
Order Geophilomorpha	*Pseudohimantarium mediterraneum*	AF000778	AF000786
	Clinopodes poseidonis	AF000777	AF000785
Class Diplopoda			
Order Polyxenida	*Polyxenus lagurus*	X	X
Order Callipodida	*Abacion magnum*	X	
Order Julida	*Cylindroiulus punctatus*	AF005448	AF005463
Order Polydesmida	*Polydesmus coriaceus*	AF005449	AF007105
Order Spirostreptida	*Thyropisthus* sp.	X*	
Class Symphyla			
F. Scutigerellidae	*Scutigerella* sp1.	AF007106	AF005464
	Scutigerella sp2.	AF005450	AF005465
Class Pauropoda			
F. Pauropodidae	Pauropodidae sp.	AF005451	AF005466
Crustacea			
Class Branchiopoda			
Order Anostraca	*Branchinecta packardi*	X	
	Artemia salina	X	X
Order Notostraca	*Lepidurus packardi*	X	
Order Cladocera	*Daphnia galeata*	X	
Order Conchostraca	*Limnadia lenticularis*	X	
Class Maxillopoda			
SubClass Ostracoda			
Superorder Myodocopa	*Euphilomedes cacharodonta*	X	
	Rutiderma sp.	X	
Superorder Podocopa	*Heterocypris* sp.	X	
	Bairdia sp.	X	
SubClass Copepoda			
Order Calanoida	*Calanus pacificus*	X	
Order Harpacticoida	*Cancrincola plumipes*	X	
Order Cyclopoida	*Encyclops serrulatus*	X	
SubClass Branchiura	*Argulus nobilis*	X	
Pentastomida	*Porocephalus crotali*	X	
SubClass Cirripedia			
Order Thoracica	*Balanus eburneus*	X	
	Balanus sp.[a]		X
	Lepas anatifera	X	
Order Ascothoracica	*Ulophysema oeresundense*	X	
Order Acrothoracica	*Berndtia purpurea*	X	
	Trypetesa lampas	X	
Order Rhizocephala	*Loxothylacus texanus*	X	
Class Malacostraca			
Order Leptostraca	*Nebalia bipes*	X	
Order Stomatopoda	*Squilla empusa*	X	
	Gonodactylus sp.	X	
Order Anaspidacea	*Anaspides tasmaniae*	X	
Order Decapoda	*Panulirus argus*	X	
	Procambarus leonensis	X	
	Procambarus clarkii[b]		X

TABLE 1—*Continued*

		18S	28S
Hexapoda			
Order Collembola	*Lepidocyrtus paradoxus*	X	
	Podura aquatica	AF005452	AF005468
Order Protura	*Acerentulus traegardhi*	AF005453	AF005469
Order Diplura	Campodeidae sp1.	AF005454	AF005470
	Campodeidae sp2.	AF005455	AF005471
	Catajapyx sp.	AF005456	
	Metajapyx sp.[c]		AF005472
Order Archaeognatha	*Dilta littoralis*	AF005457	AF005473
	Petrobius brevistylis	X*	X
	Trigoniopthalmus alternatus	X*	X
Order Zygentoma	*Lepisma* sp.	AF005458	AF005474
Order Ephemeroptera	*Ephemera* sp.	X	
	Ephemerella sp.	X*	X
Order Odonata	*Libellula pulchella*	X*	X
	Aeschna cyanea	X	
Order Plecoptera	*Cultus decisus*	X*	X
	Mesoperlina pecircai	X*	
Order Blattodea	*Blaberus* sp.	X*	
Order Mantodea	*Mantis religiosa*	X*	X
Order Dermaptera	*Forficula* sp.	X	
	Labidura riparia	X*	
Order Orthoptera	*Melanoplus* sp.	X*	X
	Acheta domesticus	X	
Order Hemiptera	*Saldula pallipes*	X*	X
	Raphigaster nebuloa	X	
Order Homoptera	*Okanagana utahensis*	X	
	Philaenus spumaris	X	
	Spissistilus festins	X	
Order Diptera	*Tipula* sp.	X	X
	Drosophila melanogaster	X	X
Order Siphonaptera	*Archaeopsylla erinacei*	X	
	Ctenocephalides canis	X*	X
Order Mecoptera	*Boreus* sp.	X	
	Boreus coloradensis[d]		X
Order Megaloptera	*Sialis* sp.	X	
Order Neuroptera	*Myrmeleon immaculatus*	X*	X
Order Coleoptera	*Tenebrio molitor*	X	X
	Meloe proscarabaeus	X	
Order Trichoptera	*Hydropsyche* sp.	X	
	Hydropsyche sparna[e]		X
Order Lepidoptera	*Galleria mellonella*	X*	X
	Papilio troilus	X*	X
Order Hymenoptera	*Dasymutilla gloriosa*	X*	X
	Leptothorax acervorum	X	
	Polistes dominulus	X	
	Polistes fuscatus[f]		X

Note. Sequences from the authors with GenBank accession codes are given. Otherwise, an X indicates the partition available at GenBank. Asterisks refer to incomplete sequences. 28S refers to the D3 region.

[a] The 28S sequence of *Balanus* sp. has been combined with the 18S sequence of *B. eburneus.*

[b] The 28S sequence of *Procambarus clarkii* has been combined with the 18S sequence of *P. leonensis.*

[c] The 28S sequence of *Metajapyx* sp. has been combined with the 18S sequence of *Catajapyx* sp.

[d] The 28S sequence of *Boreus coloradensis* has been combined with the 18S sequence of *Boreus* sp.

[e] The 28S sequence of *Hydropsyche sparna* has been combined with the 18S sequence of *Hydropsyche* sp.

[f] The 28S sequence of *Polistes fuscatus* has been combined with the 18S sequence of *P. dominulus.*

dye-labeled terminators (ABI Prism Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit) is also based on the Sanger method and was performed in a Perkin–Elmer GeneAmp PCR system 9600 or 2400. Amplification was carried out in a 20-μl volume reaction: 8 μl of Terminator Ready Reaction Mix, 10–30 ng/ml PCR product, 5 pmol of primer, and dH_2O to 20 μl. The cycle-sequencing program consisted of a previous 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-labeled PCR products were ethanol-precipitated with 0.1 volumes of 3 M NaOAc, pH 5.2, and 2 vol 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.

Sequences were divided into the smallest (unambiguously recognizable) homologous regions possible to save computation time as well as to avoid trying to "align" non-homologous DNA regions. The split was done first by using primer regions and then by identifying secondary structure features. Nomenclature of the secondary structure regions of the 18S rRNA gene followed Hendriks *et al.* (1988). When the split was not trivial for all the taxa, we decided not to divide the sequences (i.e., regions E21-1 and E21-2 were treated as one single fragment). In total, the 18S rRNA molecule was divided into 44 regions (excluding the external primers 1F and 9R). Six of the 44 regions (10, E10-2, E21-1–2, 41, 43–44, and 47b) were excluded from the analyses because of large variation in sequence length among the sampled taxa. For example, region 41 ranges between 25 and 385 nucleotides in length. The D3 fragment of the 28S rRNA gene was divided into 7 regions, from which 4 variable ones were not used due to problems in establishing primary homology. In total, 38 regions of the 18S rRNA gene and 3 regions of the 28S rRNA gene fragment were included in the analyses.

Data Analysis

"Direct optimization." Sequence data were analyzed using the "direct optimization" method described by Wheeler (1996; see also Wheeler and Hayashi, 1998) and implemented in the computer program POY (Gladstein and Wheeler, 1997). This method directly assesses the number of DNA sequence transformations (evolutionary events) required by a phylogenetic topology without the use of multiple sequence alignment. This is accomplished through a generalization of existing character optimization procedures to include insertion and deletion events (indels) in addition to base substitutions. The crux of the method is the treatment of indels as processes as opposed to the patterns implied by multiple sequence alignment. The results of this procedure are directly compatible with parsimony-based tree lengths and appear to generate more efficient (simpler) explanations of sequence variation than multiple sequence alignment (Wheeler, 1996). The method, although computationally intense, is much less demanding than parsimony-based multiple sequence alignment algorithms such as the one implemented in MALIGN (Wheeler and Gladstein, 1994, 1995). The method has also been demonstrated to yield more congruent results than multiple sequence alignments when using character congruence among partitions as a criterion (Wheeler and Hayashi, 1998), although exceptions may exist (Giribet, unpublished data).

Sensitivity analysis. Character transformations were weighted differentially to study how they affect phylogenetic conclusions (sensitivity analysis *sensu* Wheeler, 1995). A parameter space of two analytical variables was examined: insertion–deletion cost ratio and transversion–transition ratio (as in Wheeler, 1995). When the transversion–transition ratio was set at a value other than unity, the insertion–deletion cost was set according to the cost of transversions. In total, six combinations of parameters were employed in the analysis (with insertion–deletion ratios of 1, 2, 4; transversion–transition ratios of 1, 2, 4). The six parameters employed are named 111, 211, 411, 121, 141, and 221, with a maximum gap value of 4 (in the following cases: 411, 141, 221). For example, parameter set 221 (gap:tv:ts ratio) means that the gap cost is set as twice the highest change cost (in this case the tv, which is set as twice the ts cost). So, the ratio 221 implies costs for gap, tv, and ts of 4, 2, and 1, respectively. The stepmatrices were specified using the command **-molecularmatrix** with an argument for the given stepmatrix. For example, **-molecularmatrix 221** has the following format:

0	2	1	2	4
2	0	2	1	4
1	2	0	2	4
2	1	2	0	4
4	4	4	4	0

Since parameter choice is arbitrary (although explicit), the necessity of parameter exploration is stressed. A sensitivity analysis is considered a way to explore the data and to discern between robust relationships (those supported throughout a wide range of parameters) and unstable relationships (those that appear only under particular parameter sets). For arthropods, the best parameter sets have been experimentally found to be for gap:transversion:transition = 211, 411, 221 (Wheeler, 1995, 1998a,b, Wheeler and Hayashi, 1998), but in certain cases, especially for low-level relationships, higher gap costs have been found to be the most congruent ones (e.g., Giribet and Wheeler, 1999a). We consider that such a data exploration is required when analyzing molecular data and in particular when analyzing large data sets of groups as diverse as arthropods. As Wheeler (1998b:26) stated: "Arthropods are too large and diverse a group to be allied based on a single shot in the dark, whether that be due to taxonomic, empirical, or epistemological myopia."

The analyses were run in a parallel cluster of 21 processors (the command **-parallel** executes in parallel using PVM). In order to speed up the analyses, the jackboot option of POY (it does parsimony jackknifing in the sense of Farris, 1995; Farris *et al.*, 1996) was used and the 50% majority rule consensus of all the trees was used as a constraint for further searches. The following commands were used for the jackboot search:

-norandomizeoutgroup: does not allow the randomization of the outgroup in "random" and "multibuild." This should be specified for jackboot and constrained runs.

-noleading: does not count leading and trailing gaps.

-seed -1: sets seed for pseudorandom number generation. An argument of -1 will cause the system time, in seconds, to be used.

-nospr: "spr" branch swapping suppressed.

-notbr: "tbr" branch swapping suppressed.

-maxtrees 2: set maximum number of trees held in buffers to "2".

-jackboot: performs Farris' parsimony jackknifing procedure with "random n" replicates or "multibuild n" replicates. All topologies are output.

-random 25: causes "25" random addition sequence searches (build through swapping) to be performed. Since the option "norandomizeoutgroup" is also specified, the outgroup will be unaffected.

The accessory program jack2hen, available together with POY, was used to generate the 50% majority rule consensus trees: **jack2hen 50 < infile > outfile**. Then, the constrained tree (**-constrain file**) was used as a starting point for an unconstrained search (for the same molecularmatrix for which the constrained file was generated) using spr and tbr branch swapping (**-spr -tbr**). Other commands specified were **-noleading -maxtrees 20 -multibuild 10 -seed 1 -slop 1**.

-slop 1: check all cladogram lengths which are within "1" tenths of a percent of the current minimum value. A slop value of 10 would check all cladograms found within 1% of the minimum tree length. This option slows down the search, but is less affected by the heuristics of the tree-length calculation shortcuts.

-multibuild 10: causes "10" random addition sequence builds (no swapping) to be performed. The best addition(s) is submitted to branch swapping. Since **-parallel** is specified, the builds are performed remotely.

The complete command line for a given stepmatrix is presented in Appendix 1.

Some clarifications. The terms "Chelicerata" and "chelicerates" refer to Pycnogonida and Euchelicerata; "Myriapoda" and "myriapods" refer to Chilopoda, Diplopoda, Symphyla, and Pauropoda; "Crustacea" and "crustaceans" refer to Branchiopoda, Maxillopoda, and Malacostraca (Remipedia and Cephalocarida not analyzed); "Hexapoda" and "hexapods" refer to Entognatha and Ectognatha.

With respect to the parameter sets used, we refer to each analysis by a number based on the parameter set employed. For example, the analysis with an insertion–deletion ratio of 2 and a transversion–transition ratio of 2 will be named 221.

Partitioned analyses published within total evidence analyses are also cited when comparing the information between the different data partitions. Thus, the same paper can be cited for a morphological, a molecular, and a combined analysis as yielding alternative hypotheses. For example, the study of Wheeler (1998a)

suggests paraphyly of myriapods in both morphological and total evidence analyses, while it suggests monophyly of myriapods (Chilopoda and Diplopoda) based on the molecular analysis alone. However, when we cite each of the partitioned analyses, it does not necessarily mean that the authors defended such a hypothesis. We understand that for the authors using a total evidence approach, the combined analysis is the most defensible hypothesis since it is the most corroborated.

RESULTS AND DISCUSSION

Direct Optimization

The direct character optimization method has been chosen because it allows one to analyze large data sets in an objective and repeatable way. Certainly, parsimony-based multiple sequence alignments (such as MALIGN; Wheeler and Gladstein, 1994, 1995) are extremely demanding computationally, since two levels of heuristics are involved to generate the alignments and to generate the guide trees in which the alignments are diagnosed. Other multiple sequence alignment programs such as CLUSTAL (Higgins and Sharp, 1988; Thompson *et al.*, 1994, 1997) use a single guide tree, yielding suboptimal alignments. For these reasons, multiple sequence alignments for large data sets are often impractical with current technology.

Phylogenetic Trees

The results of the analyses for the combined 18S and 28S data sets are shown in Figs. 2 to 7. The first impression is that very few groups are found in common among the different parameters used. The trees were rooted on the branch that separates the nematodes from the remaining taxa.

Outgroup Relationships

Nematodes, tardigrades, and nematomorphs were monophyletic throughout the parameter space. Onychophoran monophyly, however, was not found under set 411. Onychophorans had a tendency to branch within the bulk of arthropods, related to a clade containing the myriapods (but not the symphylans), diplurans, and branchiopods (411); chelicerates (121); and diplurans as a basal pancrustacean clade (141) or again a clade of myriapods (excluding *Polyxenus*), diplurans, and *Daphnia* (221). Parameters 111 and 211 placed onychophorans outside the arthropods. Tardigrades have been placed within arthropods in certain trees based on molecular data (Giribet *et al.*, 1996; Aguinaldo *et al.*, 1997), although here, they appear only within the bulk of arthropods under parameter set 111, within a clade that contains the chelicerates, symphylans, and chilognath millipedes. Nematomorphs appeared as sister group to chelicerates under a single parameter set (141). The placement of any of these outgroups within the arthropods is not supported by morphological evidence and could be interpreted as an artifact due to random convergence of extremely divergent sequences, since the proposed relationships are unstable to parameter choice.

With respect to the relationships among the outgroup phyla, Kynorhyncha and Priapulida appeared as sister taxa under four of the six parameters studied (111, 121, 221, and 411).

Arthropod Relationships

None of the parameter sets used yielded strict monophyly of the Arthropoda. As mentioned above, in several cases some of the outgroups (tardigrades, onychophorans, or nematomorphs) appeared nested within the Arthropoda. In other cases, some arthropod taxa appeared outside of the Arthropoda, intermingled with the outgroup taxa.

Regarding internal arthropod relationships, tree 111 (Fig. 2) shows *Daphnia* as the sister group to the Onychophora, outside the bulk of arthropods. Within the arthropods, a first clade is composed of Pycnogonida, Chelicerata, and a clade that includes the chilognathan millipedes, symphylans, and tardigrades. The next clade, composed of *Polyxenus* and Chilopoda, is sister group of a "pancrustacean" clade that also contains the pauropod (nested within the crustaceans and the entognathous hexapods) and a monophyletic Ectognatha. Tree 211 (Fig. 3) does not place any of the outgroup taxa within the bulk of arthropods, but the pauropod and *Daphnia* clade is sister group to the Onychophora.

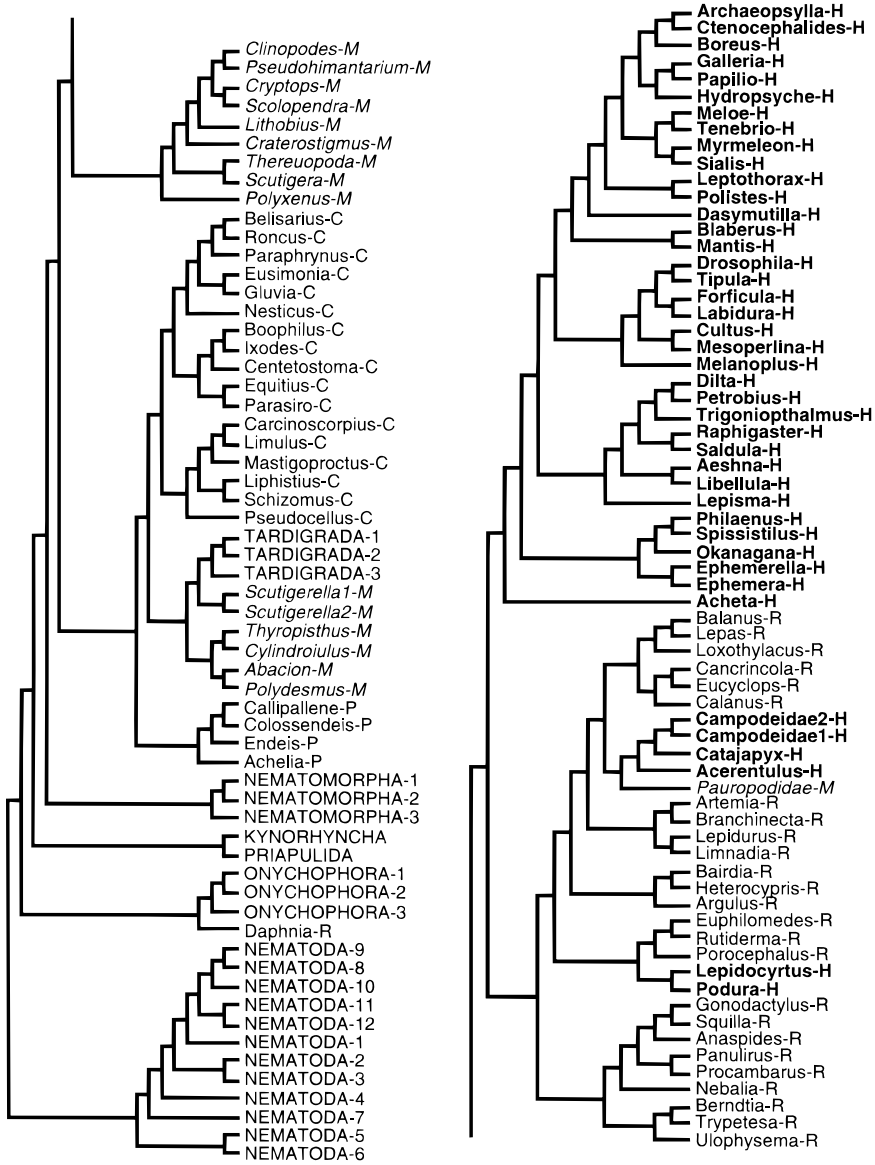

FIG. 2. Phylogenetic tree of the Arthropoda based on a combined analysis of 18S and the D3 region of the 28S rRNA loci for parameter set 111 (gap:transversion:transition ratio). Outgroup taxa are represented in capitals: Kinorhyncha (*Pycnophyes kielensis*), Priapulida (*Priapulus caudatus*), Nematomorpha 1–3 (1, *Gordius aquaticus*; 2, *G. albopunctatus*; 3, *Chordotes morgani*), Nematoda 1–12 (1, *Plectus* sp.; 2, *Desmodora ovigera*; 3, *Metachromadora* sp.; 4, *Enoplus brevis*; 5, *Longidorus elongatus*; 6, *Mermis nigrescens*; 7, *Trichinella spiralis*; 8, *Globodera pallida*; 9, *Bursaphelenchus* sp.; 10, *Haemonchus placei*; 11, *Anisakis* sp.; 12, *Brugia malayi*); Onychophora 1–3 (1, *Peripatopsis capensis*; 2, *Euperipatoides leukartii*; 3, *Epiperipatus biolleyi*), and Tardigrada 1–3 (1, *Hypsibius* sp.; 2, *Macrobiotus hufelandi*; 3, *Milnesium tardigradum*). Ingroup taxa are coded as follows: -P for Pycnogonida, -C for Chelicerata, -M for Myriapoda, -R for Crustacea, and -H for Hexapoda. Furthermore, myriapod taxa are in italics and hexapod taxa in bold.

Arthropods are divided into two main clades, one containing chelicerates and myriapods (Chelicerata (Pycnogonida (*Polyxenus* (Diplopoda + Chilopoda)))) and a "pancrustacean" clade that also includes the symphylans as sister group to Diplura.

These two trees, the ones with the lowest transformation costs, summarize many of the trends shown by the data, including those of the other parameter sets, which are: (a) a basal position of chelicerates and myriapods (with symphylans and pauropods moving

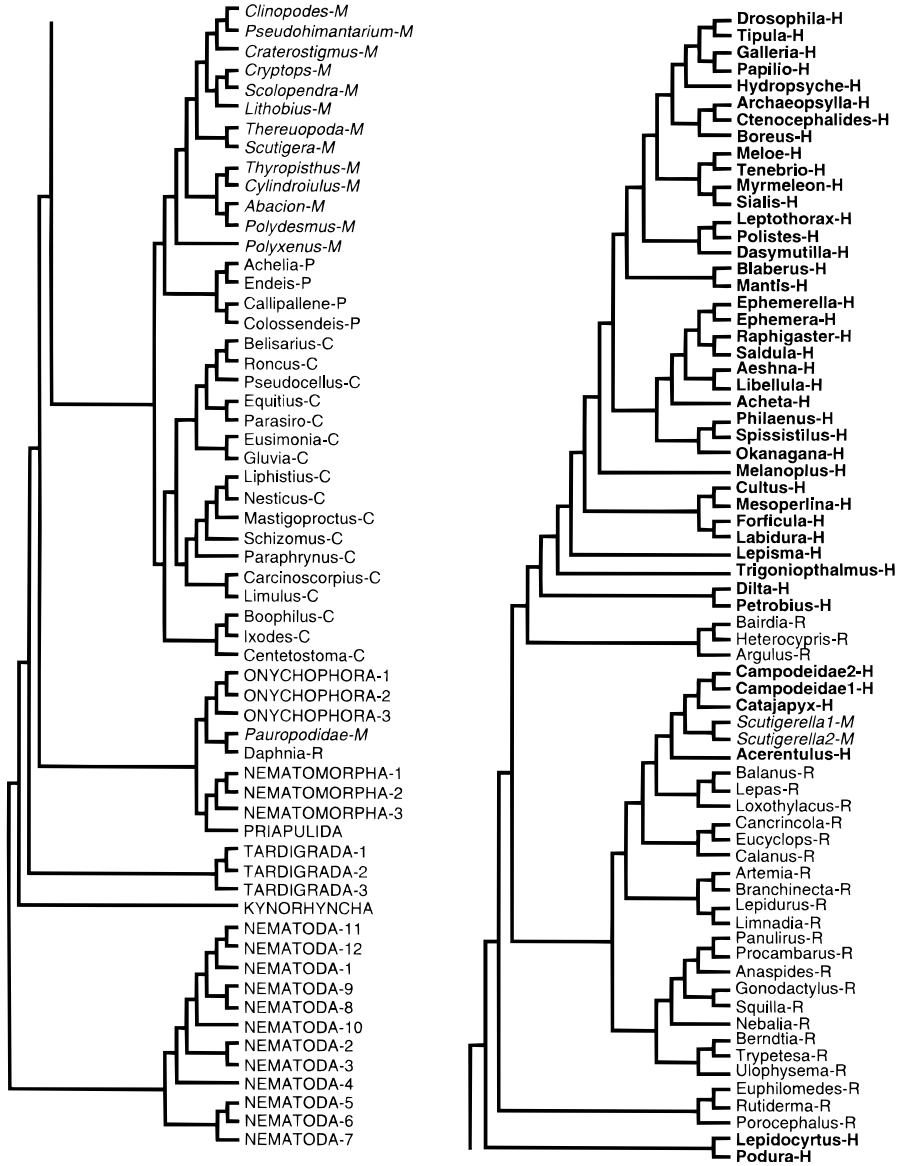

FIG. 3. Phylogenetic tree of the Arthropoda based on a combined analysis of 18S and the D3 region of the 28S rRNA loci for parameter set 211 (gap:transversion:transition ratio). Codes as in Fig. 2.

around), (b) a "pancrustacean," and (c) monophyletic Ectognatha. However, as the transformation costs increase, these general trends become less evident.

The most problematic sequences with this respect are symphylans, pauropods, and particularly the cladoceran *Daphnia*. These three taxa appeared in multiple positions in the trees obtained from our analyses and clearly disrupted certain well-established morphological groups. These sequences present major differences in both primary sequence and length with respect to other arthropod 18S rRNA sequences. This is also found in some of the outgroup taxa, especially the Onychophora. In fact, these are some of the most abnormal sequences in our analyses, which present large and/or numerous insertion–deletion events. The 18S rRNA loci of symphylans are about 1350 bp, while it is ca. 2200 bp long in pauropods, with several small insertions, and it can be even longer in some Onychophora (see further discussion below).

Clades found throughout the parameter space are Pycnogonida, Branchiopoda (excluding *Daphnia*), and Copepoda. Other groups obtained in most of the analy-

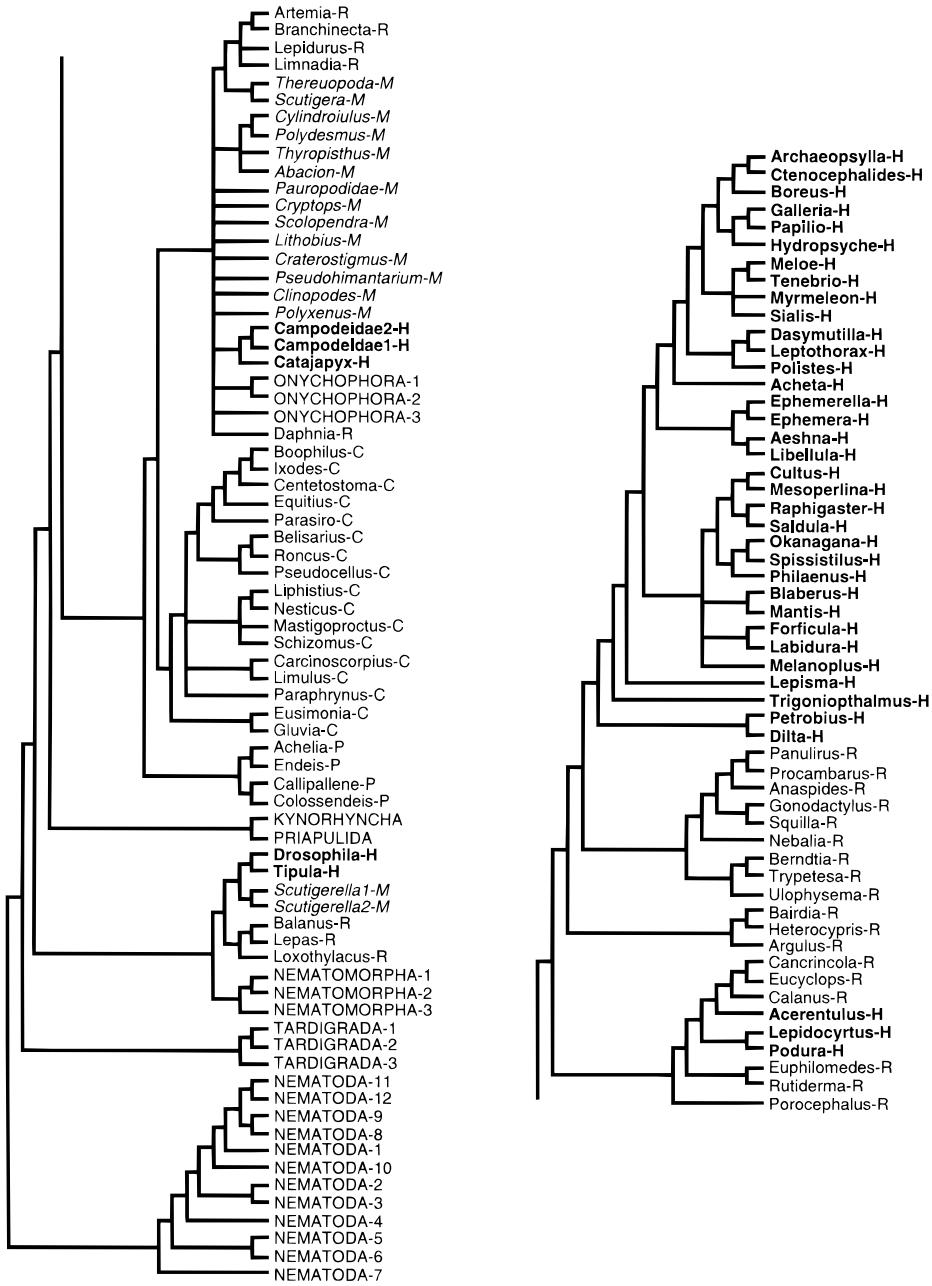

FIG. 4. Phylogenetic tree of the Arthropoda based on a combined analysis of 18S and the D3 region of the 28S rRNA loci for parameter set 411 (gap:transversion:transition ratio). Codes as in Fig. 2.

ses are Ectognatha (although in tree 411 the Diptera are placed out of the arthropods), Eucheliceratа (although in tree 121 they include the Onychophora), Chilognatha (Diplopoda excluding *Polyxenus*; although they are not monophyletic in tree 221), Chilopoda (although in trees 411 and 221 they are not), and Malacostraca.

Despite the observation that the placement of the most divergent taxa might not be possible using the 18S rRNA gene alone, we stress the necessity of data exploration using different parameters, because it is the only way to realize that some of the relationships inferred for these taxa are indeed unstable.

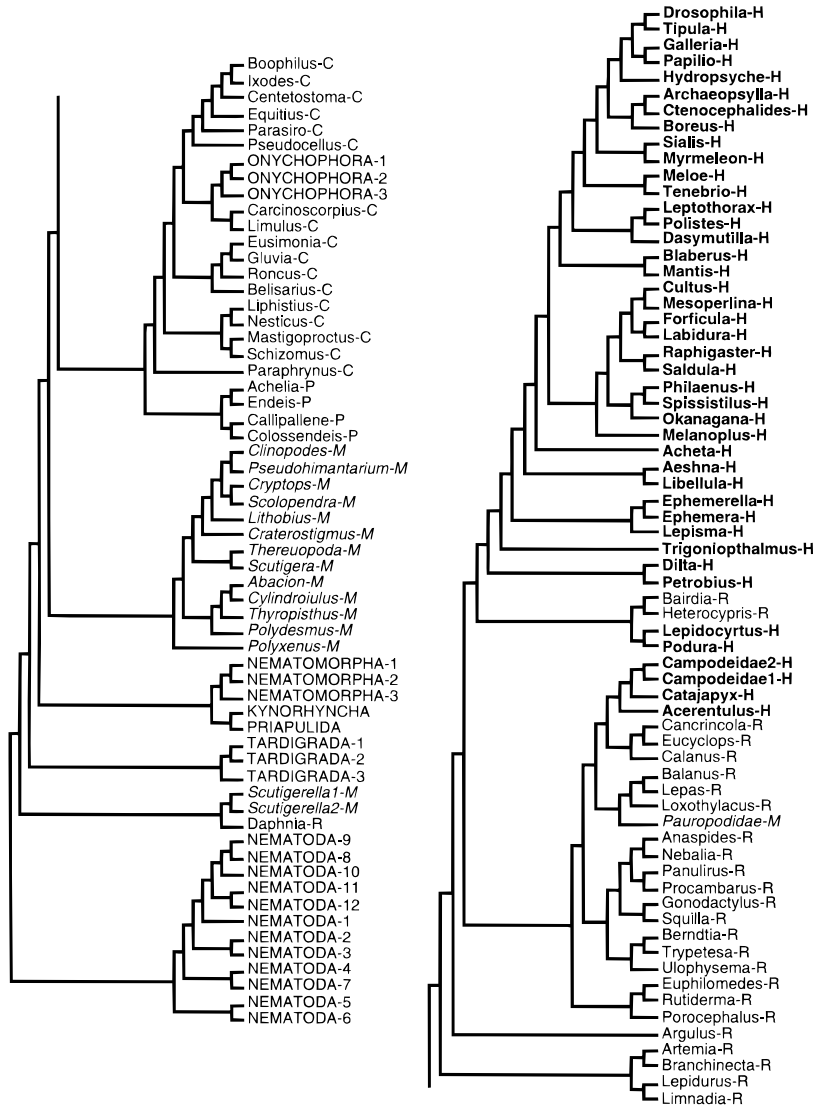

FIG. 5. Phylogenetic tree of the Arthropoda based on a combined analysis of 18S and the D3 region of the 28S rRNA loci for parameter set 121 (gap:transversion:transition ratio). Codes as in Fig. 2.

Chelicerata

The *Euchelicerata* probably constitute the most homogeneous of all the major groups of arthropods. Clearly monophyletic, the internal relationships of the Euchelicerata are challenging, with different hypotheses recently proposed based on cladistic analyses (Weygoldt and Paulus, 1979a,b; Shultz, 1990; Wheeler and Hayashi, 1998). The monophyly of Euchelicerata has been challenged by only few authors based on morphological grounds (e.g., van der Hammen, 1977, 1985, 1986), but molecular data are consistent with the monophyly of the group (Turbeville *et al.*, 1991; Wheeler *et al.*, 1993; Giribet *et al.*, 1996; Regier and Shultz, 1997, 1998; Giribet and Ribera, 1998; Wheeler, 1998a,b; Wheeler and Hayashi, 1998), with the exception of the "experimental" genes of Colgan *et al.* (1998), histone H3 and small nRNA U2.

In the present study the chelicerates were represented by 21 terminal taxa, distributed as Pycnogonida (4), Xiphosura (2), Scorpiones (1), Acari (2), Pseudoscorpiones (1), Opiliones (3), Amblypygi (1), Ricinulei (1), Solifugae (2), Schizomida (1), Uropygi (1), and

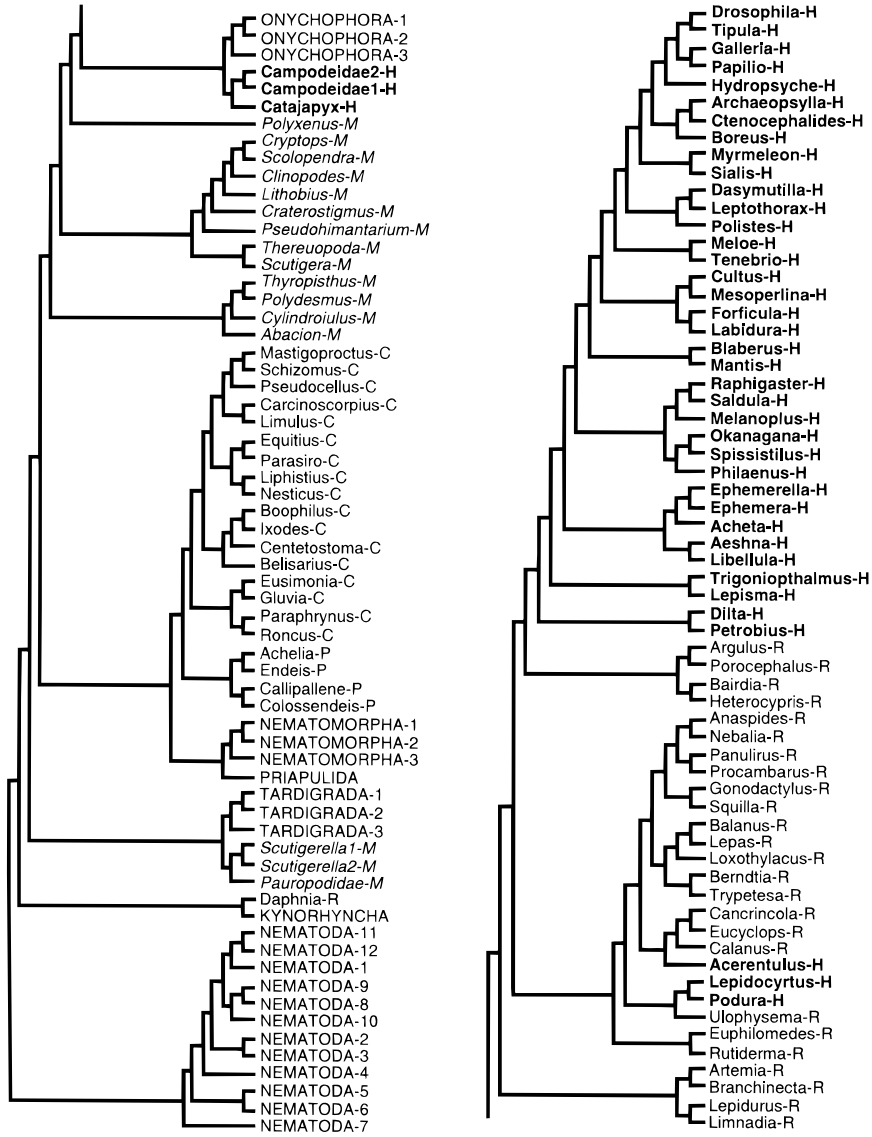

FIG. 6. Phylogenetic tree of the Arthropoda based on a combined analysis of 18S and the D3 region of the 28S rRNA loci for parameter set 141 (gap:transversion:transition ratio). Codes as in Fig. 2.

Araneae (2), constituting all extant orders except Palpigradi. The four pycnogonids formed a monophyletic group throughout the parameters examined, while the euchelicerates were monophyletic for all parameter sets except for 121, because onychophorans appeared nested within the chelicerates. Other relationships found throughout the analyses are the monophyly of the Xiphosura, Acari, and Solifugae.

Euchelicerates present very conserved ribosomal sequences, which caused them to be supported as monophyletic throughout most of the parameter space. However, this sequence conservation may affect the inference of chelicerate internal relationships based solely on molecular ribosomal characters.

The relationship between pycnogonids and the euchelicerates was unstable. Parameter set 141 yielded monophyly of (Pycnogonida + Euchelicerata), parameter set 221 was unresolved, parameter set 121 yielded a sister-group relationship between pycnogonids and euchelicerates, but included onychophorans (!). The rest of parameters did not yield chelicerate monophyly. Regier and Shultz (1998) did not find a

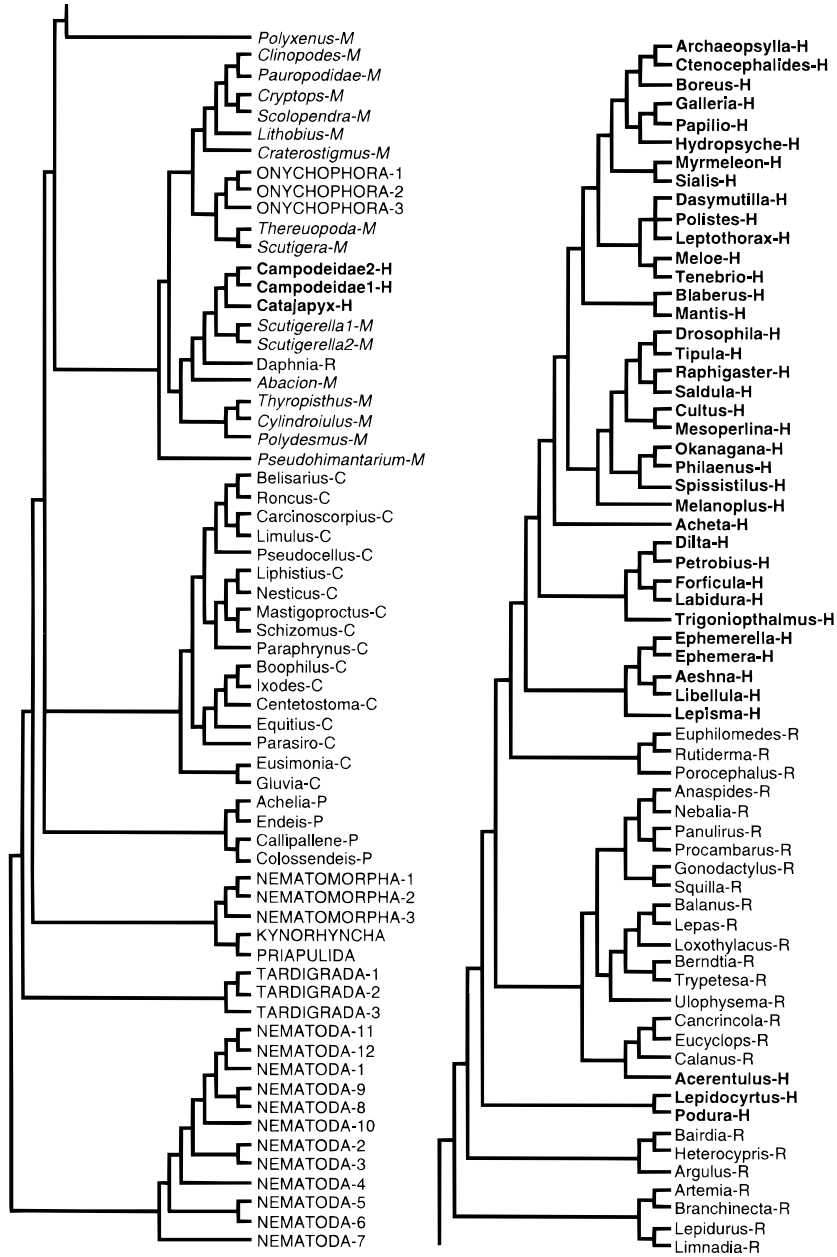

FIG. 7. Phylogenetic tree of the Arthropoda based on a combined analysis of 18S and the D3 region of the 28S rRNA loci for parameter set 221 (gap:transversion:transition ratio). Codes as in Fig. 2.

sister-group relationship for pycnogonids and euchelicerates based on EF1-α data. H3 data did not support a relationship between pycnogonids and euchelicerates either, but the U2 data suggested a relationship of pycnogonids to some other chelicerates (Colgan *et al.*, 1998).

Myriapoda

Myriapods play a pivotal position in the arthropod phylogenetic tree, although they constitute one of the most problematic groups in morphological analyses and the worst represented group in molecular analyses published so far, as mentioned above. Based on mor-

phological data, almost all modern authors agree in the monophyly of the *Atelocerata* (= Hexapoda + Myriapoda), although myriapods have been widely considered a paraphyletic group that includes the Hexapoda.

Pocock (1893) first proposed that myriapods were paraphyletic and presented a classification for the Atelocerata with two main groups, *Progoneata* (= Symphyla, Pauropoda, and Diplopoda) and *Opisthogoneata* (= Chilopoda and Hexapoda). Snodgrass (1938) proposed the name *Labiata* to group the Diplopoda, Pauropoda, Symphyla, and Hexapoda, leaving the Chilopoda as the sister group of Labiata. Kaestner (1963) kept the class Myriapoda divided into three groups: Chilopoda, *Dignatha* (= Diplopoda and Pauropoda), and *Trignatha* (= Symphyla), although he assumed a relationship between Symphyla and Hexapoda, according to the Labiata hypothesis of Snodgrass (1938). Thus, he directly questioned myriapod monophyly. Other authors have considered the paraphyly of myriapods based on different morphological characters: ((Diplopoda + Pauropoda) (Chilopoda (Symphyla + Hexapoda))) (Sharov, 1966) and (Chilopoda ((Symphyla (Diplopoda + Pauropoda)) Hexapoda)) (Dohle, 1980; Kraus and Kraus, 1994, 1996; Borucki, 1996; Kraus, 1998; Wheeler, 1998b).

The monophyly of Myriapoda, with different internal relationships, has been proposed by several authors (Manton, 1972, 1977; Anderson, 1973; Bacceti, 1979; Jamieson, 1987; Wills *et al.*, 1995, 1998; Zrzavý *et al.*, 1998a). Boudreaux (1979b, 1987) also considered myriapods a clade, divided into *Collifera* (= Dignatha) and *Atelopoda* (Symphyla and Chilopoda), proposed a sister-group relationship between myriapods and hexapods, and provided several putative synapomorphies for the Myriapoda. Brusca and Brusca (1990) also provided a number of synapomorphies for the Myriapoda, although several of these characters were criticized by Shear (1998). According to Kristensen (1991), the only clear synapomorphy for myriapods is the architecture of the cephalic endoskeleton. Baccetti (1979) and Jamieson (1987) considered myriapods monophyletic based on the monolayered acrosomic complex, which lacks a perforatorium in the myriapods. Further discussion of these and other characters in favor and against the monophyly of myriapods can be found in Giribet *et al.* (1999b).

In the present study, myriapods are represented by 16 terminal taxa: Chilopoda [Scutigeromorpha (2), Lithobiomorpha (1), Craterostigmomorpha (1), Scolopendromorpha (2), and Geophilomorpha (2)], Diplopoda [Polyxenida (1), Callipodida (1), Julida (1), Spirostreptida (1), and Polydesmida (1)], Symphyla (2), and Pauropoda (1). Myriapod sequences are among the most unusual metazoan sequences. For example, within the Chilopoda, the members of the order Geophilomorpha (except the family Mecistocephalidae) have insertions of about 300 bp in a given loop of the 18S rRNA locus (Giribet *et al.*, 1999a; Edgecombe *et al.*, 1999). Within the Diplopoda, members of the family Polyzonidae have 18S rRNA sequences longer than 2700 bp (Ribera, unpublished data). The only 18S rRNA sequence available for the Pauropoda has ca. 2200 bp, with several insertions (this study). But the most abnormal sequences are found within symphylans. Amplification of symphylan 18S rRNA loci of two species of the genus *Scutigerella* yielded a product band size of about 1350 nt. Sequencing of this PCR fragment demonstrates a deletion of about 500 bp in the central region of the molecule (this study). This result might seem unique; however, the presence of the deletion in a third species of symphylan of the genus *Hanseniella* (Giribet and Wheeler, unpublished results) clearly demonstrates that such event has occurred in a common ancestor of these three symphylan species. Obviously, such abnormalities in primary sequence data on many myriapod species affected their phylogenetic position. Symphylans and pauropods appeared in different unrelated positions in most of the analyses (see Fig. 2–7). In the few cases that they grouped with other myriapods, they always appeared to group with other divergent sequences. The two symphylans grouped with the tardigrades and the chilognathan millipedes (111), with diplurans (211), with the Diptera (411), with *Daphnia* (121), with tardigrades and the pauropod (141), or with *Daphnia* and the diplurans (221). The pauropod grouped with proturans and diplurans (111), with *Daphnia* (211), within an unresolved clade containing the centipedes, millipedes, diplurans, branchiopods, and onychophorans (411), with some cirripedes (121), with symphylans and tardigrades (141), or with centipedes (as sister taxon to the geophilomorphan *Clinopodes*) and onychophorans (221). Obviously, the relationships proposed for symphylans and pauropods are extremely fragile and in general involve taxa that differ considerably from the typical arthropod sequences.

Certainly the position of these enigmatic taxa is far from resolved using ribosomal genes, and other molecular markers are needed. Elongation factor-1α data (Regier and Shultz, 1997, 1998) suggested monophyly of (Diplopoda + Chilopoda + Symphyla). Molecular data for the histone H3 and small nRNA U2 are available for symphylans and pauropods (Colgan *et al.*, 1998), although the results obtained for these genes are not easily interpreted in the context of arthropod evolution.

The Chilopoda appeared as a monophyletic group under most of the parameters explored (111, 211, 121, and 141). In the other two cases, the clade containing the centipedes was unresolved (411) or included the onychophorans as sister group to Scutigeromorpha (221). In the cases that centipedes were monophyletic, the Scutigeromorpha were sister group to the remaining centipedes (= Pleurostigmophora), as found in previous morphological (i.e., Dohle, 1985; Shear and Bonamo, 1988; Borucki, 1996; Prunescu, 1996), molecular (Giribet *et al.*, 1999a), and combined (Edgecombe *et al.*, 1999) analyses. The Diplopoda appeared non-monophyletic in all parameter sets, since *Polyxenus* (Pselaphognatha) never grouped with the Chilognatha (remaining millipedes). Chilognatha were monophyletic in all but one parameter set (221), in which *Abacion* (Callipodida) did not group with the remaining Chilognatha.

The relationship between centipedes and millipedes was parameter-dependent, contrary to the study of Wheeler (1995) in which the only group found throughout all the parameter space there explored was (*Scutigera* + *Spirobolus*). The monophyly of (Chilopoda + Diplopoda) was also obtained in other molecular analyses (e.g., Wheeler *et al.*, 1993; Friedrich and Tautz, 1995; Giribet and Ribera, 1998; Wheeler, 1998a, 1998b; Giribet and Wheeler, 1999b). This result contrasts with the paraphyletic status proposed in many morphological analyses. In our analyses, we found different hypotheses of relationships between Chilopoda and Diplopoda. (Chilopoda + Diplopoda) were monophyletic under two parameter sets (211, 121) but non-monophyletic under four parameter sets (111, 141, 411, and 221).

Considering the instability about the monophyly of Diplopoda, (Chilopoda + Diplopoda), and the uncertain positions of symphylans and pauropods, the status of myriapods appears to be one of the most difficult issues to resolve based solely on ribosomal sequence data. Furthermore, taxonomic sampling within the Diplopoda, Symphyla, and Pauropoda needs to be improved. Consequently, taxon sampling deficiencies and the large degree of divergence in primary sequence allow us to conclude very little about the relationships of this interesting group based on ribosomal DNA data. From our analyses, it cannot be concluded that myriapods are monophyletic or that they are paraphyletic with respect to the Hexapoda, as proposed by several authors (e.g., Dohle, 1980; Kraus and Kraus, 1994, 1996; Borucki, 1996; Kraus, 1998; Wheeler, 1998b).

Crustacea

Crustaceans constitute the extant arthropod group with the highest diversity of body plans. Five classes are widely recognized (Remipedia, Malacostraca, Maxillopoda, Cephalocarida, and Branchiopoda), although certain authors grouped Phyllocarida, Cephalocarida, and Branchiopoda into the class Phyllopoda (Schram, 1986). Relationships among the crustacean classes have remained complicated. Morphological data (e.g., Schram, 1986; Wilson, 1992; Briggs and Fortey, 1989; Briggs *et al.*, 1993; Schram and Hof, 1998; Wills, 1998; Wills *et al.*, 1998) and molecular data (Spears and Abele, 1998) are extremely discordant. Little consensus has been achieved for their internal relationships, as well as for their monophyletic status (see Schram, 1986; Wägele, 1993; Schram and Hof, 1998). A paraphyletic origin of Crustacea with respect to Atelocerata (Lauterbach, 1983), or to Chelicerata + Trilobitomorpha (Briggs and Fortey, 1989), has been previously suggested, although current morphological data seem to agree with crustacean monophyly (Wheeler *et al.*, 1993; Schram and Hof, 1998; Wheeler, 1998a,b).

Non-monophyly of crustaceans has been recently obtained in molecular data analyses (Garey *et al.*, 1996; Giribet *et al.*, 1996; Giribet and Ribera, 1998; Giribet and Wheeler, 1999b; see also the molecular partitions of Wheeler *et al.*, 1993; Wheeler, 1998a,b), although these studies included few crustacean samples. Another study including most higher crustacean lineages using 18S rRNA sequence data also resulted in crustacean polyphyly (Spears and Abele, 1998). EF-1α data (Regier and Shultz, 1997, 1998) seem to indicate a polyphyletic origin of crustaceans, with four groups: (1)

Maxillopoda + Malacostraca, (2) Remipedia, (3) Ostracoda + Branchiopoda, and (4) Cephalocarida. H3 and U2 data (Colgan *et al.*, 1998) did not obtain monophyly of crustaceans, either. However, the combined analyses of morphological and molecular data resolved crustaceans as a clade (Wheeler *et al.*, 1993; Wheeler, 1998a,b; Zrzavý *et al.*, 1998a).

The scarce molecular data available on certain crustacean lineages has made it difficult to draw conclusions on their monophyletic status. Molecular data on Remipedia and Cephalocarida have been published recently. For the Remipedia, there are 18S rRNA (Spears and Abele, 1998) and EF-1α (Regier and Shultz, 1997, 1998) data for the species *Speleonectes tulumensis* and H3 and U2 data for *Lasionectes exleyi* (Colgan *et al.*, 1998). Data for the cephalocarid species *Hutchinsoniella macracantha* are also available for 18S rRNA (Spears and Abele, 1998), EF-1α (Regier and Shultz, 1997, 1998), and H3 and U2 (Colgan *et al.*, 1998). In our analyses, we have included 18S rRNA data for representatives of the Branchiopoda, Maxillopoda, and Malacostraca. We have avoided the use of some particular crustacean sequences available in the literature because they are questionable in origin. The cladoceran branchiopod *Bosmina longirostris* and the cumacean malacostracan *Diastylis* sp. seemed to be contaminations, probably from Plathelminthes. Unfortunately, the published 18S rRNA sequence of the singular *Speleonectes tulumensis* is also extremely divergent from other crustacean or arthropod sequences. We used several fragments of this sequence to do BLAST searches in GenBank (Altschul *et al.*, 1997), and many of the fragments scored with fungi. Since the original authors described some problems sequencing *Speleonectes* due to the existence of multiple gene copies, we avoided the use of this sequence. The sequence of *Hutchinsoniella macracantha* was not included in the present analysis since it was not available in GenBank when the present analyses were completed. Within the three represented crustacean classes, taxonomic sampling is poor for the Malacostraca, but it is broad for the Branchiopoda and Maxillopoda. Furthermore, the 28S rRNA data set for the crustaceans includes only three taxa. Poor taxonomic sampling and missing data in the 28S partition do not constitute the best scenario for addressing questions such as crustacean monophyly, but may still be useful for a broad study on higher arthropod relationships.

The monophyly of the represented malacostracans was supported throughout the parameter space examined, as was also found by Spears and Abele (1998). The Branchiopoda were also monophyletic, to the exclusion of *Daphnia*. The Maxillopoda were not monophyletic under any parameter set, as was again also found by Spears and Abele (1998). Malacostraca and Branchiopoda are thus well-supported groups of crustaceans, while the status of Maxillopoda will require further study.

The phylogenetic position of the parasitic Pentastomida has been claimed to be one of the successes of molecular phylogenetics, positioning them as extremely modified branchiurans (which are also parasitic) (Abele *et al.*, 1989; Spears and Abele, 1998). Data on sperm morphology have also added corroboration to the phylogenetic position of this enigmatic group (Wingstrand, 1972; Riley *et al.*, 1978; Jamieson and Storch, 1992). However, the discovery of fossil pentastomids from the Lower Paleozoic (Walossek and Müller, 1994; Walossek *et al.*, 1994) has been interpreted as a falsification of such a hypothesis, although pentastomids are still considered to be more closely related to arthropods than to other "Pararthropoda" (Walossek *et al.*, 1994). In our analyses, the pentastomid (*Porocephalus*) appeared to be related to other maxillopod crustaceans, generally to the myodocopoid ostracods, although it was related to the branchiuran *Argulus* under parameter set 141. The conclusion that Pentastomids are derived maxillopods, not necessarily derived branchiurans, seems to be more reasonable in accord with our ribosomal data.

Hexapoda

Hexapods constitute the largest animal group in terms of described species. Most modern morphological analyses seem to agree with the monophyly of the Hexapoda (i.e., Hennig, 1969; Boudreaux, 1979b; Kristensen, 1981, 1991, 1998; Kukalová-Peck, 1991; Štys *et al.*, 1993; Wheeler *et al.*, 1993; Kraus and Kraus, 1994, 1996; Bitsch and Bitsch, 1998; Kraus, 1998; Wheeler, 1998a,b; Willmann, 1998). Monophyly of *Ellipura* (or *Parainsecta*) (= Protura and Collembola), as well as of *Ectognatha* (= Archaeognatha, Zygentoma and Pterygota), is also well accepted. However, certain aspects such as the monophyly of the *Entognatha* (= Ellipura and Diplura) and the monophyly of the Diplura have been questioned (see a review of hypotheses in Štys

and Zrzavý, 1994; Bitsch and Bitsch, 1998; Kristensen, 1998).

Molecular analyses by themselves have contributed very little to this issue, because until the publication of Wheeler's (1998a) analysis, no data were available for Protura nor for both groups of Diplura (Campodeidae and Japygidae), and these were partial sequences. Most arthropod molecular analyses concluded that the ectognathous hexapods are monophyletic (Carmean *et al.*, 1992; Chalwatzis *et al.*, 1996; Giribet *et al.*, 1996; Whiting *et al.*, 1997; Giribet and Ribera, 1998). However, the monophyly of Hexapoda ("Entognatha" + Ectognatha) depended on which groups of crustaceans were included in the analyses [see monophyly of Collembola + Entognatha in Friedrich and Tautz, 1995, versus non-monophyly in Giribet *et al.*, 1996; Giribet and Ribera, 1998; Spears and Abele, 1998]). The molecular partition of Wheeler (1998a) was not congruent with the monophyly of Hexapoda or Ectognatha, and the three groups of entognathous hexapods (Collembola, Protura, and Diplura) appeared "related" to certain crustacean groups.

Our data set included 42 hexapod terminal taxa, represented by Protura (1), Collembola (2), Diplura [Campodeidae (2) and Japygidae (1)], Archaeognatha (3), Zygentoma (1), and 32 sequences of Pterygota, representing a total of 23 orders of insects. The Ectognatha (= Archaeognatha + Zygentoma + Pterygota) were monophyletic in all the analyses except for parameter set 411, in which *Drosophila* + *Tipula* grouped with other taxa with divergent sequences. The monophyly of Dicondylia (= Zygentoma + Pterygota) and of Pterygota was parameter-dependent, both groups being monophyletic under parameter sets 211 and 411. In other cases only Pterygota were monophyletic (141).

Monophyly of Hexapoda, Entognatha, or Ellipura was not supported by the data. The entognathous hexapods appeared to be spread among the crustacean taxa or related to some myriapod sequences in different positions in every analysis. The Diplura (Campodeidae + Japygidae) were monophyletic through all parameter sets as proposed by several authors (e.g., Kristensen, 1998), not supporting the hypotheses of dipluran paraphyly (Štys and Bilinski, 1990; Štys *et al.*, 1993; Štys and Zrzavý, 1994; Bitsch and Bitsch, 1998). However, the sister-group relationships of the Diplura are ambiguous. Under certain parameters, Diplura were sister group to Protura (111, 121) or to Symphyla (211, 221). But the positions of both Protura and Collembola were equally unstable. Protura and Collembola did not constitute a clade under any of the parameter sets here explored, which contrasts with the traditional hypothesis of ellipuran monophyly (e.g., Hennig, 1969; Kukalová-Peck, 1987; Štys and Bilinski, 1990; Štys *et al.*, 1993; Kraus and Kraus, 1994; Štys and Zrzavý, 1994; Bitsch and Bitsch, 1998; Kraus, 1998; Kristensen, 1998; Wheeler, 1998a). Certainly, the ectognathous hexapods have unusual sequences that make their positioning by means of ribosomal data a hard task.

The polyphyletic status of hexapods is certainly unacceptable from a morphological point of view, although it has been repeatedly obtained in molecular analyses of ribosomal sequence data (Giribet *et al.* 1996; Giribet and Ribera, 1998; Spears and Abele, 1998; Wheeler, 1998a,b), while they are always monophyletic in morphological (e.g., Hennig, 1969; Bitsch and Bitsch, 1998; Kristensen, 1998; Wheeler, 1998a,b; Zrzavý *et al.*, 1998) and combined analyses (Wheeler, 1998a,b; Zrzavý *et al.*, 1998). Hexapods were also monophyletic in the molecular analyses of EF1-α and RNA polymerase II sequence data (Regier and Shultz, 1997, 1998), although only four hexapod taxa were used in the most inclusive analysis: one Collembola, one Archaeognatha, one Zygentoma, and one Blattodea. Hexapods were non-monophyletic in the analyses of Colgan *et al.* (1998).

Atelocerata or Pancrustacea?

Two alternative hypotheses concerning hexapod sister-group relationships are being debated in the most modern literature of arthropod phylogeny: the classical hypothesis of *Atelocerata* being a natural group (hexapods and myriapods constituting a clade), and the most modern, and molecular-based hypothesis of *Pancrustacea* (hexapods and crustaceans forming a clade). The term Pancrustacea was introduced by Zrzavý and Štys (1997) indicating a putative crustacean origin of hexapods (crustaceans paraphyletic with respect to hexapods) or a sister-group relationship between both groups (if crustaceans and hexapods were each monophyletic). This term was coined in the molecular literature, in which crustaceans and hexapods grouped together to the exclusion of myriapods in many analyses of ribosomal sequence data (Field *et al.*, 1988; Turbeville *et al.*, 1991; Ballard *et al.*, 1992; Friedrich and Tautz,

1995; Giribet *et al.*, 1996; Giribet and Ribera, 1998). The clade Pancrustacea is also supported by a combined analysis of molecular and morphological data (Zrzavý *et al.*, 1998a) and data derived from mitochondrial gene order (Boore *et al.*, 1995, 1998). Ribosomal and mitochondrial gene order data seemed thus to support the Pancrustacea hypothesis, but other molecular studies based on EF-1α sequence data seem to propose that crustaceans are polyphyletic (Regier and Shultz, 1998), with only the cephalocarid *Hutchinsoniella* branching between Myriapoda and Hexapoda, a result completely incongruent with the ribosomal sequence data.

The alternative hypothesis, monophyly of Atelocerata, is supported by most morphological analyses (Snodgrass, 1938, 1950, 1951; Briggs and Fortey, 1989; Schram and Emerson, 1991; Bergström, 1992; Briggs *et al.*, 1992; Wägele, 1993; Wheeler *et al.*, 1993; Kraus and Kraus, 1994, 1996; Wills *et al.*, 1994, 1995, 1998; Emerson and Schram, 1998; Kraus, 1998; Wheeler, 1998a,b). Some of the putative synapomorphies for Atelocerata are the absence of second antennae, presence of Malpighian tubules, presence of postantennal organs, and presence of tracheae (summarized in Dohle, 1998).

In addition to the molecular evidence, the arguments used in favor of Pancrustacea are mainly based on the idea that the characters supporting the group Atelocerata are prone to convergence due to a terrestrial mode of life (see Averof and Akam, 1995; Friedrich and Tautz, 1995; Dohle, 1998). We could agree *a priori* that both Malpighian tubules and the presence of tracheal respiration could be convergences due to the terrestrial habitat of myriapods and insects [also supported by the presence of both non-homologous (?) structures in terrestrial chelicerates]. The absence of the second antenna in myriapods and hexapods is more difficult to explain due to convergence, although Dohle (1998) considered it to be a weak argument due to the differences between the intercalary segments in insects and in myriapods. Furthermore, terrestrial isopods have lost the first antenna (or antennula), which may indicate that two antennae are unnecessary in terrestrial habitats. Postantennal organs (Tömosvary organs) are present in anamorphic centipedes, in symphylans, in several millipede orders, and in collembolans (Haupt, 1979; Dohle, 1998), but their differences in fine structure made Altner *et al.* (1971) conclude that they might have had an independent origin in collembolans.

Recently, certain synapomorphies for Pancrustacea have been proposed, although they all seem to be shared only by insects and the malacostracan crustaceans. These putative synapomorphies (according to Dohle, 1998) are brain structure, axonogenesis in early differentiating neurons, presence of neuroblasts, structure of the ommatidia, and expression of certain segmentation genes. Studies on the genetic control of segmentation and nervous system formation in different groups of arthropods seem to suggest a relationship between insects and malacostracan crustaceans (Dohle and Scholtz, 1988; Whitington *et al.*, 1991, 1993; Patel, 1994; Whitington, 1996; Dohle, 1998). The processes of segmentation, neurogenesis, and axon formation in the centipede *Ethmostigmus rubripes* differ from the pattern shared by insects and malacostracan crustaceans (Whitington *et al.*, 1993, 1996). The similarities observed in the cellular pattern of the eyes and the nervous system of insects and crustaceans have not been observed in chelicerates or myriapods (Paulus, 1979; Osorio and Bacon, 1994; Averof and Akam, 1995; Osorio *et al.*, 1995; Nilsson and Osorio, 1998). Malacostracan crustaceans and insects present also some similarities in the brain structure (Osorio *et al.*, 1995; Nilsson and Osorio, 1998). Studies on developmental genetics also suggested the existence of a common ancestor for insects and crustaceans with a primitive tagmosis in three body regions with head, trunk, and a caudal region (Osorio *et al.*, 1995).

The hypothesis about the homology of the mandibles of insects and myriapods, and that they are not homologous to the mandibles of crustaceans (e.g., Manton, 1973), has been recently refuted by studies of the expression pattern of the homeotic gene *Distal-less* (*Dll*) in the mandibles of insects and crustaceans (Panganiban *et al.*, 1995; Popadic *et al.*, 1996, 1998; Scholtz *et al.*, 1998). The expression pattern of *Dll* in the mandibles of different arthropod groups has been also used as an argument favoring the Pancrustacea hypothesis (Panganiban *et al.*, 1995; Popadic *et al.*, 1996), although these conclusions were subsequently refuted after adding data for more taxa (Popadic *et al.*, 1998; Scholtz *et al.*, 1998).

Which hypotheses, Atelocerata or Pancrustacea, is favored by our data? In general, our trees agree with the Pancrustacea theory, despite the unstable position of the myriapods Symphyla and Pauropoda (which under certain parameters branch within the "Pancrustacea") and the hexapods Diplura and Diptera (which

branch outside the "Pancrustacea" under certain parameters). This result is not surprising, since the Pancrustacea hypothesis was primarily based on ribosomal data analyses. However, this hypothesis had not been previously tested using a large taxon sampling of crustaceans and hexapods (with the exception of Wheeler, 1998a). The present more rigorous test shows instability of the data and especially of certain taxa. Major incongruence with morphological and EF-1α data does not allow us to conclude that Pancrustacea is a robust group. On the contrary, the Atelocerata hypothesis is stable to analysis of neontological morphological data (Wheeler *et al.*, 1993; Wheeler, 1998a,b; Zrzavý *et al.*, 1998) and fossil data (Briggs and Fortey, 1989; Schram and Emerson, 1991; Briggs *et al.*, 1992; Wills *et al.*, 1994, 1995, 1998; Emerson and Schram, 1998), as well as to the combined analyses of morphological and ribosomal data (Wheeler *et al.*, 1993; Wheeler, 1998a,b), but not to the combined morphological and ribosomal data analysis of Zrzavý *et al.* (1998).

One of the strongest arguments that has been used for defending the ancestral status of the Crustacea with respect to Myriapoda and Hexapoda, or what is tantamount to the Atelocerata hypothesis, is the fossil record. First, unequivocal myriapod fossils (Chilopoda and Diplopoda) come from the Late Silurian and Early Devonian (Almond, 1985; Jeram *et al.*, 1990; Shear *et al.*, 1996, 1998; Shear, 1998) or from the Early Silurian (Mikulic *et al.*, 1985a,b). In contrast, crustaceans are known from the Cambrian (Müller and Walossek, 1988; Walossek, 1995; Walossek and Müller, 1998). Other groups related to Atelocerata have been described from Late Silurian, Arthropleurida (Shear and Selden, 1995) and Kampecarida (Almond, unpublished, in Shear, 1998), and other possible myriapod taxa have been described for the Early Devonian (Tesakov and Alekseev, 1992, 1998; Edgecombe, 1998b,c). Euthycarcinoidea dating from the Ordovician or Early Silurian are problematic, but they could be a basal lineage of Atelocerata (Edgecombe and Morgan, 1999). No fossils of possible myriapods are known from the Ordovician, but Johnson *et al.* (1993) reported trace fossils of locomotion, attributed to a myriapod, although it seems very speculative. Finally, the earliest fossil attributed to a myriapod-like animal from marine deposits in the Middle Cambrian (Robison, 1990) may represent evidence for the early origin of the myriapods, although it has been said that the interpretation of such fossil is highly speculative (Wägele, 1993; Shear, 1998).

One hypothetical scenario for Pancrustaceas is that the Cambrian ancestors of the actual mandibulates were a poorly diversified group, which remained like that until one of its descent lineages diversified in the sea, originating the crustaceans. But the stem group would have been too rare to be preserved in the fossil record until it conquered land, later in the Silurian. In a recent paper, Fortey *et al.* (1997) have pointed out that "it may not be generally appreciated by biologists that first occurrence in the fossil record is not necessarily the same as time of origination" and concluded that "the fossil record cannot be taken literally as a chronology of phylogenesis." They also provided an example of copepod crustaceans, an extremely important component in oceanic biomass, yet they are very rarely preserved in marine sediments. Their entire pre-Tertiary fossil record is confined to a parasitic form preserved inside a fossilized Cretaceous fish from Brazil (Cressey and Boxshall, 1989). Obviously, this example could be applicable to the "marine myriapods," if they ever existed.

Mandibulata or Schizoramia?

An older, but still active debate, is whether the arthropods with mandibles (Mandibulata) constitute a monophyletic clade or if, in contrast, arthropods with primitively polyrameous appendages (Schizoramia) are monophyletic and sister group to Atelocerata (with uniramous appendages). The concept of Schizoramia assumes the monophyly of Pycnogonida, Euchelicerata, Crustacea, and several groups called collectively "Trilobitomorpha." Basically, paleontologists and carcinologists support the monophyly of Schizoramia (i.e., Cisne, 1974; Hessler and Newman, 1975; Briggs and Fortey, 1989; Schram and Emerson, 1991; Bergström, 1992; Briggs *et al.*, 1992; Budd, 1993; Wills *et al.*, 1994, 1995, 1998; Fortey *et al.*, 1996, 1997; Emerson and Schram, 1998; Walossek and Müller, 1998; Zrzavý *et al.*, 1998). Neontologists, either morphologists (i.e., Snodgrass, 1938, 1950, 1951; Boudreaux, 1987; Kukalová-Peck, 1992, 1998; Wägele, 1993; Wheeler *et al.*, 1993; Wheeler, 1998a,b; Zrzavý *et al.*, 1998) or molecular biologists (i.e., Wheeler *et al.*, 1993; Giribet and Ribera,

1998; Wheeler 1998a,b), suggest monophyly of Mandibulata. The morphological partition of Zrzavý *et al.* (1998a) is the only neontological data matrix suggesting Schizoramia, although this hypothesis was not obtained when the morphological data were analyzed in combination with the ribosomal sequence data.

According to the most recent morphological and total evidence analyses the concept of Mandibulata (in the sense of Snodgrass) is the best corroborated hypothesis, although the inclusion of extinct arthropod taxa could be crucial in understanding arthropod evolution. Zrzavý *et al.* (1998), however, obtained a total evidence tree with Mandibulata being monophyletic, but with the myriapods as sister group to Pancrustacea. Some molecular analyses including a broad taxonomic sampling of arthropod lineages agreed with this result (Giribet and Ribera, 1998; Giribet and Wheeler, 1999b), although some kind of sensitivity analysis should be done to scrutinize such a result. Previous molecular analyses based on ribosomal sequence data were not able to discern between the monophyly of the Mandibulata versus the monophyly of (Chelicerata + Myriapoda) (Turbeville *et al.*, 1991; Wheeler *et al.*, 1993; Giribet *et al.*, 1996) or concluded that chelicerates and myriapods are sister taxa (Friedrich and Tautz, 1995). This is not the case, however, for EF1-α sequence data, which did not support either the Mandibulata or the Schizoramia hypothesis (Regier and Shultz, 1997, 1998). A very restricted data set (RNA polymerase II) supported Mandibulata (and Atelocerata) (Regier and Shultz, 1997), but again neither of the two hypotheses was supported by the histone H3 and U2 snRNA (Colgan *et al.*, 1998).

Our data exploration suggests that the lack of consensus in previous ribosomal sequence data analyses is not an artifact and that neither the monophyly of mandibulate arthropods nor the monophyly of chelicerates + myriapods is an easily discernible issue based solely on ribosomal sequence data.

CONCLUSIONS

From the results obtained here, we realize that ribosomal DNA sequence data by itself may not contain enough information to give a satisfactory explanation for the large and complicated evolutionary history of arthropods. This might be based on three main reasons: (1) for the same reason that explanatory power favors combined analyses of several partitions over the analysis of the individual partitions (see Remsen and DeSalle, 1998); (2) for the large rate of extinction accounted in arthropods and arthropod-like creatures (see Gould, 1989), which cannot be explored using molecular data; and (3) for the large rates of ribosomal sequence divergence that occur within arthropods. Clearly, combination of our data with information from other genes as well as the morphology of extant and extinct arthropod groups in the near future should contribute to drawing a firmer picture of arthropod relationships.

Combined analyses of different gene regions and morphology of several arthropod taxa have already been published (Wheeler *et al.*, 1993; Wheeler, 1998a,b; Zrzavý *et al.*, 1998a), although in these studies the molecular data had some important gaps, particularly within the myriapods, basal hexapods, and crustaceans. These groups have been extensively represented in the present study, and many of the results here obtained could not be obtained without examining such a large taxon sampling as the one included in the present study. Also taxon stability could not be tested without performing a sensitivity analysis. Thus many of the results of former strictly molecular analyses are here refuted by the inclusion of new data on many important arthropod groups. Others are refuted by showing their instability to parameter variation in data analysis. This instability indeed stresses the necessity of such explorations.

Despite the molecular analysis neatness, and the methodology used to analyze large data sets, especially of nonconserved molecular data, the phylogenetic conclusions of our study are not very encouraging per se. Those taxa that are difficult to position based on their morphology, for their unusual body plans, present the most unusual sequences as well. Onychophorans, pycnogonids, symphylans, pauropods, proturans, collembolans, diplurans, and many crustaceans are clear examples. However, we cannot exclude these taxa from our analyses in order to gain "resolution" or "reliability" or simply to "present a nice tree," because these will probably be the key taxa to explain arthropod relationships. Perhaps newer methods of sequence data analyses able to accommodate a large sequence length variation as in that presented by arthropods will be the way to proceed in the future.

APPENDIX 1

Complete command line used for one stepmatrix (111). The files "345" to "c" represent the input files for the 18S rRNA gene, and "s1", "s2", and "s7" represent the input files for the D3 fragment of the 28S rRNA gene.

```
poy -parallel -norandomizeoutgroup -noleading -molecularmatrix 111 -maxtrees
2 -jackboot -random 20 -seed −1 -nospr -notbr 345 6 7 8 9 11 12 13 14 15 16
17 18 19 20-21 E21-4 E21-5 E21-6 22 23 24 25 27 28 29 31 33 35 36 38 40 42
36b 34-32 45 46 a c s1 s2 s7 > art111.out

jack2hen 50 < art111.out > art111.con

poy -parallel -noleading -molecularmatrix 111 -maxtrees 20 -multibuild 10
-seed −1 -slop 1 345 6 7 8 9 11 12 13 14 15 16 17 18 19 20-21 E21-4 E21-5
E21-6 22 23 24 25 27 28 29 31 33 35 36 38 40 42 36b 34-32 45 46 a c s1 s2 s7
-constrain art111.con > art111.tre
```

ACKNOWLEDGMENTS

Greg Edgecombe, Jyrki Muona, and Ward Wheeler read early versions of the manuscript and helped to improve it considerably. This work could not have been possible without the help and resources provided by Ward Wheeler. The invaluable comments of Greg Edgecombe are also acknowledged. The sequencing for this project was undertaken at the laboratories of Jaume Baguñá (Universitat de Barcelona), Ward Wheeler (American Museum of Natural History, New York), and Richard Thomas (The Natural History Museum, London). M. A. Arnedo, H. Enghoff, G. S. Hunt, E. Mateos, A. Muñoz-Cuevas, P. Oromí, C. Palacín, L. Prendini, J. Pujade, M. Rambla, P. J. Schwendinger, A. Serra, W. A. Shear, J. W. Shultz, N. Tsurusaki, W. Wheeler, and J. Zrzavý provided specimens. This research was supported by a DGICYT grant and a CIRIT grant. G. Giribet was supported by a fellowship from the "Direcció General de Recerca" (Generalitat de Catalunya), by a FPI fellowship from the MEC (Spain), and by a Lerner-Gray Research fellowship from the AMNH (U.S.A.).

REFERENCES

Abele, L. G., Kim, W., and Felgenhauer, B. E. (1989). Molecular evidence for inclusion of the phylum Pentastomida in the Crustacea. *Mol. Biol. Evol.* **6,** 685–691.

Aguinaldo, A. M. A., Turbeville, J. M., Lindford, L. S., Rivera, M. C., Garey, J. R., Raff, R. A., and Lake, J. A. (1997). Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* **387,** 489–493.

Almond, J. E. (1985). The Silurian–Devonian fossil record of the Myriapoda. *Philos. Trans. R. Soc. London B Biol. Sci.* **309,** 227–238.

Altner, H., Karuhize, G., and Ernst, K.-D. (1971). Untersuchungen am Postantennalorgan der Collembolen (Apterygota). II. Cuticulärer Apparat und Dendritenendigung bei *Onychiurus* spec. *Rev. Écol. Biol. Sol* **8,** 31–35.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25,** 3389–3402.

Anderson, D. T. (1973). "Embryology and Phylogeny in Annelids and Arthropods." Pergamon, Oxford.

Averof, M., and Akam, M. (1995). Insect–crustacean relationships: Insights from comparative developmental and molecular studies. *Philos. Trans. R. Soc. London B Biol. Sci.* **347,** 293–303.

Baccetti, B. (1979). Ultrastructure of sperm and its bearing on arthropod phylogeny. *In* "Arthropod Phylogeny" (A. P. Gupta, Ed.), pp. 609–644. Van Nostrand–Reinhold, New York.

Ballard, J. W. O., Ballard, O., Olsen, G. J., Faith, D. P., Odgers, W. A., Rowell, D. M., and Atkinson, P. (1992). Evidence from 12S ribosomal RNA sequences that onychophorans are modified arthropods. *Science* **258,** 1345–1348.

Bergström, J. (1992). The oldest Arthropoda and the origin of the Crustacea. *Acta Zool.* **73,** 287–291.

Bitsch, C., and Bitsch, J. (1998). Internal anatomy and phylogenetic relationships among apterygote insect clades (Hexapoda).*Ann. Soc. Entomol. Fr. (N. S.)* **34,** 339–363.

Boore, J. L., Collins, T. M., Stanton, D., Daehler, L. L., and Brown, W. M. (1995). Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. *Nature* **376,** 163–165.

Boore, J. L., Lavrow, D. V., and Brown, W. M. (1998). Gene translocation links insects and crustaceans.*Nature* **392,** 667–668.

Borucki, H. (1996). Evolution und Phylogenetisches System der Chilopoda (Mandibulata, Tracheata). *Verh. Naturwiss. Ver. Hamburg* **35,** 95–226.

Boudreaux, H. B. (1979a). Significance of intersegmental tendon system in arthropod phylogeny and a monophyletic classification of Arthropoda.*In* "Arthropod Phylogeny" (A. P. Gupta, Ed.), pp. 551–586. Van Nostrand–Reinhold, New York.

Boudreaux, H. B. (1979b). "Arthropod Phylogeny with Special Reference to Insects." Wiley, New York.

Boudreaux, H. B. (1987). Phylogeny of the arthropod classes.*In* "Arthropod Phylogeny, with Special Reference to Insects," pp. 82–122. Krieger, Malabar, FL.

Briggs, D. E. G., and Fortey, R. A. (1989). The early radiation and relationships of the major Arthropod groups. *Science* **246,** 241–243.

Briggs, D. E. G., Fortey, R. A., and Wills, M. A. (1992). Morphological disparity in the Cambrian. *Science* **256,** 1670–1673.

Brusca, R. C., and Brusca, G. J. (1990). "Invertebrates." Sinauer, Sunderland, MA.

Budd, G. E. (1993). A Cambrian gilled lobopod from Greenland. *Nature* **364,** 709–711.

Budd, G. E. (1996). The morphology of *Opabinia regalis* and the reconstruction of the arthropod stem-group. *Lethaia* **29,** 1–14.

Carmean, D., Kimsey, L. S., and Berbee, M. L. (1992). 18S rDNA sequences and the holometabolous insects. *Mol. Phylogenet. Evol.* **1,** 270–278.

Chalwatzis, N., Hauf, J., van de Peer, Y., Kinzelbach, R., and Zimmermann, F. K. (1996). 18S ribosomal RNA genes of insects: Primary structure of the genes and molecular phylogeny of the Holometabola. *Ann. Entomol. Soc. Am.* **89,** 788–803.

Cisne, J. L. (1974). Trilobites and the origin of arthropods. *Science* **186,** 13–18.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18,** 5294–5299.

Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., Cassis, G., and Gray, M. R. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution.*Aust. J. Zool.* **46,** 419–437.

Cressey, R., and Boxshall, G. (1989). *Kabatarina pattersoni*, a fossil parasitic copepod (Dichelesthiidae) from a Lower Cretaceous fish. *Micropalaeontology* **35,** 150–167.

Dohle, W. (1980). Sind die Myriapoden eine monophyletische Gruppe? *Abh. Naturwiss. Ver. Hamburg* **23,** 45–104.

Dohle, W. (1985). Phylogenetic pathways in the Chilopoda. *Bijdr. Dierkd.* **55,** 55–66.

Dohle, W. (1998). Myriapod–insect relationships as opposed to an insect–crustacean sister group relationship. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 305–315. Chapman & Hall, London.

Dohle, W., and Scholtz, G. (1988). Clonal analysis of the crustacean segment: The discordance between genealogical and segmental borders.*Development* **104,** 147–160.

Edgecombe, G. D. (1998a). "Arthropod Fossils and Phylogeny." Columbia Univ. Press, New York.

Edgecombe, G. D. (1998b). Devonian terrestrial arthropods from Gondwana. *Nature* **394,** 172–175.

Edgecombe, G. D. (1998c). Early myriapodous arthropods from Australia: *Maldybulakia* from the Devonian of New South Wales.*Rec. Aust. Mus.* **50,** 293–313.

Edgecombe, G. D., Giribet, G., and Wheeler, W. C. (1999). Filogenia de Chilopoda: Combinando secuencias de los genes ribosómicos 18S y 28S, y morfología [Phylogeny of Chilopoda: Combining 18S and 28S rDNA sequences and morphology]. *In* "Evolución y Filogenia de Arthropoda" (A. Melic, J. J. de Haro, M. Méndez, and I. Ribera), pp. 293–331. Bol. S.E.A. Monogr. 26. Soc. Entomol. Aragonesa, Zaragoza, Spain.

Edgecombe, G. D., and Morgan, H. (1999). *Synaustrus* and the euthycarcinoid puzzle. *Alcheringa* **23,** 193–213.

Emerson, M. J., and Schram, F. R. (1998). Theories, patterns, and reality: Game plan for arthropod phylogeny. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 67–86. Chapman & Hall, London.

Farris, J. S. (1995) The Parsimony Jackknifer, version 4.22. Naturhistoriska riksmuseet, Stockholm.

Farris, J. S., Albert, V. A., Kallersjo, M., Lipscomb, D., and Kluge, A. G. (1996). Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12,** 99–124.

Field, K. G., Olsen, G. J., Lane, D. J., Giovannoni, S. J., Ghiselin, M. T., Raff, E. C., Pace, N. R., and Raff, R. A. (1988). Molecular phylogeny of the animal kingdom. *Science* **239,** 748–753.

Fortey, R. A., Briggs, D. E. G., and Wills, M. A. (1996). The Cambrian evolutionary 'explosion': Decoupling cladogenesis from morphological disparity. *Biol. J. Linn. Soc.* **57,** 13–33.

Fortey, R. A., Briggs, D. E. G., and Wills, M. A. (1997). The Cambrian evolutionary 'explosion' recalibrated. *BioEssays* **19,** 429–434.

Fortey, R. A., and Thomas, R. H. (1998). "Arthropod Relationships." Chapman & Hall, London.

Friedrich, M., and Tautz, D. (1995). Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods. *Nature* **376,** 165–167.

Fryer, G. (1996). Reflections on arthropod evolution. *Biol. J. Linn. Soc.* **58,** 1–55.

Fryer, G. (1998). A defence of arthropod polyphyly. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 23–33. Chapman & Hall, London.

Giribet, G. (1997). Filogenia molecular de Artrópodos basada en la secuencia de genes ribosomales. Univ. de Barcelona. [Ph.D. thesis]

Giribet, G. (1999). Ecdysozoa *versus* Articulata, dos hipótesis alternativas sobre la posición de los Artrópodos en el reino Animal. *In* "Evolución y Filogenia de Arthropoda" (A. Melic, J. J. de Haro, M. Méndez, and I. Ribera), pp. 197–212. Bol. S.E.A. 26. Soc. Entomol. Aragonesa, Zaragoza, Spain.

Giribet, G., Carranza, S., Baguñà, J., Riutort, M., and Ribera, C. (1996). First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Mol. Biol. Evol.* **13,** 76–84.

Giribet, G., Carranza, S., Riutort, M., Baguñà, J., and Ribera, C.

(1999a). Internal phylogeny of the Chilopoda (Arthropoda, 'Myriapoda'): A combined approach using complete 18S rDNA and partial 28S rDNA sequences. *Philos. Trans. R. Soc. London B* **354,** 215–222.

Giribet, G., Distel, D. L., Polz, M., Sterrer, W., and Wheeler, W. C. (2000). Triploblastic relationships with emphasis on the acoelomates, and the position of Gnathostomulida, Cycliophora, Plathelminthes and Chaetognatha: A combined approach of 18S rDNA sequences and morphology. *Syst. Biol.* **49.**

Giribet, G., Edgecombe, G. D., and Wheeler, W. C. (1999b). Sistemática y filogenia de artrópodos; estado de la cuestión con énfasis en análisis de datos moleculares. *In* "Evolución y Filogenia de Arthropoda" (A. Melic, J. J. de Haro, M. Méndez, and I. Ribera), pp. 197–212. Bol. S.E. A. 26. Soc. Entomol. Aragonesa, Zaragoza, Spain.

Giribet, G., Rambla, M., Carranza, S., Baguñà, J., Riutort, M., and Ribera, C. (1999c). Internal phylogeny of the order Opiliones (Arthropoda, Arachnida) based in a combined approach of complete 18S rDNA, partial 28S rDNA sequences and morphology. *Mol. Phylogenet. Evol.* **11,** 296–307.

Giribet, G., and Ribera, C. (1998). The position of arthropods in the animal kingdom: A search of a reliable outgroup for internal arthropod phylogeny. *Mol. Phylogenet. Evol.* **9,** 481–488.

Giribet, G., and Wheeler, W. C. (1999a). On gaps.*Mol. Phylogenet. Evol.* **13,** 132–143.

Giribet, G., and Wheeler, W. C. (1999b). The position of arthropods in the animal kingdom: Ecdysozoa, islands, trees, and the "parsimony ratchet." *Mol. Phylogenet. Evol.* **13,** 619–623.

Gladstein, D. S., and Wheeler, W. C. (1997). "POY: The Optimization of Alignment Characters." American Museum of Natural History, New York.

Gould, S. J. (1989). "Wonderful Life." Norton, New York.

Haupt, J. (1979). Phylogenetic aspects of recent studies on myriapod sense organs. *In* "Myriapod Biology" (M. Camatini, Ed.), pp. 391–406. Academic Press, London.

Hendriks, L., van Broeckhoven, C., Vandenberghe, A., van de Peer, Y., and De Wachter, R. (1988). Primary and secondary structure of the 18S ribosomal RNA of the bird spider *Eurypelma californica* and evolutionary relationships among eukaryotic phyla. *Eur. J. Biochem.* **177,** 15–20.

Hennig, W. (1969). "Die Stammesgeschichte der Insekten." Kramer, Frankfurt.

Hessler, R. R., and Newman, W. A. (1975). A trilobitomorph origin for the Crustacea. *Fossils Strata* **4,** 437–459.

Higgins, D. G., and Sharp, P. M. (1988). CLUSTAL: A package for performing multiple sequence alignment on a microcomputer.*Gene* **73,** 237–244.

Jamieson, B. G. M. (1987). "The Ultrastructure and Phylogeny of Insect Spermatozoa." Cambridge Univ. Press, Cambridge, UK.

Jamieson, B. G. M., and Storch, V. (1992). Further spermatological evidence for including the Pentastomida in the Crustacea. *Int. J. Parasitol.* **22,** 95–108.

Jeram, A. J., Selden, P. A., and Edwards, D. (1990). Land animals in the Silurian: Arachnids and myriapods from Shropshire, England. *Science* **250,** 658–661.

Johnson, E. W., Briggs, D. E. G., Suthern, R. J., Wright, J. L., and Tunnicliff, S. P. (1993). Non-marine arthropod traces from the subaerial Borrowdale Volcanic Group, English Lake District. *Geol. Mag.* **17,** 395–406.

Kaestner, A. (1963). "Lehrbuch der Speziellen Zoologie," VCH, Jena.

Kraus, O. (1998). Phylogenetic relationships between higher taxa of tracheate arthropods.*In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 295–303. Chapman & Hall, London.

Kraus, O., and Kraus, M. (1994). Phylogenetic system of the Tracheata (Mandibulata): On "Myriapoda": Insecta interrelationships, phylogenetic age and primary ecological niches. *Verh. Naturwiss. Ver. Hamburg* **34,** 5–31.

Kraus, O., and Kraus, M. (1996). On myriapod/insect interrelationships. *Mém. Mus. Natl. Hist. Nat.* **169,** 283–290.

Kristensen, N. P. (1981). Phylogeny of insect orders. *Annu. Rev. Entomol.* **26,** 135–157.

Kristensen, N. P. (1991). Phylogeny of extant Hexapods.*In* "The Insects of Australia" (I. D. Nauman, Ed.), pp. 125–140. Cornell Univ. Press, Ithaca, NY.

Kristensen, N. P. (1998). The groundplan and basal diversification of the hexapods.*In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 281–293. Chapman & Hall, London.

Kukalová-Peck, J. (1987). New carboniferous Diplura, Monura, and Thysanura, the hexapod groundplan, and the role of thoracic side lobes in the origin of wings (Insecta). *Can. J. Zool.* **65,** 2327–2345.

Kukalová-Peck, J. (1991). Fossil history and the evolution of hexapod structures.*In* "The Insects of Australia" (I. D. Nauman, Ed.), pp. 141–179. Cornell Univ. Press, Ithaca, NY.

Kukalová-Peck, J. (1992). The "Uniramia" do not exist: The ground plan of the Pterygota as revealed by permian Diaphanopterodea from Russia (Insecta: Paleodictyopteroidea). *Can. J. Zool.* **70,** 236–255.

Lankester, E. R. (1904). The structure and classification of the Arthropoda. *Q. J. Microsc. Sci.* **38,** 523–582.

Lauterbach, K.-E. (1983). Zum Problem der Monophylie der Crustacea. *Verh. Naturwiss. Ver. Hamburg* **26,** 293–320.

Manton, S. M. (1972). The evolution of arthropodan locomotory mechanisms. Part 10. Locomotory habits, morphology and the evolution of the hexapod classes. *Zool. J. Linn. Soc.* **51,** 203–400.

Manton, S. M. (1973). Arthropod phylogeny—A modern synthesis. *J. Zool. London* **171,** 11–130.

Manton, S. M. (1977). "The Arthropoda: Habits, Functional Morphology, and Evolution." Clarendon Press, Oxford.

McNamara, K. J., and Trewin, N. H. (1993). A euthycarcinoid arthropod from the Silurian of western Australia. *Palaeontology* **36,** 319–335.

Melic, A., De Haro, J. J., Méndez, M., and Ribera, I. (1999). "Evolución y Filogenia de Arthropoda," Bol. S.E.A. Monogr. 26. Soc. Entomol. Aragonesa, Zaragoza, Spain.

Mikulic, D. G., Briggs, D. E. G., and Klussendorf, J. (1985a). A Silurian soft-bodied biota. *Science* **228,** 715–717.

Mikulic, D. G., Briggs, D. E. G., and Klussendorf, J. (1985b). A new exceptionally preserved biota from the Lower Silurian of Wisconsin, U. S. A. *Philos. Trans. R. Soc. London B Biol. Sci.* **311,** 75–85.

Müller, K. J., and Walossek, D. (1988). External morphology and larval development of the Upper Cambrian maxillopod *Bredocaris admirabilis*. *Fossils Strata* **23,** 1–70.

Nielsen, C. (1995). "Animal Evolution, Interrelationships of the Living Phyla," Oxford Univ. Press, Oxford.

Nielsen, C., Scharff, N., and Eibye-Jacobsen, D. (1996). Cladistic analyses of the animal kingdom. *Biol. J. Linn. Soc.* **57,** 385–410.

Nilsson, D.-E., and Osorio, D. (1998). Homology and parallelism in arthropod sensory processing. *In* "Oarthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 3333-. Chapman & Hall, London.

Osorio, D., Averof, M., and Bacon, J. P. (1995). Arthropod evolution: Great brains, beautiful bodies. *Trends Ecol. Evol.* **10,** 449–454.

Osorio, D., and Bacon, J. P. (1994). A good eye for arthropod evolution. *BioEssays* **16,** 419–424.

Panganiban, G., Nagy, L., and Caroll, S. B. (1994). The role of the *Distal-less* gene in the development and evolution of insect limbs. *Curr. Biol.* **4,** 671–675.

Panganiban, G., Sebring, A., Nagy, L., and Carroll, S. (1995). The development of crustacean limbs and the evolution of arthropods. *Science* **270,** 1363–1366.

Patel, N. H. (1994). Developmental evolution: Insights from studies of insect segmentation. *Science* **266,** 581–590.

Paulus, H. F. (1979). Eye structure and the monophyly of the Arthropoda. *In* "Arthropod Phylogeny" (A. P. Gupta, Ed.), pp. 299–383. Van Nostrand–Reinhold, New York.

Pocock, R. J. (1893). On the classification of the tracheate Arthropoda. *Zool. Anz.* **16,** 271–275.

Popadic, A., Rusch, D., Peterson, M., Rogers, B. T., and Kaufman, T. C. (1996). Origin of the arthropod mandible. *Nature* **380,** 395.

Popadic, A., Panganiban, G., Rusch, D., Shear, W. A., and Kaufman, T. C. (1998). Molecular evidence for the gnathobasic derivation of arthropod mandibles and for the appendicular origin of the labrum and other structures. *Dev. Genes Evol.* **208,** 142–150.

Prunescu, C. C. (1996). Plesiomorphic and apomorphic characters states in the class Chilopoda. *Mém. Mus. Natl. Hist. Nat.* **169,** 299–306.

Regier, J. C., and Shultz, J. W. (1997). Molecular phylogeny of the major arthropod groups indicates polyphyly of crustaceans and a new hypothesis for the origin of hexapods. *Mol. Biol. Evol.* **14,** 902–913.

Regier, J. C., and Shultz, J. W. (1998). Molecular phylogeny of arthropods and the significance of the Cambrian "explosion" for molecular systematics. *Am. Zool.* **38,** 918–928.

Remsen, J., and DeSalle, R. (1998). Character congruence of multiple data partitions and the origin of the Hawaiian Drosophilidae. *Mol. Phylogenet. Evol.* **9,** 225–235.

Riley, J., Banaja, A. A., and James, J. L. (1978). The phylogenetic relationships of the Pentastomida: The case for their inclusion within the Crustacea. *Int. J. Parasitol.* **8,** 245–254.

Robison, R. A. (1990). Earliest-known uniramous arthropod. *Nature* **343,** 163–164.

Sanger, F., Nicklen, S., and Coulsen, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74,** 5463–5468.

Scholtz, G., Mittmann, B., and Gerberding, M. (1998). The pattern of *Distal-less* expression in the mouthparts of crustaceans, myriapods and insects: New evidence for a gnathobasic mandible and the common origin of Mandibulata. *Int. J. Dev. Biol.* **42,** 801–810.

Schram, F. R. (1986). "Crustacea," Oxford Univ. Press, New York/Oxford.

Schram, F. R., and Emerson, M. J. (1991). Arthropod pattern theory: A new approach to arthropod phylogeny. *Mem. Qld Mus.* **31,** 1–18.

Schram, F. R., and Hof, C. H. J. (1998). Fossils and the interrelationships of major crustacean groups. *In* "Arthropod Fossils and Phylogeny" (G. D. Edgecombe, Ed.), pp. 233–302. Cambridge Univ. Press, New York.

Sharov, A. G. (1966). "Basic Arthropodan Stock with Special Reference to Insects," Pergamon Press, Oxford.

Shear, W. A. (1998). The fossil record and evolution of the Myriapoda. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 211–219. Chapman & Hall, London.

Shear, W. A., and Bonamo, P. M. (1988). Devonobiomorpha, a new order of centipeds (Chilopoda) from the middle Devonian of Gilboa, New York State, USA, and the phylogeny of centiped orders. *Am. Mus. Novitates* **2927,** 1–30.

Shear, W. A., Gensel, P. G., and Jeram, A. J. (1996). Fossils of large terrestrial arthropods from the Lower Devonian of Canada. *Nature* **384,** 555–557.

Shear, W. A., Jeram, A. J., and Selden, P. A. (1998). Centipede legs (Arthropoda, Chilopoda, Scutigeromorpha) from the Silurian and Devonian of Britain and the Devonian of North America. *Am. Mus. Novitates* **3231,** 1–16.

Shear, W. A., and Selden, P. A. (1995). *Eoarthropleura* (Arthropoda, Arthropleurida) from the Silurian of Britain and the Devonian of North America. *N. Jb. Geol. Paläont. Abh.* **196,** 347–375.

Shultz, J. W. (1990). Evolutionary morphology and phylogeny of Arachnida. *Cladistics* **6,** 1–38.

Snodgrass, R. E. (1938). Evolution of the Annelida, Onychophora and Arthropoda. *Smithsonian Misc. Collect.* **97,** 1–159.

Snodgrass, R. E. (1950). Comparative studies on the jaws of mandibulate arthropods. *Smithsonian Misc. Collect.* **116,** 1–85.

Snodgrass, R. E. (1951). "Comparative Studies on the Head of Mandibulate Arthropods," Comstock, Ithaca, NY.

Snodgrass, R. E. (1952). "A Textbook of Arthropod Anatomy," Cornell Univ. Press, Ithaca, NY.

Spears, T., and Abele, L. G. (1998). Crustacean phylogeny inferred from 18S rDNA. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 169–187. Chapman & Hall, London.

Štys, P., and Biliński, S. (1990). Ovariole types and the phylogeny of hexapods. *Biol. Rev. Cambridge Phil. Soc.* **65,** 401–429.

Štys, P., and Zrzavý, J. (1994). Phylogeny and classification of extant Arthropoda: Review of hypotheses and nomenclature. *Eur. J. Entomol.* **91,** 257–275.

Štys, P., Zrzavý, J., and Weyda, F. (1993). Phylogeny of the Hexapoda and ovarian metamerism. *Biol. Rev.* **68,** 365–379.

Tesakov, A. S., and Alekseev, A.S. (1992). Myriapod-like arthropods from the Lower Devonian of central Kazakhstan. *Paleontol. J.* **26,** 18–23.

Tesakov, A. S., and Alekseev, A. S. (1998). *Maldybulakia*—New name for *Lophodesmus* Tesakov & Alekseev, 1992 (Arthropoda). *Paleontol. J.* **32,** 49.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25,** 4876–4882.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22,** 4673–4680.

Tiegs, O. W., and Manton, S. M. (1958). The evolution of the Arthropoda. *Biol. Rev.* **33,** 255–337.

Turbeville, J. M., Pfeifer, D. M., Field, K. G., and Raff, R. A. (1991). The phylogenetic status of arthropods, as inferred from 18S rRNA sequences. *Mol. Biol. Evol.* **8,** 669–686.

van der Hammen, L. (1977). A new classification of Chelicerata. *Zool. Meded. (Leiden)* **51,** 307–319.

van der Hammen, L. (1985). A structural approach in the study of evolution and classification. *Zool. Meded. (Leiden)* **59,** 392–409.

van der Hammen, L. (1986). Acarological and arachnological notes. *Zool. Meded. (Leiden)* **60,** 217–230.

Waggoner, B. M. (1996). Phylogenetic hypotheses of the relationships of arthropods to Precambrian and Cambrian problematic fossil taxa. *Syst. Biol.* **45,** 190–222.

Walossek, D. (1995). The Upper Cambrian *Rehbachiella*, its larval development, morphology and significance for the phylogeny of Branchiopoda and Crustacea. *Hydrobiologia* **298,** 1–13.

Walossek, D., and Müller, K. J. (1994). Pentastomid parasites from the Lower Palaeozoic of Sweden. *Trans. R. Soc. Edinburgh Earth Sci.* **85,** 1–37.

Walossek, D., and Müller, K. J. (1998). Cambrian 'Orsten'-type arthropods and the phylogeny of Crustacea. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 139–143. Chapman & Hall, London.

Walossek, D., Repetski, J. E., and Müller, K. J. (1994). An exceptionally preserved parasitic arthropod, *Heymonsicambria taylori* n. sp. (Arthropoda *incertae sedis*: Pentastomida), from Cambrian–Ordovician boundary beds of Newfoundland, Canada. *Can. J. Earth Sci.* **31,** 1664–1671.

Wägele, J. W. (1993). Rejection of the 'Uniramia' hypothesis and implication of the Mandibulata concept. *Zool. Jb. Syst.* **120,** 253–288.

Wägele, J. W., and Stanjek, G. (1995). Arthropod phylogeny inferred from partial 12S rRNA revisited: Monophyly of the Tracheata depends on sequence alignment. *J. Zool. Syst. Evol. Res.* **33,** 75–80.

Weygoldt, P. (1979). Significance of later embryonic stages and head development in arthropod phylogeny. *In* "Arthropod Phylogeny" (A. P. Gupta, Ed.), pp. 107–136. Van Nostrand, New York.

Weygoldt, P., and Paulus, H. F. (1979a). Untersuchungen zur Morphologie, Taxonomie und Phylogenie der Chelicerata. I. Morphologische Untersuchungen. *Z. Zool. Syst. Evol. Forsch.* **17,** 85–116.

Weygoldt, P., and Paulus, H. F. (1979b). Untersuchungen zur Morphologie, Taxonomie und Phylogenie der Chelicerata. II. Cladogramme und die Entfaltung der Chelicerata. *Z. Zool. Syst. Evol. Forsch.* **17,** 177–200.

Wheeler, W. C. (1993). The triangle inequality and character analysis. *Mol. Biol. Evol.* **10,** 707–712.

Wheeler, W. C. (1995). Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. *Syst. Biol.* **44,** 321–331.

Wheeler, W. C. (1996). Optimization alignment: The end of multiple sequence alignment in phylogenetics? *Cladistics* **12,** 1–9.

Wheeler, W. C. (1998a). Sampling, groundplans, total evidence and the systematics of arthropods. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 87–96. Chapman & Hall, London.

Wheeler, W. C. (1998b). Molecular systematics and arthropods. *In* "Arthropod Fossils and Phylogeny" (G. D. Edgecombe, Ed.), pp. 9–32. Cambridge Univ. Press, New York.

Wheeler, W. C. (1999). Fixed character states and the optimization of molecular sequence data. *Cladistics* **15,** 379–386.

Wheeler, W. C., Cartwright, P., and Hayashi, C. Y. (1993). Arthropod phylogeny: A combined approach. *Cladistics* **9,** 1–39.

Wheeler, W. C., and Gladstein, D. S. (1994). MALIGN: A multiple sequence alignment program. *J. Hered.* **85,** 417–418.

Wheeler, W. C., and Gladstein, D. (1995) MALIGN, version 2.7. American Museum of Natural History, New York.

Wheeler, W. C., and Hayashi, C. Y. (1998). The phylogeny of extant chelicerate orders. *Cladistics* **14,** 173–192.

Whiting, M. F., Carpenter, J. M., Wheeler, Q. D., and Wheeler, W. C. (1997). The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Syst. Biol.* **46,** 1–68.

Whitington, P. M. (1996). Evolution of neural development in the arthropods. *Semin. Cell Dev. Biol.* **7,** 605–614.

Whitington, P. M., Leach, D., and Sandeman, R. (1993). Evolutionary change in neural development within the arthropods: Axonogenesis in the embryos of two crustaceans. *Development* **118,** 449–461.

Whitington, P. M., Meier, T., and King, P. (1991). Segmentation, neurogenesis and formation of early axonal pathways in the centipede, *Ethmostigmus rubripes* (Brandt). *Roux's Arch. Dev. Biol.* **199,** 349–363.

Willmann, R. (1998). Advances and problems in insect phylogeny. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 269–279. Chapman & Hall, London.

Wills, M. A. (1998). A phylogeny of recent and fossil Crustacea derived from morphological characters. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 189–209. Chapman & Hall, London.

Wills, M. A., Briggs, D. E. G., and Fortey, R. A. (1994). Disparity as an evolutionary index: A comparison of Cambrian and recent arthropods. *Paleobiology* **20,** 93–130.

Wills, M. A., Briggs, D. E. G., and Fortey, R. A. (1998). Evolutionary correlates of arthropod tagmosis: Scrambled legs. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 57–65. Chapman & Hall, London.

Wills, M. A., Briggs, D. E. G., Fortey, R. A., and Wilkinson, M. (1995). The significance of fossils in understanding arthropod evolution. *Verh. Dtsch. Zool. Gesamte* **88,** 203–215.

Wilson, G. D. F. (1992). Computerized analysis of crustacean relationships. *Acta Zool.* **73,** 383–389.

Wingstrand, K. G. (1972). Comparative spermatology of a pentastomid, *Raillietiella hemidactyli*, and a branchiuran crustacean, *Argulus foliaceus*, with a discussion of pentastomid Relationships. *K. Dan. Vidensk. Selsk. Biol. Skr.* **19,** 1–72.

Zrzavý, J., Hypša, V., and Vlášková, M. (1998a). Arthropod phylogeny: Taxonomic congruence, total evidence and conditional combination approaches to morphological and molecular data sets. *In* "Arthropod Relationships" (R. A. Fortey and R. H. Thomas, Eds.), pp. 97–107. Chapman & Hall, London.

Zrzavý, J., Mihulka, S., Kepka, P., Bezdék, A., and Tietz, D. (1998b). Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. *Cladistics* **14,** 249–285.

Zrzavý, J., and Štys, P. (1997). The basic body plan of arthropods: Insights from evolutionary morphology and developmental biology. *J. Evol. Biol.* **10,** 353–367.