

First Molecular Evidence for the Existence of a Tardigrada + Arthropoda Clade

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The complete 18S rDNA gene sequence of *Macrobiotus* group *hufelandi* (Tardigrada) was obtained and aligned with 18S rDNA and rRNA gene sequences of 24 metazoans (mainly protostomes). Discrete character (maximum-parsimony) and distance (neighbor-joining) methods were used to infer their phylogeny. The evolution of bootstrap proportions with sequence length (pattern of resolved nodes, PRN) was studied to test the resolution of the nodes in neighbor-joining trees. The results show that arthropods are monophyletic. Tardigrades represent the sister group of arthropods (in parsimony analyses) or they are related with crustaceans (distance analysis and PRN). Arthropoda are divided into two main evolutionary lines, the Hexapoda + Crustacea line (weakly supported), and the Myriapoda + Chelicerata line. The Hexapoda + Crustacea line includes Pentastomida, but the internal resolution is far from clear. The Insecta (Ectognatha) are monophyletic, but no evidence for the monophyly of Hexapoda is found. The Chelicerata are a monophyletic group and the Myriapoda cluster close to Arachnida. Overall, the results obtained represent the first molecular evidence for a Tardigrada + Arthropoda clade. In addition, the congruence between molecular phylogenies of the Arthropoda from other authors and this obtained here indicates the need to review those obtained solely on morphological characters.

Introduction

Controversy about arthropod phylogeny has dragged on for more than a century. Several hypotheses have been entertained, but the outcome is still far from clear. Wheeler, Cartwright, and Hayashi (1993) provide a comprehensive summary of these hypotheses, and a general discussion on monophyletic versus polyphyletic theories is given in Willmer (1990).

Onychophora, Pentastomida, and Tardigrada are three enigmatic groups somehow related to arthropods. They have been placed in an artificial group named 'Pararthropoda,' due to their unclear anatomical affinities. Sperm morphology (Wingstrand 1972; Jamieson and Storch 1992) and 18S rRNA sequences (Abele, Kim, and Felgenhauer 1989) relate Pentastomida to Crustacea. Data for 12S rRNA (Ballard et al. 1992) and 18S rDNA (Wheeler, Cartwright, and Hayashi 1993) sequences strongly support Onychophora as an early offshoot of early arthropod radiation.

Traditionally the tardigrades (water bears) have been considered a small phylum that appears to be closely tied to the annelid–arthropod line. The first tardigrade was recorded in 1773, and over 400 species from marine, freshwater, and terrestrial habitats are known. Most species are widespread and many are cosmopolitan. Some authors consider tardigrades a phylum (Pearse et al. 1987; Brusca and Brusca 1990; Willmer

1990; Meglitsch and Schram 1991; Ruppert and Barnes 1994). Instead, the phylogenies of the animal kingdom based on morphological data reported by Hadži (1956, 1963), Nielsen (1985), and Meglitsch and Schram (1991) include tardigrades within the arthropods.

Molecular characters may be used to test arthropod relationships independently of morphological or developmental characters. Molecular phylogenies based on 18S rRNA or rDNA have been inferred below the phylum level in several groups: Platyhelminthes (Riutort et al. 1992, 1993), Arthropoda (Turbeville et al. 1991; Wheeler, Cartwright, and Hayashi 1993), Crustacea (Kim and Abele, 1990; Spears, Abele, and Kim 1992), Insecta (Carmean, Kimsey, and Berbee 1992), Tunicata (Wada et al. 1992), Echinodermata (Wada and Satoh 1994), etc. Thus far, molecular data bearing on the phylogenetic position of Tardigrada are not available.

In this paper we present the first 18S rDNA sequence data from a tardigrade species (*Macrobiotus hufelandi* group) and those of two arachnid orders (Solifugae and Opiliones), a Chilopoda (*Scolopendra*) and a Nemertinea (*Prostoma*). We have aligned these sequences to those from the principal arthropod groups, other protostomes, a deuterostome, and a platyhelminth reported in the literature. The aim of this study is to infer the phylogenetic position of tardigrades within the protostome animals and to comment on arthropod phylogeny.

Materials and Methods

Biological Material

Specimens of one species of the *M. hufelandi* group (Tardigrada, Eutardigrada, Macrobiotidae) were obtained from moss samples (Collserola, Barcelona,

Key words: Tardigrada, Arthropoda, Protostomata, 18S rDNA, phylogeny, pattern of resolved nodes (PRN).

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Mol. Biol. Evol. 13(1):76–84. 1996

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Table 1
Primers Used in Amplification and Sequencing

Primer	5'-3'
1F	TACCTGGTTGATCCTGCCAGTAG
3R	AGGCTCCCTCTCCGGAATCGAAC
3F	GTTTCGATTCCGGAGAGGGA
4R	GAATTACCGCGGCTGCTGG
4F	CCAGCAGCCGCGCTAATTC
5R	CTTGGCAAATGCTTTTCGC
5F	GCGAAAGCATTTGCCAAGAA
6R	ATTCCTTTAAGTTT
6F	AAACTTAAAGGAAT
7R	GCATCACAGACCTGTTATTGC
7F	GCAATAACAGGTCTGTGATGCCC
8R	ACGGGCGGTGTGTAC
8F	GTACACACCGCCCGT
9R	GATCCTTCCGAGGTTACCTAC

NOTE.—Primer pairs 1F-3R, 3F-5R, and 5F-9R have been used in amplification of *Macrobiotus* and *Scolopendra*. Primer pairs 1F-5R and 5F-9R have been used in amplification of the remaining species. All primers have been used in sequencing 18S rDNA gene.

Spain). Identification follows Bertolani and Rebecchi (1993).

We also obtained material for the following species: *Odiellus troguloides* (Arachnida, Opiliones, Phalangidae), *Eusimonia wunderlichi* (Arachnida, Solifugae, Karschiidae), *Scolopendra cingulata* (Myriapoda, Chilopoda, Scolopendridae), and *Prostoma eilhardi* (Nemertini, Hoplonemertini, Tetrastemmatidae).

Sample Preparation

Genomic DNA samples from *Macrobiotus* were obtained by direct lysis of tardigrade tissues in 400 μ l of polymerase chain reaction (PCR) buffer w/nonionic detergents (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.45% NP40, 0.45% Tween-2) and 0.6 μ l of Proteinase K (10 mg/ml)/100 μ l buffer (modified from Higuchi 1989). The 18S rDNA was PCR amplified in three fragments of about 400, 550, and 850 bp each, using primer pairs 1F-3R, 3F-5R, and 5F-9R, respectively (table 1). DNA from *Odiellus*, *Eusimonia*, *Scolopendra*, and *Prostoma* were obtained using a guanidinium thiocyanate protocol. Amplification was performed with DynaZyme™ polymerase in 100 μ l total reaction volume, using 50 μ l of the DNA extraction PCR buffer. Samples were amplified for 35 cycles (94°C for 45s, 49°C for 45s, 72°C for 1 min) with a previous step of 95°C for 5 min. Negative controls were carried out in amplification.

Amplified DNA was electrophoresed on an agarose gel containing ethidium bromide to verify product band size and then purified by DEAE-cellulose transfer. DNA samples were redissolved in 400 μ l of eluting buffer (10 mM Tris-HCl, 1mM EDTA [TE], pH 7.5 and NaCl 1.5 M) at 65°C for 2 h, extracted in phenol, and precipitated

with isopropanol. The pellet was redissolved in 15 μ l of sterile water.

Each of the three PCR products was ligated into pUC 18 *Sma*I/BAP dephosphorylated vector using the SureClone™ Ligation Kit (Pharmacia P-L Biochemicals). Constructs were transformed into competent *Escherichia coli* JM 109 cells. The alkaline lysis method for small-scale preparations of plasmid DNA (Sambrook, Fritsch, and Maniatis 1989), was used to yield DNA for sequencing. Product band size was again tested on an agarose gel after restriction enzyme (*Eco*RI–*Bam*HI) digestion.

Sequencing was performed by the dideoxy termination method (Sanger, Nicklen, and Coulson 1977) using T7 DNA polymerase (T7Sequencing™ Kit from Pharmacia Biotech). Plasmid and internal 18S primers were used for sequencing (table 1). Full-length sequences of both strands from three clones were obtained and a consensus sequence was derived. Differences in the consensus sequence among the three clones are indicated by an N.

All sequences have been deposited in the EMBL, GenBank, and DDBJ Nucleotide Sequence Database with the following accession codes: *M. hufelandi* group (X81442), *O. troguloides* (X81441), *E. wunderlichi* (U29492), *S. cingulata* (U29493), and *P. eilhardi* (U29494).

Sequence Analysis

Sequences of 25 metazoa (including several arthropods and other non-arthropod protostomes) (table 2) were aligned by hand using the sequence editor of the GDE (Genetic Data Environment, version 2.2) package. Highly divergent regions that could not be aligned reliably were excluded from analysis. When possible, at least two complete sequences representative of each taxonomic group were included in the analyses. The taxonomic position and number of base pairs sequenced for each species are given in table 2. The platyhelminth *Lobatostoma manteri* was used as an outgroup.

Maximum parsimony analyses were performed using PAUP: Phylogenetic Analysis Using Parsimony, version 3.1 (Swofford 1993). Two tree-constructing options were used, and a consistency index (CI) (Kluge and Farris 1969) as well as a retention index (RI) (Farris 1989a, 1989b) were estimated. A preliminary tree using 25 taxa was constructed with the heuristic search option to give a general overview. According to these results the number of taxa was reduced to 11, to use more accurate algorithms. The branch and bound search option was then used with these taxa to obtain a set of maximum-parsimonious cladograms. Successive character weighting (Farris 1969) was used to choose a cladogram from the set of the most equally parsimonious cladograms. A

Table 2
Taxa Used in the Analyses, Number of bp Sequenced, and Accession Code to GenBank

Species Name	Systematic Position	bp	Accession Code
<i>Lobatostoma manteri</i>	(Platyhelminthes , Trematoda)	1,976	L16911
<i>Branchiostoma floridae</i>	(Chordata , Cephalochordata)	1,778	M97571
<i>Acanthopleura japonica</i>	(Mollusca, Polyplacophora)	1,817	X70210
<i>Limicolaria kambeul</i>	(Mollusca, Gastropoda)	1,839	X66374
<i>Glycera americana</i>	(Annelida , Polychaeta)	1,814	U19519
<i>Phoronis vancouverensis</i>	(Lophophorata, Phoronidea)	1,758	U12648
<i>Glottidia pyramidata</i>	(Lophophorata, Brachiopoda, Inarticulata)	1,743	U12647
* <i>Prostoma eilhardi</i>	(Nemertinea , Hoplonemertinea)	1,840	U29494
* <i>Macrobitus</i> group <i>hufelandi</i>	(Tardigrada , Eutardigrada)	1,808	X81442
<i>Procambarus leonensis</i>	(Arthropoda, Crustacea, Decapoda)	1,869	M34363
<i>Artemia salina</i>	(Arthropoda, Crustacea, Branchiopoda)	1,809	X01723
<i>Balanus eburneus</i>	(Arthropoda, Crustacea, Cirripedia)	1,836	L26510
<i>Porocephalus crotali</i>	(Arthropoda, Pentastomida)	1,830	M29931
<i>Hypogastrura</i> sp.	(Arthropoda, Hexapoda, Collembola 1)	1,812	Z26765
<i>Crossodonthina koreana</i>	(Arthropoda, Hexapoda, Collembola 2)	1,811	Z36893
<i>Tenebrio molitor</i>	(Arthropoda, Insecta, Coleoptera)	2,016	X07801
<i>Meloe proscarabaeus</i>	(Arthropoda, Insecta, Coleoptera 2)	1,934	X77786
<i>Polistes dominulus</i>	(Arthropoda, Insecta, Hymenoptera)	1,919	X77785
<i>Spissistilus festinus</i>	(Arthropoda, Insecta, Homoptera)	1,900	U06477
<i>Lygus hesperus</i>	(Arthropoda, Insecta, Heteroptera)	1,922	U06476
* <i>Scolopendra cingulata</i>	(Arthropoda, Myriapoda, Chilopoda)	1,888	U29493
<i>Eurypelma californica</i>	(Arthropoda, Arachnida, Araneae)	1,957	X13457
<i>Androctonus australis</i>	(Arthropoda, Arachnida, Scorpionida)	1,812	X77908
* <i>Eusimonia wunderlichi</i>	(Arthropoda, Arachnida, Solifugae)	1,811	U29492
* <i>Odiellus trogluoides</i>	(Arthropoda, Arachnida, Opiliones)	1,806	X81441

NOTE.—New sequences reported in this paper are marked with an asterisk. Bold letters indicate the taxonomic categories represented in the trees.

bootstrap method with heuristic search of 1,000 replicates was applied.

PHYLIP (Phylogeny Inference Package) version 3.5c (Felsenstein 1993) included in the GDE package was used for distance analyses. The Kimura two-parameter distance was estimated and neighbor-joining (Saitou and Nei 1987) trees were constructed with the same 11 taxa that were used in the branch and bound analysis.

PRN (Pattern of Resolved Nodes) Analysis

Instead of simply examining the bootstrap proportions (BP) at important nodes as a criterion of robustness of the corresponding nodes, Lecointre et al. (1994) have introduced a procedure of BP analysis that involves following the values of BP as a function of increasing number of nucleotides.

The method described by Lecointre et al. (1994) was used with the same 11 taxa used in the distance analysis plus the Chordata, under the following conditions. The alignments of the 12 species were each submitted to random sampling (jack-knife) of a given number of sites through the use of the program PRN, running on UNIX platforms. Ten different sequence lengths were chosen (25, 50, 75, 125, 250, 375, 600, 900, 1,200, and 1,500 sites) and for each, 200 samples were drawn.

Thus a total of 2,000 subsets of sequence alignments were obtained, each including all 12 species. Each of these subsets was used to construct a neighbor-joining tree, which was submitted to 1,000 bootstrap replicates. Selection of the nodes was then carried out using the program AFT-PRN according to the following criteria: the node should correspond to a BP with an ascending tendency, it should be present in more than 200 of the subsets of sequences, and it should reach a maximum BP of at least 800. At a given node, one could therefore display graphically the evolution of BP as a function of the number of nucleotides that were used to generate the tree (COMP_BOO program of the MUST package [Philippe 1993]).

Lecointre et al. (1994) have shown that mean bootstrap proportions (BP) can be related to the number of nucleotides, x , through the function $BP = 100(1 - e^{-b(x-x')})$. The parameters b and x' are specific for each node and they are estimated by nonlinear regression using the GENSTAT package.

Results

Data Analyses

The consensus 18S rDNA gene sequence of the *M. hufelandi* group is 1,808 bp long (461 A; 445 T; 403 C;

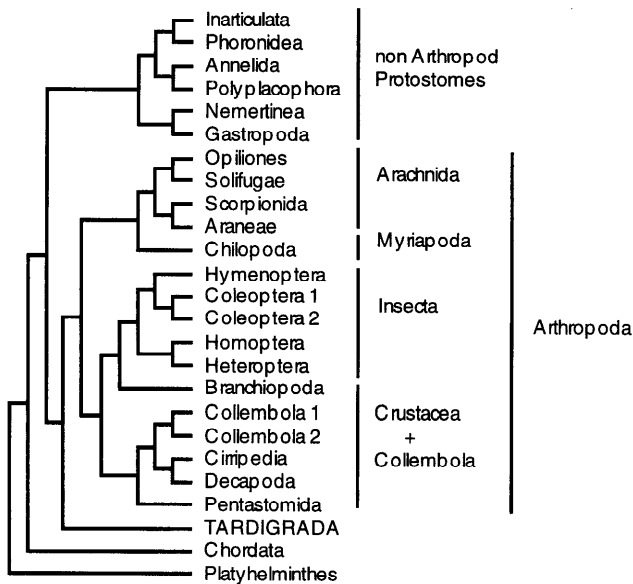


FIG. 1.—The most-parsimonious tree of 1,451 steps (CI 0.571; RI 0.468), obtained by heuristic search option using PAUP (1,638 sites; 330 parsimony-informative sites) when all taxa are included.

496 G; 3 N). Amplified primer sequences, 98 bp, are not included in the analyses. Areas of the molecule that could not be aligned unambiguously were excluded.

The alignment of the 18S rDNA sequences of the 25 taxa yields 1,638 comparable sites, with 635 variable sites and 330 parsimony-informative sites. The 11 sequences used in branch and bound analysis and in neighbor-joining analysis yield 445 variable sites, 181 of which are parsimony informative.

Phylogenetic Trees

The heuristic-search option performed in PAUP yields a single most-parsimonious cladogram of 1,451 steps (CI 0.571; RI 0.468) shown in figure 1. Several points are worth mentioning. First, the tardigrade appears to be the sister group of the monophyletic Arthropoda, and non-arthropod protostomes appear to be a monophyletic clade, as well. Second, arthropods branch in two principal evolutionary lines: (Hexapoda [Insecta and Collembola] + Crustacea) and (Myriapoda + Arachnida). Third, Pentastomida clusters within the Hexapoda + Crustacea lineage close to Crustacea. However, some inconsistencies appear within this line, such as the branching of *Artemia* (Branchiopoda) with the Insecta and the grouping of both Collembola with the Crustacea. Fourth, the Insecta and the Arachnida are both monophyletic groups.

The brand and bound option using 11 18S sequences yields two trees of 708 steps (CI 0.754; RI 0.490). Both cladograms (fig. 2A and B) differ in the position of Chilopoda, which clusters with Arachnida in tree A and in the base of Hexapoda + Crustacea in tree B. After successive character weighting, a single cladogram is retained, corresponding to tree A. The results are stable after the second iteration. This cladogram is completely compatible with the general cladogram obtained in the heuristic search option, with the tardigrade as the sister group of Arthropoda. The bootstrap results obtained (represented in fig. 2A) support the group Tardigrada + Arthropoda (80% BP) and define four other monophyletic groups: Insecta (90%), Crustacea (98%),

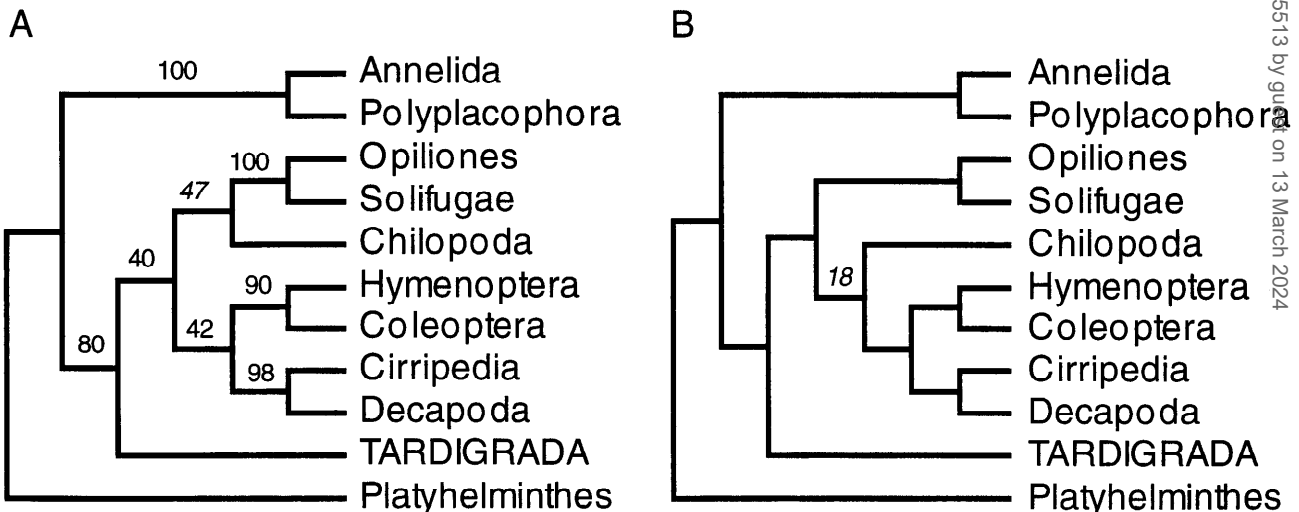


FIG. 2.—The two most-parsimonious trees of 708 steps (CI 0.754; RI 0.490) obtained by branch and bound search option performed in PAUP when 11 taxa are used in the analysis (1,638 sites; 202 parsimony-informative sites). Tree A coincides with the single one obtained by successive character weighting. Numbers at nodes indicate the bootstrap proportions. Note that BP for the clade Chilopoda + Hexapoda + Crustacea is very low.

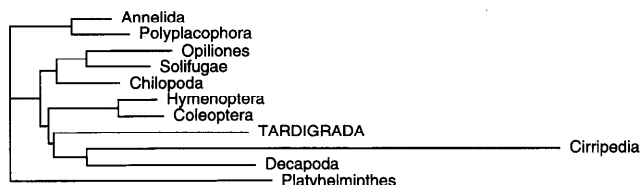


FIG. 3.—Neighbor-joining tree using the Kimura two-parameter distance performed in PHYLIP using the same 11 taxa as in the branch and bound trees (1,638 sites; 445 variable sites).

Arachnida (100%), and Annelida + Mollusca (100%). Even though the most-parsimonious solutions yield tardigrades as the sister group of arthropods, the node defining the monophyletic Arthropoda shows a low bootstrap value (40%).

The neighbor-joining tree (fig. 3) performed in PHYLIP differs from the parsimony tree from figure 2A in the position of Tardigrada. In this case Tardigrada appears as the sister group of Crustacea. No other differences from the parsimony tree are found.

Overall, these results represent the first molecular evidence placing the Tardigrada as the sister group of Arthropoda (in parsimony analyses) or within the Arthropoda (in distance analysis), always separated from non-arthropod protostomes.

PRN

Figure 4 shows the PRN of two nodes with data referring to Tardigrada; figure 4A shows an almost resolved node showing the monophyly of all arthropods and the tardigrade. Figure 4B shows the node uniting crustaceans with the tardigrade. This is a promising node, meaning a node with an ascending shape of the PRN that may correspond to early stages of its ascending part (Lecointre et al. 1994). This kind of node could become more robust by increasing the sequence length (in this case using another molecule).

The PRNs of different resolved and unresolved nodes are shown in figure 5. An almost resolved node grouping the Chilopoda with both Arachnida is depicted in figure 5A and an unresolved node (displaying low BP) grouping the Hexapoda with Crustacea is shown in figure 5B. Other resolved nodes represent the monophyly of non-arthropod protostomes, crustaceans, arachnids, and insects and are shown in figure 5C, D, E, and F, respectively.

Discussion

Comparison of 18S rDNA sequences between a species of Tardigrada and a large number of sequences from arthropods, other protostomes, a deuterostome, and a platyhelminth clearly indicates that Tardigrada are closely related with the Arthropoda lineage. The PRN

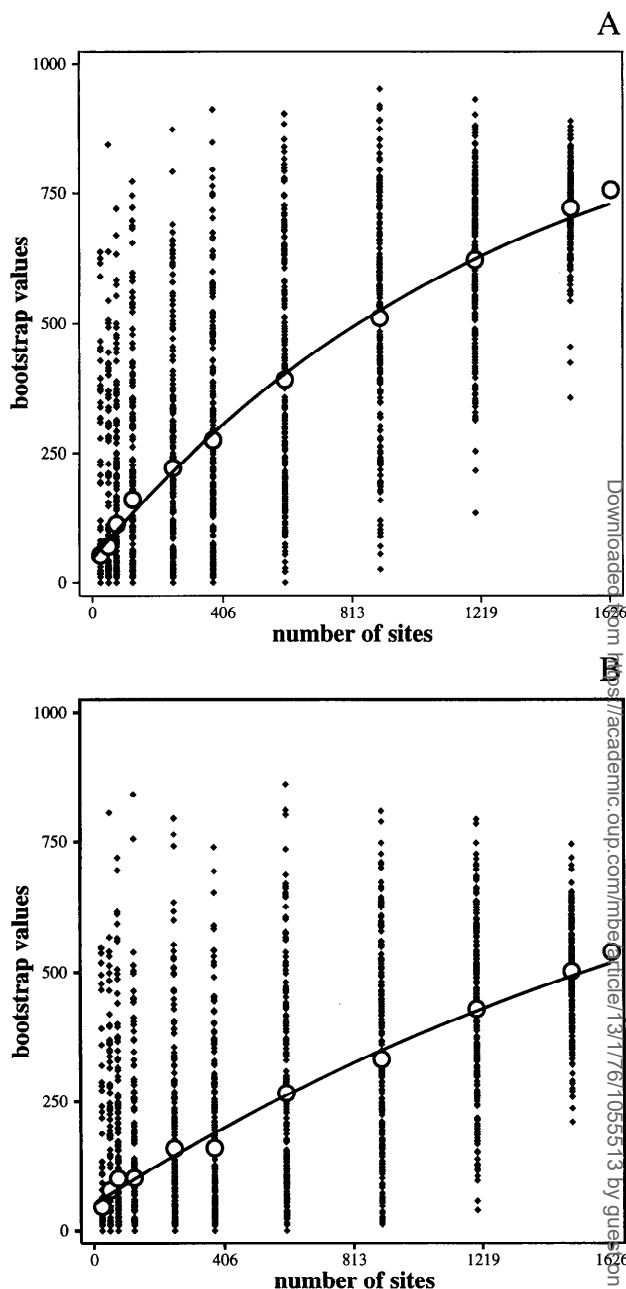


FIG. 4.—BP distributions plotted against sequence length, with the PRN representation for two nodes concerning the tardigrades in 2 taxa neighbor-joining trees. In each graph, the 200 bootstrap proportions obtained for a given number of nucleotides (25, 50, 75, 125, 250, 375, 600, 900, 1,200, and 1,500 sites) are plotted vertically as a function of increased number of nucleotides. The open circles correspond to the average value of all the BP. The general shape of the curve has been estimated through nonlinear regression that fits to the function $BP = 100(1 - e^{-b(x-x')})$. The graphs correspond to a resolved node for the monophyly of tardigrade + arthropods (A) and to a promising node for the monophyly of the tardigrade with crustaceans (B).

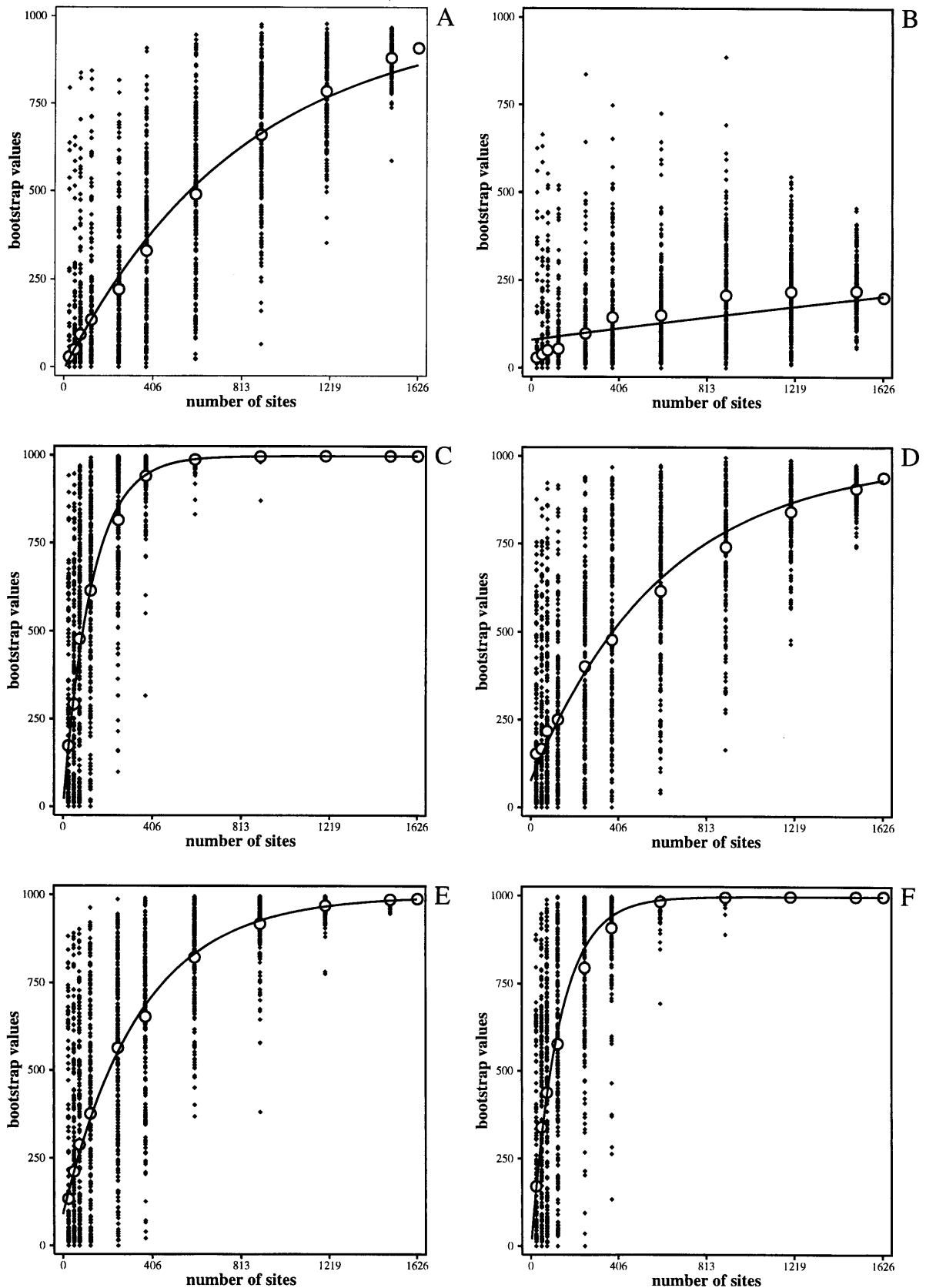


FIG. 5.—PRN for six nodes from the neighbor-joining trees. The graphs correspond to the monophyly of the chilopod + arachnids (A), annelid + mollusc (C), crustaceans (D), arachnids (E), and insects (F). Node B is a nonresolved node for the monophyly of hexapods and crustaceans.

results strongly support the idea that tardigrades and arthropods form a monophyletic clade (fig. 4A), as do the bootstrap results in the parsimony analyses. Empirical studies of bootstrap analyses (Hillis and Bull 1993), under some specific conditions, show that BPs $\geq 70\%$ usually correspond to a probability of $\geq 95\%$ that the corresponding clade is an historical lineage, so we can consider our BP good enough to ensure the monophyly of tardigrades + arthropods. Tardigrada are found as the sister group of Arthropoda in the parsimony analyses performed in PAUP or as a derivative arthropod within a group formed by Hexapoda + Crustacea in distance analyses. The PRN for the node uniting the tardigrade and crustaceans (fig. 4B) is a promising node that could probably be confirmed with the addition of more data. Consequently, if they are secondarily derived arthropods, they should be closer to crustaceans than to any other arthropod. The addition of more tardigrade sequences from nonrelated groups could help to place them more accurately, but we have not been able to obtain tardigrade samples other than those of the family Macrobiotidae.

Commenting on other aspects about arthropod phylogeny, Abele, Kim, and Felgenhauer (1989) used 18S rRNA sequence data to place the Pentastomida into the Crustacea, though the few taxa included in their analysis was not enough to establish clear relations with other Arthropoda. In our analysis Pentastomida occurs within the Arthropoda, within the Hexapoda + Crustacea group (fig. 1). Hence, it seems that Pentastomida are true arthropods, albeit modified due to their parasitic life, and belong to the Crustacea.

Onychophora have not been included in the analyses because only partial sequence data (662 bp of two species of Onychophora) are available (Wheeler, Cartwright, and Hayashi 1993). However, preliminary results using these partial sequences in the alignment (results not shown) place Onychophora within non-arthropod protostomes, related to the polychaete annelid. On morphological grounds, Onychophora has always been considered the sister group of Atelocerata (Myriapoda + Hexapoda), constituting the Unirramia (Tiegs and Manton 1958), or a group of uncertain position between Annelida and Arthropoda (Snodgrass 1938), which would represent an early stage of arthropodization. Using the partial 18S rDNA sequences, Wheeler, Cartwright, and Hayashi (1993) found that Onychophora appeared to be the sister group of Arthropoda. In contrast 12S rRNA data showed Onychophora to be modified Arthropoda (Ballard et al. 1993). These conflicting data and the availability of only partial 18S rDNA sequences leave this issue unresolved.

Arthropoda monophyly is consistent with all molecular phylogenies reported so far (Turbeville et al.

1991; Ballard et al. 1992; Wheeler, Cartwright, and Hayashi 1993) and with most hypotheses based on morphological characters. No support for an 'Articulata' (Annelida + Arthropoda) group is found either in parsimony or in distance and PRN analyses.

Furthermore, the molecular data do not support 'Unirramia' lineage (Onychophora + Myriapoda + Hexapoda) or the 'Atelocerata' lineage (Myriapoda + Hexapoda). Within the Arthropoda, the existence of an Hexapoda + Crustacea lineage agrees with previous molecular data analyses by Turbeville et al. (1991), Wheeler, Cartwright, and Hayashi (1993), and Ballard et al. (1992). However, this clade results in a nonresolved node in the PRN analysis (fig. 5B), and some internal inconsistencies appear.

Within the Hexapoda group, Insecta (Ectognatha) always group together, so they should be considered as a monophyletic group. It should be noted, however, that Collembola (Endognatha) never groups with the Ectognatha (fig. 1). This results agrees with the hypothesis based on morphological characters from Hennig (1953) and other authors that consider two evolutionary lines within the Hexapoda: Endognatha (Diplura + Protura + Collembola) and Ectognatha (Microcoryphia + Zygentoma + Pterygota). Complete 18S data on Diplura, Protura, Zygentoma, and Microcoryphia are currently being obtained to resolve the phylogenetic relationships between different groups of Hexapoda (Giribet et al., work in progress).

The other evolutionary line obtained within the Arthropoda, (Chelicerata + Myriapoda) is more supported than the Hexapoda + Crustacea group (fig. 5, A and B). Turbeville et al. (1991) also found these two lines with parsimony analysis. Our results do not allow us to confirm this branching pattern, due to the lack of data (complete 18S rDNA sequences) on Diplopoda, Symphyla, and Pauropoda, and do not let us establish their phylogenetic relationships with other arthropods. Other molecular studies on arthropod phylogeny show Myriapoda and Chelicerata as the result of the first branching processes in arthropods phylogeny (Lake 1990; Ballard et al. 1992; Wheeler, Cartwright, and Hayashi 1993). As first shown by Turbeville et al. (1991) and Wheeler, Cartwright, and Hayashi (1993), Chelicerata turns out to be a monophyletic group.

These data suggest that phylogenetic relationships derived from morphological data within arthropods should be reviewed, and the existence of a sole arthropodization process as the only way to Arthropoda should be questioned.

Acknowledgments

We would like to thank Phillip Newmark (Departament de Genètica, Universitat de Barcelona), G. Hor-

miga (Department of Entomology, NMNH, Washington), M. A. Arnedo (Departament de Biologia Animal, Universitat de Barcelona), and two anonymous reviewers for their helpful comments and discussion. We acknowledge E. Dominguez-Puigjaner (CID, CSIC, Barcelona) for her help in cloning. This research was supported from DGICYT PB90-0477 and from 2192-PGC 94A (Generalitat de Catalunya).

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- MANOLO GOUY, reviewing editor
- Accepted August 9, 1995