Molecular Phylogeny of the Major Arthropod Groups Indicates Polyphyly of Crustaceans and a New Hypothesis for the Origin of Hexapods

Jerome C. Regier and Jeffrey W. Shultz†*

*Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute; and †Department of Entomology, University of Maryland at College Park

A phylogeny of the arthropods was inferred from analyses of amino acid sequences derived from the nuclear genes encoding elongation factor-1α and the largest subunit of RNA polymerase II using maximum-parsimony, neighbor-joining, and maximum-likelihood methods. Analyses of elongation factor-1α from 17 arthropods and 4 outgroup taxa recovered many arthropod clades supported by previous morphological studies, including Diplopoda, Myriapoda, Insecta, Hexapoda, Branchiopoda (Crustacea), Araneae, Tetrapulmonata, Arachnida, Chelicerata, and Malacostraca (Crustacea). However, counter to previous studies, elongation factor-1α placed Malacostraca as sister group to the other arthropods. Branchiopod crustaceans were found to be more closely related to hexapods and myriapods than to malacostracan crustaceans. Sequences for RNA polymerase II were obtained from 11 arthropod taxa and were analyzed separately and in combination with elongation factor-1α. Results from these analyses were concordant with those derived from elongation factor-1α alone and provided support for a Hexapoda/Branchiopoda clade, thus arguing against the monophyly of the traditionally defined Atelocerata (Hexapoda + Myriapoda).

Introduction

Arthropods offer many opportunities for addressing fundamental issues in evolutionary biology, as they encompass an unparalleled range of structural and taxonomic diversity (Manton 1977), have a rich and ancient fossil record (Gould 1989; Wills, Briggs and Fortey 1994), and have emerged as a favored model system for studies of morphogenesis (Patel 1994; Averof and Akam 1995; Panganiban et al. 1995). Exploration and synthesis of such information requires a reliable phylogenetic framework, but evolutionary relationships among the major arthropod lineages remain controversial. Our review of recent work on the morphological and molecular systematics of arthropods reveals substantial disagreement in the phylogenetic reconstructions offered by the two types of data (fig. 1) as well as nearly exclusive dependence by molecular systematists on ribosomal nucleotides, especially nuclear small-subunit ribosomal DNA. In an attempt to generate additional molecular characters for use in resolving arthropod phylogeny, we developed two conserved nuclear protein-coding genes, namely elongation factor-1α (EF-1α) and the largest subunit of RNA polymerase II (POLII). Recent studies have indicated that amino acid sequences of EF-1α and POLII have evolved at rates appropriate for resolving ancient phylogenetic events, such as those that gave rise to the extant metazoan phyla and classes (Cammarano et al. 1992; Friedlander, Regier, and Mitter 1992, 1994; Hasegawa et al. 1993; Kojima et al. 1993). Consequently, we generated 1,093 bp of EF-1α-coding sequence from each of 17 arthropod taxa and 4 nonarthropods, and added the published sequence from the branchiopod crustacean *Artemia salina.* We also generated 582 bp of POLII-coding sequence from 10 of the above arthropods and, again, combined them with the published sequence from *Artemia salina.* The alignments of EF-1α and POLII were unambiguous, and indels were completely absent in the arthropods. Our phylogenetic analyses were based on 364 inferred amino acids of EF-1α and 194 inferred amino acids of POLII. EF-1α was analyzed separately and in combination with the more rapidly evolving POLII using several tree-building algorithms, all of which yielded highly concordant results regarding the relationships among myriapods, hexapods, and branchiopod crustaceans. We conclude that hexapods may be more closely related to branchiopod crustaceans than to myriapods and, from analyses of the EF-1α data set, that Crustacea may be polyphyletic, as the malacostracan crustaceans appear to form the sister group to the other arthropods in the study.

Abbreviations: EF-1α, elongation factor-1α; POLII, largest subunit of RNA polymerase II; PCR, polymerase chain reaction; RT-PCR, reverse transcription/polymerase chain reaction.

Key words: Arthropoda, Atelocerata, Crustacea, molecular systematics, phylogeny, elongation factor-1α, RNA polymerase II.

Address for correspondence and reprints: Jerome C. Regier, Center for Agricultural Biotechnology, University of Maryland, Plant Sciences Building, College Park, Maryland 20742. E-mail: regier@glue.umd.edu.

Mol. Biol. Evol. 14(9):902–913. 1997

Background

The main phylogenetic problems left by the last century of morphological research on arthropods include a long-standing debate about arthropod polyphyly and the precise relationships among the traditionally recognized arthropod subphyla, namely Chelicerata, Crustacea, and Atelocerata. The possibility that Arthropoda are polyphyletic was promoted most aggressively by Manton (1973, 1977), who argued that the arthropod body plan evolved several times from nonarthropod ancestors and, thus, that arthropods represent a grade rather than a monophyletic group. Specifically, Manton placed Atelocerata (=Hexapoda + Myriapoda) and Onychophora in the "phylum" Uniramia based on the supposition that these groups lack multiramous appendages and have mandibles derived from "whole limbs" rather than limb bases. She also erected separate "phyla" to accommodate chelicerates and crustaceans. Manton's scenario-

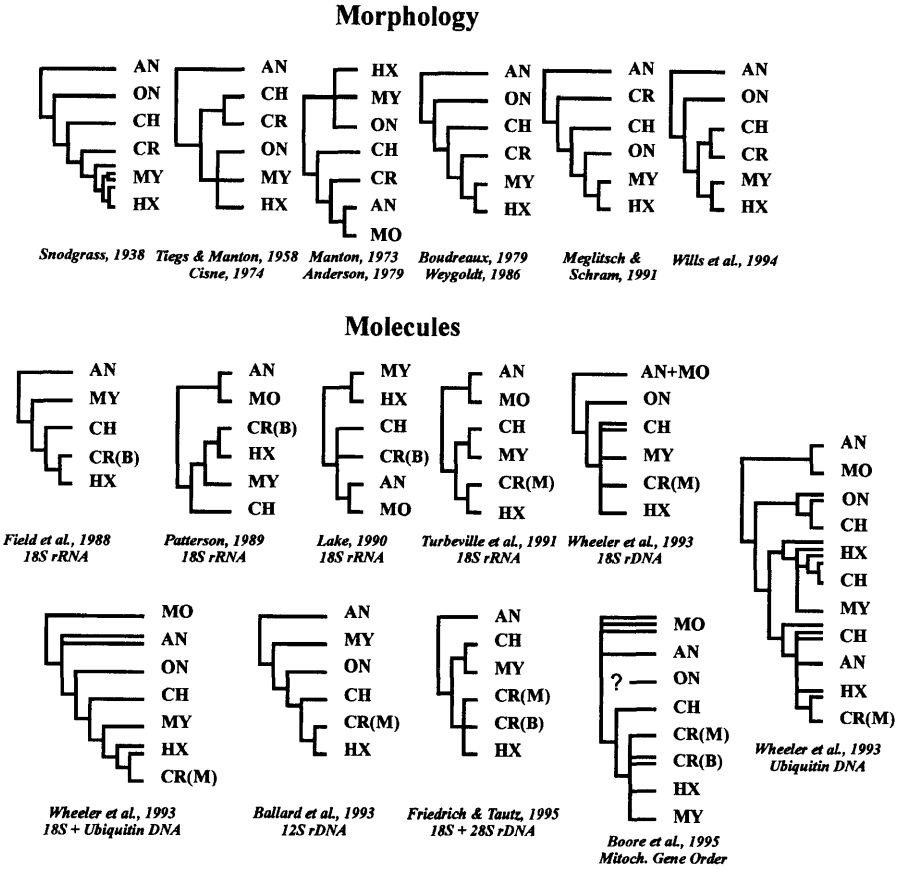

FIG. 1.—Hypotheses of relationships among arthropods as presented in previous studies. AN, Annelida; ON, Onychophora; CH, Chelicerata; CR, Crustacea (including Malacostraca and Branchiopoda); CR(B), Crustacea represented by Branchiopoda only; CR(M), Crustacea represented by Malacostraca only; MY, Myriapoda; HX, Hexapoda; MO, Mollusca.

based phylogenetic hypotheses and the evidence used to support them have been criticized repeatedly (e.g. Platnick 1978; Boudreaux 1979; Kristensen 1981; Weygoldt 1986; Kukalová-Peck 1992; Shear 1992; Wheeler, Cartwright, and Hayashi 1993), but arthropod polyphyly, in one form or another, retains support among many workers (Anderson 1979; Bergström 1979; Whittington 1985; Willmer 1990; Ballard et al. 1992; Budd 1993). Indeed, reconstructions of fossils from the Burgess Shale and related faunas indicate that many supposed arthropod synapomorphies (e.g., compound lateral and multiple medial eyes, claws, sclerites, jointed legs, multiramous appendages) occurred in a mosaic of wormlike and arthropodlike taxa (Whittington 1985; Gould 1989; Hou, Ramsköld, and Bergström 1991; Ramsköld 1992; Budd 1993). Although Wheeler, Cartwright, and Hayashi (1993) dismissed hypotheses of arthropod polyphyly as being based on "single characters, non-empirical notions of character transformation and plesiomorphy," paleontological evidence is not inconsistent with multiple pathways of arthropodization, if not arthropod polyphyly per se, even if Manton used what would now be regarded as questionable methods when first proposing her versions of these hypotheses.

The contentious issue of polyphyly aside, the question remains as to the relationships of the arthropod subphyla. Assuming that each traditionally recognized subphylum is monophyletic, three relationships are possible. Monophyly of Atelocerata plus Chelicerata was proposed in the 19th century based on characters associated with terrestriality (tracheae, malpighian tubules, etc.), but this scenario was gradually abandoned and now has little support, although Meglitsch and Schram (1991) have resurrected the hypothesis using a different set of characters. Most neontologists follow Snodgrass (1938) in uniting Atelocerata and Crustacea within a monophyletic Mandibulata, but many paleontologists and some neontologists combine Chelicerata and Crustacea within a monophyletic Schizoramia based on the supposition that these lineages have multiramous appendages and that this condition is derived rather than primitive (Cisne 1974; Bergström 1979; Wills, Briggs, and Fortey 1994). However, given inadequate demonstration of atelocerata or crustacean monophyly, it is possible that relationships of the traditional subphyla are even more complex than is generally supposed. The possibility that certain elements of Crustacea are more closely related to elements of Atelocerata than to other crustaceans or that Hexapoda alone is sister to Crustacea cannot be convincingly eliminated by current morphological, developmental genetic or molecular evidence (Wägele 1993; Averof and Akam 1995; Friedrich and Tautz 1995; Osorio, Averof, and Bacon 1995; Telford and Thomas 1995).

Given the paucity of noncontroversial morphological characters, many workers are now turning to molecular sequence data to resolve arthropod relationships. Most attempts to resolve subphylum relationships within Arthropoda using molecular evidence have focused on small-subunit nuclear (18S) ribosomal nucleotides. These sequences have both highly conserved and highly variable regions, but exclusion of ambiguously aligned regions prior to phylogenetic analysis has been a typical procedure. Field et al. (1988) examined 18S rRNA in four arthropods within a broad study of metazoan phylogeny. Their distance-based analysis indicated that Arthropoda are monophyletic, and they presented a pectinate tree (fig. 1) with a millipede (*Spirobolus*) emerging basally followed by a chelicerate (*Limulus*), a crustacean (*Artemia*), and a hexapod (*Drosophila*), suggesting that Atelocerata are not monophyletic. Field et al. (1988) and others (e.g., Lake 1990) regarded these results as unreliable due to rapid evolution in the mandibulate lineages, inadequate taxon sampling, and incongruence with traditional relationships. Patterson (1989) reanalyzed the data using maximum-parsimony and found a pectinate topology in Arthropoda (fig. 1) with the chelicerate emerging basally followed by the millipede, the crustacean, and the hexapod, which also suggests that Atelocerata are not monophyletic. Rate-invariant analysis conducted by Lake (1990) on the same data indicated that Arthropoda are paraphyletic within protostomes, but he questioned this result due to problems of inadequate taxon sampling and long branches in mandibulates. Turbeville et al. (1991) added 18S sequences from several taxa, excluded the long-branched *Artemia* and *Drosophila,* and analyzed the expanded data set with a variety of methods. They concluded that Arthropoda and Chelicerata are monophyletic, but relationships among the other arthropod lineages were only weakly supported. Friedrich and Tautz (1995) expanded the taxon sample, combined 18S and 28S rDNA, and analyzed the data with a variety of methods, including maximum likelihood, maximum parsimony, and neighbor-joining. Their results indicated that Chelicerata are sister to Myriapoda and that Crustacea and Hexapoda form a monophyletic group, but it was unclear whether all crustaceans or only branchiopod crustaceans (*Artemia*) were sister to hexapods.

Ballard et al. (1992) examined arthropod phylogeny using small-subunit mitochondrial (12S) ribosomal DNA. Their analysis of 34 species included an intensive sampling of flies (Diptera) and Australian onychophorans, but other major lineages were represented by one to three species. Maximum-parsimony analysis discovered 144 minimal-length trees, and a nonparametric statistical method (T-PTP) (Faith 1991; Faith and Cranston 1991) was used to determine which topologies showed statistically significant covariation among characters with respect to a population of trees derived from multiple randomizations of the data. The T-PTP tree is pectinate with Myriapoda arising basally followed by Onychophora, Chelicerata, Crustacea, and Hexapoda (fig. 1). This topology suggests that Arthropoda are paraphyletic and that Atelocerata are not a natural group. The results from the analysis conducted by Ballard et al. (1992) are problematic because (1) 12S rDNA is a rapidly evolving gene that is generally regarded as useful only for more recent phylogenetic divergences (Mindell and Honeycutt 1990), (2) the tree was selected using a controversial statistical procedure (Carpenter 1992; Källersjö et al. 1992), (3) the result is highly sensitive to the specific alignment procedures used to establish orthology (Wägele and Stanjek 1995), and (4) the taxon sample is highly unbalanced (over 60% of arthropods sampled were dipterans), a situation favorable to long-branch attraction among the underrepresented lineages.

In an attempt to resolve phylogenetic relationships among the main arthropod lineages using all available evidence, Wheeler, Cartwright, and Hayashi (1993) compared and combined evidence from morphology with sequence data from 18S rDNA and ubiquitin-coding DNA. Morphological characters were gleaned from the literature review (especially Weygoldt 1986), and parsimony analysis revealed the topology (consistency index [CI] = 0.84) favored by many recent morphology-based studies (i.e., Chelicerata are sister to Mandibulata, Crustacea are sister to Atelocerata). The cladogram resulting from analysis of 18S rDNA (CI = 0.60) was consistent with monophyly of Arthropoda, Chelicerata, Crustacea, Myriapoda, and Hexapoda but did not resolve relationships among these lineages (fig. 1). Relationships expressed in the ubiquitin-based tree (CI = 0.31) were regarded as essentially unresolved (fig. 1), a result that is perhaps not surprising given the existence of concerted evolution within the gene (Sharp and Li 1987; Tan, Bishoff, and Riley 1993). Results from maximum-parsimony analysis of the molecular evidence alone are largely congruent with results from other molecular analyses in reconstructing hexapods and crustaceans as a monophyletic group exclusive of myriapods. The cladogram resulting from combining all data was consistent with the morphology-based tree, which is largely congruent with previous morphology-based hypotheses, especially Snodgrass (1938), Boudreaux (1979), and Weygoldt (1986) (fig. 1). Wheeler, Cartwright, and Hayashi (1993) reasoned that phylogenetic history is the only feature common to such diverse data sets and thus regarded their result as supporting the total-evidence approach (Kluge 1989). However, it is also possible that homoplasy within the morphological characters is artificially low given the typological approaches used in the original studies, thus giving undue influence to the morphological data in the combined analysis.

Materials and Methods

Taxon Sampling

Seventeen species of arthropods representing the four major groups (Chelicerata, Crustacea, Hexapoda, Myriapoda) and two species each from two outgroup phyla (Annelida, Mollusca) were sampled for analysis of EF-1α sequences, and 11 of the arthropods were sampled for POLII. Linnean names, common names, and classifications of these taxa are listed in table 1. Specimens either were alive until frozen at −85°C or were

Table 1
Species Sampled

Species Name	Common Name	Higher Classification	GenBank Accession No. EF-1α	GenBank Accession No. POLII
Arthropods				
Hanseniella sp.	Symphylan	Myriapoda/Symphyla	U90049	
Scutigera coleoptrata	Centipede	Myriapoda/Chilopoda	U90057	U90042
Narceus americanus	Millipede	Myriapoda/Diplopoda	U90053	U90039
Polyxenus fasciculatus	Millipede	Myriapoda/Diplopoda	U90055	
Periplaneta americana	Cockroach	Hexapoda/Insecta	U90054	U90040
Pedetontus saltator	Bristletail	Hexapoda/Insecta	U90056	U90041
Tomocerus sp.	Springtail	Hexapoda/Collembola	U90059	
Artemia salina[a]	Brine shrimp	Crustacea/Branchiopoda	X03349	U10331
Triops longicaudatus	Tadpole shrimp	Crustacea/Branchiopoda	U90058	U90043
Aphonopelma chalcodes	Tarantula	Chelicerata/Arachnida	U90045	U90035
Dysdera crocata	Spider	Chelicerata/Arachnida	U90047	U90036
Mastigoproctus giganteus	Vinegaroon	Chelicerata/Arachnida	U90052	U90038
Dinothrombium pandorae	Velvet mite	Chelicerata/Arachnida	U90048	
Vonones ornata	Harvestman	Chelicerata/Arachnida	U90060	U90044
Limulus polyphemus	Horseshoe crab	Chelicerata/Xiphosura	U90051	U90037
Armadillidium vulgare	Pillbug	Crustacea/Malacostraca	U90046	
Libinia emarginata	Crab	Crustacea/Malacostraca	U90050	
Nonarthropods				
Acmaea testudinalis	Limpet	Mollusca/Gastropoda	U90061	
Chaetopleura apiculata	Chiton	Mollusca/Polyplacophora	U90062	
Hirudo medicinalis	Leech	Annelida/Hirudinea	U90063	
Nereis virens	Clam worm	Annelida/Polychaeta	U90064	

[a] Analyzed but not sequenced in this study.

stored in 100% ethanol at ambient temperature for up to 2 weeks prior to final storage at −85°C.

Primer Development, PCR Amplification, and DNA Sequencing

The 20 new EF-1α amino acid sequences (approximately 364 residues each, 79% of total coding sequence) analyzed for this study were inferred from the nucleotide sequences of an approximately 1,063-bp DNA fragment amplified by the polymerase chain reaction (PCR). GenBank accession numbers for these sequences are listed in table 1, together with the already available sequence for *Artemia salina.* Nine different oligonucleotide primers, defined by comparison with published sequences, were used for template amplification by PCR (table 2). Templates consisted of preparations of total nucleic acids (DNA/RNA Isolation Kit, Amersham Corp., Arlington Heights, Ill.). Initially, the entire 1,093-bp fragment (PCR primer sequences not included) or a slightly larger, 1,102-bp, fragment was amplified by reverse transcription/polymerase chain reaction (RT-PCR) (Perkin-Elmer, Foster City, Calif.) using primer pairs 40.71F/41.21RC and 40.6F/41.21RC, respectively. RT-PCR typically followed a touchdown protocol (Hecker and Roux 1996), in which the annealing temperature decreased from 55°C to 45°C over 25 cycles, followed by 14 cycles at 45°C. The desired fragment was gel-isolated (Wizard PCR Preps, Promega Corp., Madison, Wisc.). Subsequently, nested subfragments that together span the entire 1,063 bp were reamplified by PCR using the following primer pairs: 40.71F/45.71RC, 40.71F/52RC, 45.71F/53.5RC, 52F/41.2RC, and 52.4F/41.2RC. PCR followed a standard three-step protocol in which annealing temperatures were constant, usually 50–55°C, depending on particular templates. The desired fragment was again gel-isolated.

The 10 new POLII amino acid sequences (194 residues each, ~10% of total coding sequence) analyzed for this study were inferred from the nucleotide sequences of a 583-bp DNA fragment amplified by PCR. GenBank accession numbers for these sequences are listed in table 1, together with the already available sequence for *Artemia salina.* Six different primer pairs were used for PCR amplification. Initially, highly overlapping 604-, 637-, and 658-bp fragments (PCR primer sequences not included) were amplified by RT-PCR as for EF-1α, using primer pairs 29.3F/29.82RC, 29.21F/29.8RC, and 29.21F/29.82RC, respectively. The desired fragment was gel-isolated from the best of the three initial reactions. Using this as a template, a nested 583-bp fragment was amplified by PCR using primer pair 29.3F/29.8RC. The appropriately sized fragment was gel-isolated and sequenced from both ends. Internal primers 29.6F and 29.6RC were used for amplifying subfragments and for confirming internal sequences. All primer sequences also included either M13REV or M13-21 sequences (not shown) at their 5′ ends to facilitate automated sequencing on an Applied Biosystems DNA Sequencer model 373A with Stretch upgrade.

Data Analysis

Automated DNA sequencer chromatograms were edited and contigs were assembled using the TED and XDAP software programs within the Staden package (Dear and Staden 1991). Sequences from multiple species were aligned, and amino acid data sets were con-

Table 2
Sequences of Oligonucleotide Primers (5′–3′) Used in this Study

Primer	Sequence
	EF-1α
40.6F	AT(CT) GA(AG) AA(AG) TT(CT) GA(AG) AA(AG) GA(AG) GC [206]
40.71F	TCN TT(TC) AA(AG) TA(TC) GCN TGG GT [245]
45.71F	GTN G(GC)N GTI AA(CT) AA(AG) ATG GA [536]
45.71RC	TCC AT(TC) TT(GA) TTN ACN (CG)CI AC [517]
52F	CA(AG) GA(CT) GTN TA(CT) AA(AG) AT(ACT) GG [839]
52RC	CC(AGT) AT(CT) TT(AG) TAN AC(AG) TC(CT) TG [820]
52.4F	TCN GTN GA(AG) ATG CA(CT) CA(CT) G [958]
53.5RC	AT(AG) TG(ACG) G(AC)I GT(AG) TG(AG) CA(AG) TC [1153]
41.2RC	TG(CT) CTC AT(AG) TC(AGT) CG(ACG) AC(AG) GC(AG) AA [1339]
	POLII
29.21F	TT(CT) CA(CT) GCN ATG GGN GG [2264]
29.3F	GCN GA(AG) ACN GGI TA(CT) ATI CA [2318]
29.6F	TGG AA(CT) G(CT)I CA(AG) AA(AG) AT(ACT) TT [2717]
29.6RC	AA(AGT) AT(CT) TT(CT) TGI (AG)C(AG) TTC CA [2698]
29.8RC	GAN A(AG)I C(GT)(AG) AA(CT) TC(CT) TC [2902]
29.82RC	A(AG)C CAN TC(AG) AAI GC(CT) TC [2923]

NOTE.—N, all four naturally occurring nucleotides; I, inosine. All primers included an M13 sequence (not shown) at the 5′ end to facilitate automated sequencing (Cho et al. 1994). Primer names ending in F identify forward primers, which bind to the antisense strand of DNA. Primer names ending in RC identify reverse-complement primers. The number in brackets at the 3′ end of each primer sequence refers to its nucleotide position relative to the EF-1α or POLII sequence (sense strand) from *Artemia salina* (GenBank accession nos. X03349 and U10331, respectively).

structed using the Genetic Data Environment software package (version 2.2; Smith et al. 1994). Optimal alignment of both EF-1α and POLII sequences required no indels in the ingroup. However, the EF-1α sequence from *Nereis* contained a 6-nt in-frame segment not present in the ingroup, and *Acmaea* contained a 6-nt and a 9-nt in-frame segment not present in the ingroup. Interestingly, both 6-nt segments were in the same location. The segments not present in arthropods were removed from the data set for purposes of phylogenetic analysis. Ambiguous amino acid characters were coded as "X" and represent only 10 out of 7,644 characters for the EF-1α data set and 4 out of 2,134 characters for POLII.

Parsimony analysis performed with PAUP 3.2 (Swofford 1993) employed a heuristic search, using TBR branch swappings with random (100 sequence-addition replications), simple, and closest taxon additions. Bootstrap values (3,000 replicates) were calculated in a test version of PAUP* 4.0 (test versions kindly provided by D. L. Swofford), using 10 random sequence-addition replicates and TBR branch-swapping. Decay indices (Bremer 1988; Donoghue et al. 1992) were calculated by constructing constraint trees in PAUP 3.2. Neighbor-joining analyses with bootstrap values (3,000 replicates) were also calculated from PAUP* 4.0. Maximum-likelihood estimates were performed on a Sun Sparcstation using the *protml* program within the software package MOLPHY (version 2.2) (Adachi and Hasegawa 1994). An exhaustive search strategy was not possible with the number of taxa in our analysis. Instead, we used a protocol described in the MOLPHY documentation as "star decomposition." Six different evolutionary models of amino acid substitution were tested. The Dayhoff and JTT models assume a Markov model for amino acid substitutions based on the empirical transition matrices compiled by Dayhoff, Schwartz, and Orcutt (1978) and by Jones, Taylor, and Thornton (1992), respectively. The Poisson model assumes that amino acids are replaced by all other amino acids with equal probability. The "F" option in MOLPHY for each of these three models further specifies that the equilibrium frequencies of amino acids match the protein under analysis rather than the average of the databases (Dayhoff and JTT models) or being equally distributed (Poisson model). For the EF-1α only data set, all six models yield identical topologies, although the Dayhoff model yields the lowest Akaike Information Criterion (AIC = $-2 \times$ (log-likelihood) $+ 2 \times$ (no. of free parameters)) (Sakamoto, Ishiguro, and Kitagawa 1986). Hasegawa et al. (1993) prefer a model that minimizes the Akaike Information Criterion. Thus, log-likelihood values shown for the EF-1α data set in table 3 are based on the Dayhoff model. For the combined EF-1α + POLII data set, all six models yield identical topologies except for the relative order of two arachnids—*Vonones* and *Mastigoproctus*. The JTT model has the lowest Akaike Information Criterion, and log-likehood values shown for the combined data set in table 3 are based on this model. Bootstrap resampling probabilities are based on the RELL (resampling of estimated log-likelihood) method (Hasegawa and Kishino 1994), as implemented in MOLPHY.

Results

Phylogenetic Analysis of EF-1α

EF-1α amino acid sequences from 17 arthropods plus outgroups were analyzed by maximum parsimony with all characters uniformly weighted (fig. 2*A*). Two minimum-length trees were recovered that differ only in their outgroup relationships (fig. 2). Arthropod groups recovered by EF-1α that are strongly supported by morphology included Diplopoda, Insecta, Hexapoda, Atel-

Table 3
Maximum-Likelihood (ML) Estimates of EF-1α and EF-1α + POLII Gene Trees

Gene Tree	Difference from ML Tree	Δl_i + SE	P_i
EF-1α	=ML tree (Branchiopoda sister to Atelocerata, Malacostraca sister to other Arthropoda)	(−4,288.7)	0.549
EF-1α	Branchiopoda sister to Hexapoda	−2.0 ± 8.2	0.385
EF-1α	Malacostraca sister to Chelicerata	−12.7 ± 8.0	0.025
EF-1α	Crustacea sister to Hexapoda	−25.0 ± 16.0	0.034
EF-1α	Crustacea sister to Atelocerata	−22.8 ± 11.6	0.007
EF-1α	Crustacea sister to other Arthropoda	−32.5 ± 12.5	0.000
EF-1α	Crustacea sister to Chelicerata	−32.5 ± 12.5	0.000
EF-1α + POLII ...	=ML tree (Branchiopoda sister to Hexapoda)	(−4,362.9)	0.993
EF-1α + POLII ...	Branchiopoda sister to Atelocerata	−34.3 ± 13.9	0.007
EF-1α + POLII ...	Branchiopoda sister to Myriapoda	−38.2 ± 12.7	0.000

NOTE.—Topologies with the highest likelihoods ("ML tree") are briefly described in parentheses (column 2) along with their log-likelihood values (column 3). The topology of the ML tree for EF-1α is identical to the maximum-parsimony (MP) tree shown in figure 2*A* except that *Vonones* and *Dinothrombium* are reversed. The topology of the ML tree for EF-1α + POLII is identical to that of the MP and neighbor-joining (NJ) trees shown in figure 4. Log-likelihood differences from the ML estimate along with their standard errors (column 3) and bootstrap resampling probabilities for varying topologies (column 4) are listed. A log-likelihood value that differs from another likelihood value by less than the variance of that difference is considered indistinguishable (for example, that of the first two EF-1α gene trees).

ocerata, Branchiopoda (Crustacea), Araneae, Tetrapulmonata, Arachnida, Chelicerata, and Malacostraca (Crustacea). EF-1α also recovered Myriapoda, supported by many but not all previous morphological studies. Within Myriapoda, EF-1α placed Symphyla as sister to Chilopoda, with this group in turn sister to Diplopoda. The most novel finding based on analysis of EF-1α is that Crustacea are reconstructed as being polyphyletic. Malacostracan crustaceans form a sister group to all other arthropods sampled and are separated from branchiopod crustaceans by two nodes on the parsimony tree.

Two measures of branch support on the parsimony tree are displayed (fig. 2*A*)—bootstrap values and decay indices. The following groups are strongly supported, with bootstrap values above 90% and decay indices equal to approximately 1% of total tree length or greater: Diplopoda, Myriapoda, Branchiopoda, Arachnida, Chelicerata, and Malacostraca. Symphyla + Chilopoda and Araneae have moderate support (88% bootstrap values), while support for Atelocerata is very low (27%). In fact, Atelocerata become paraphyletic in parsimony trees only one step longer, with Branchiopoda now sister to Hexapoda (unpublished observation). A branchiopod/hexapod grouping is recovered by neighbor-joining (fig. 2*B*). This and a reversed placement of *Dinothrombium* and *Vonones* (both arachnids) are the only differences between the parsimony and neighbor-joining trees. The maximum-likelihood tree is identical to the parsimony tree with the exception that *Dinothrombium* and *Vonones* are resolved as for neighbor-joining (table 3). However, the likelihood value for the neighbor-joining tree, in which Branchiopoda are sister to Hexapoda, is not significantly different from the maximum-likelihood result. Thus, while EF-1α resolves many relationships, it is not strongly informative of relationships among myriapods, hexapods, and branchiopods.

Pairwise divergence values across groups generally increase with increasing phylogenetic depth (fig. 2*A*), as would be expected of a sequence data set well removed from saturation. Pairwise divergence values across arthropods increase from 13.8% when Malacostraca are excluded to 22.1% when they are included. This latter distance approximately matches that among the three phyla, consistent either with EF-1α approaching saturation at that taxonomic level or with rapid radiation of the phyla. The distance separating Branchiopoda and Malacostraca (22%) is much greater than that separating the other major arthropod groups (table 4, unpublished observation).

Phylogenetic Analysis of POLII

Partial POLII amino acid sequences have been analyzed for a subset of 11 arthropod taxa (table 1). The POLII amino acid sequence evolves several times faster than EF-1α, based on a direct comparison of pairwise divergence values across groups of different taxonomic ranks (table 4). Analyzed by maximum parsimony, POLII alone resolves several arthropod groups and strongly supports Branchiopoda + Hexapoda and Branchiopoda + Hexapoda + Myriapoda with 98% bootstrap values (fig. 3*A*). These same groups are recovered by neighbor-joining analysis, although *Limulus,* which represents the earliest branching in chelicerates, now splits arachnids (fig. 3*B*). Basal positioning of *Limulus* within Chelicerata is included among the most parsimonious solutions (fig. 3*A,* unpublished observation).

Combined Analysis and EF-1α and POLII

When the EF-1α and POLII amino acid data sets are combined and analyzed by parsimony (fig. 4*A*), neighbor-joining (fig. 4*B*), and maximum likelihood (table 3), EF-1α + POLII yield trees of identical topology. Groups recovered are Myriapoda, Insecta, Branchiopoda, Araneae, Thelyphonida, Arachnida, and Chelicerata. All three methods strongly support grouping Insecta with Branchiopoda rather than with Myriapoda.

Discussion

Comparison of Results from EF-1α, POLII, and rDNA

While rDNA has been widely used for resolving ancient phylogenetic splits (Field et al. 1988; Patterson

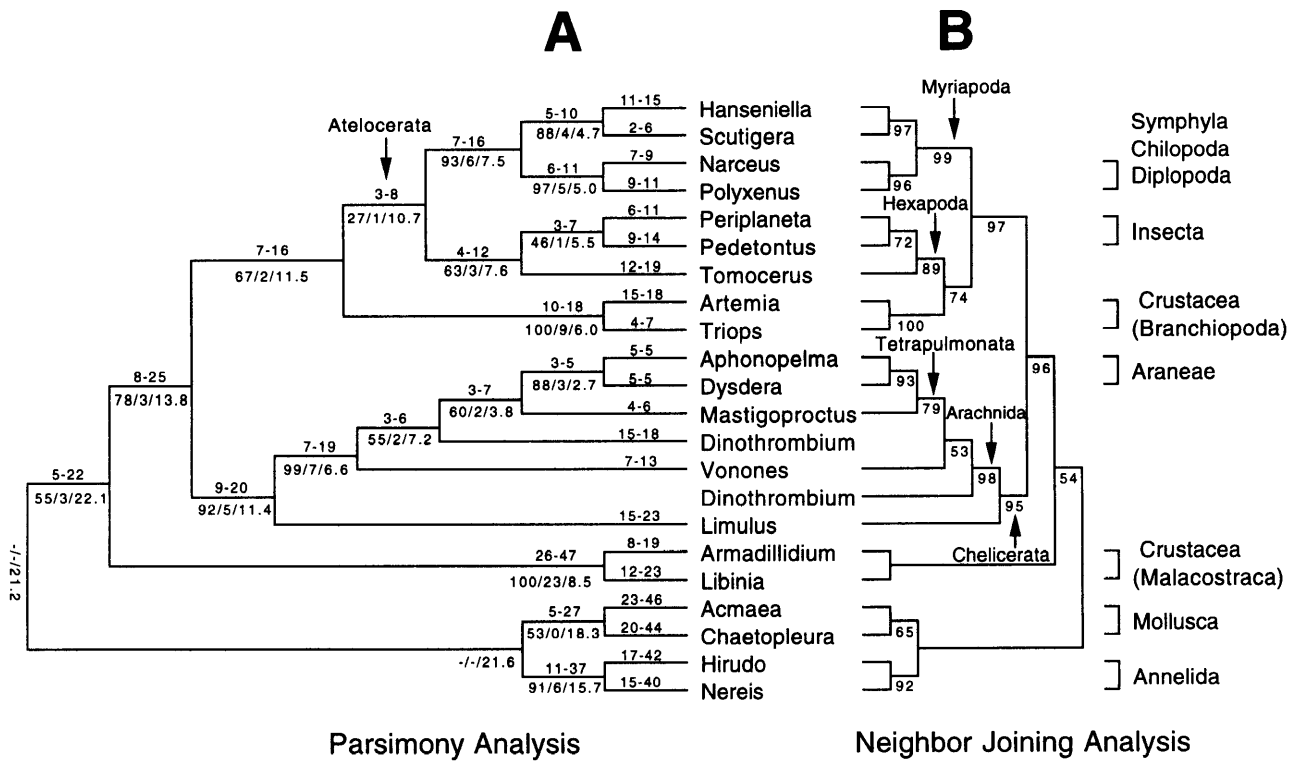

FIG. 2.—Phylogenetic relationships of arthropod taxa based on maximum-parsimony and neighbor-joining analyses of EF-1α amino acid sequences. *A*, One of two most-parsimonious trees (consistency index = 0.6331, retention index = 0.6255, tree length = 537, total number of characters = 364, number of parsimony-informative characters = 112). The other maximum-parsimony tree (not shown) placed *Chaetopleura* as sister to all other outgroup taxa. Terminal taxa are identified by genus only. Minimum numbers of character changes followed by maximum numbers of character changes under all character optimizations are placed above branches. Bootstrap values followed by decay indices followed by average, uncorrected pairwise amino acid divergence values across pairs of clades are placed below branches. *B*, The neighbor-joining tree with bootstrap values placed below branches.

1989; Lake 1990; Wheeler, Cartwright, and Hayashi 1993; Friedrich and Tautz 1995; Giribet et al. 1996), recent studies indicate that amino acid sequences of EF-1α and POLII may also be useful (Cammarano et al. 1992; Hasegawa et al. 1993; Kojima et al. 1993; Friedlander, Regier, and Mitter 1994). The current study supports this contention within arthropods by demonstrating that EF-1α and POLII recover separately (figs. 2 and 3) and in combination (fig. 4) numerous clades strongly supported by morphological and developmental characters. Clade recovery is robust to varying methods of analysis (parsimony, neighbor-joining, maximum likelihood), and sequence alignments are unambiguous. Bootstrap support for many clades is high for at least one of the two data sets, suggesting that conflicting alternative resolutions are not strongly supported. Overall high signal quality is further supported by the observation that, within Arthropoda, average pairwise amino acid divergence values generally increase with phylogenetic depth (figs. 2 and 4).

The ability of EF-1α and POLII to capture phylogenetic signals within Arthropoda can be compared rather directly with rDNA based on a recent study (Friedrich and Tautz 1995) of 10 arthropod taxa plus outgroups

Table 4
Comparison of Pairwise Divergence Values for Amino Acid Sequences from EF-1α and POLII

Taxonomic Group[a]	EF-1α	POLII
Myriapoda	6.9	18.3
Hexapoda	5.5	13.9
Branchiopoda	6.0	22.7
Araneae	2.7	16.6
Chelicerata	12.0	17.3
Hexapoda + Branchiopoda	10.2	22.3
Hexapoda + Branchiopoda + Myriapoda	10.9	26.5
Hexapoda + Branchiopoda + Myriapoda + Chelicerata	13.8	29.9

NOTE.—Pairwise divergence values are uncorrected for multiple hits.

[a] Myriapoda = *Scutigera, Narceus*; Hexapoda = *Periplaneta, Pedetontus*; Branchiopoda = *Triops, Artemia*; Araneae = *Aphonopelma, Dysdera*; Chelicerata = *Aphonopelma, Dysdera, Limulus.*

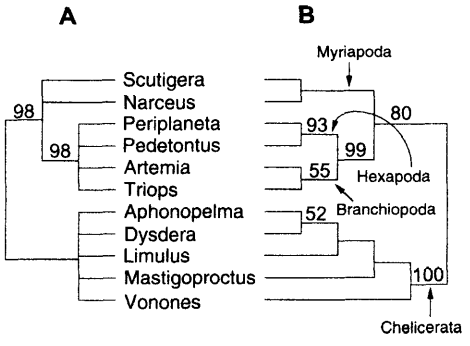

FIG. 3.—Phylogenetic relationships of arthropod taxa based on maximum-parsimony and neighbor-joining analyses of POLII amino acid sequences. *A*, A strict consensus of 12 minimum-length parsimony trees (consistency index = 0.7621, retention index = 0.7078, tree length = 269, total number of characters = 194). Bootstrap values greater than 50% are placed above branches. *B*, The neighbor-joining tree with bootstrap values placed above branches. Chelicerata are used as outgroup for the other arthropod groups.

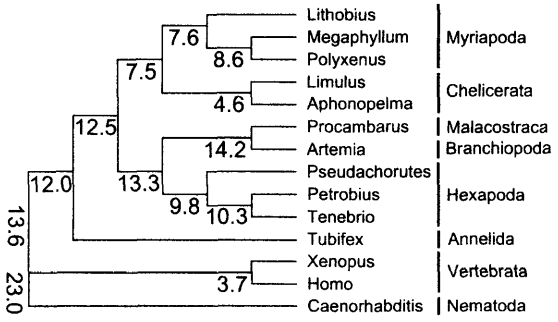

FIG. 5.—Maximum-likelihood tree obtained by Friedrich and Tautz (1995) upon analysis of an 18S + 28S data set. Pairwise divergence values across groups are placed below branches subtending the groups (our calculations) and are uncorrected for multiple hits. The genus name *Eurypelma*, used by Friedrich and Tautz, has been replaced by *Aphonopelma*, used elsewhere in this report.

with taxonomic distributions similar to those in the present study. The analyzed data set consisted of 1,853 nucleotides from 18S and 28S, conservatively aligned from a total of 3,211 nucleotides so as to eliminate gaps and ambiguous regions. The published tree for maximum-likelihood analysis (redrawn in fig. 5) recovers Diplopoda, Myriapoda, Chelicerata, Insecta, and Hexapoda. While this tree, (figure 1 in Friedrich and Tautz 1995), displays a monophyletic Crustacea (one branchiopod, one malacostracan), their legend states that bootstrap analysis supports a paraphyletic Crustacea and a sister group relationship between Branchiopoda and Hexapoda, the latter result being similar to our finding with EF-1α + POLII (figs. 3 and 4, table 3). Our parsimony analysis of the Friedrich and Tautz (1995) data set, with transversions weighted as twice transitions as recommended by the authors, yields two trees, one matching their maximum-likelihood tree (fig. 5). The second tree places a paraphyletic Myriapoda at the base of a paraphyletic Crustacea. When transversions and transitions are weighted equally, maximum parsimony recovers the second tree rather than the maximum-likelihood tree. When pairwise divergence values for the rDNA data are mapped on the preferred tree, they do not show a clear increase with phylogenetic depth (fig. 5), as was observed in the EF-1α and POLII data (figs. 2 and 4).

Implications for Arthropod Phylogeny

The conclusions that crustaceans may be polyphyletic and that hexapods may be closer to branchiopods than to myriapods are novel findings (figs. 2–4 and table 3). To date, most higher classifications of Arthropoda have assumed monophyly of Crustacea and of Atelocerata, although systematists differ in their placement of these arthropods (fig. 1). These alternative topologies are less parsimonious (table 5) and have lower likelihood (table 3) when EF-1α and POLII characters are con-

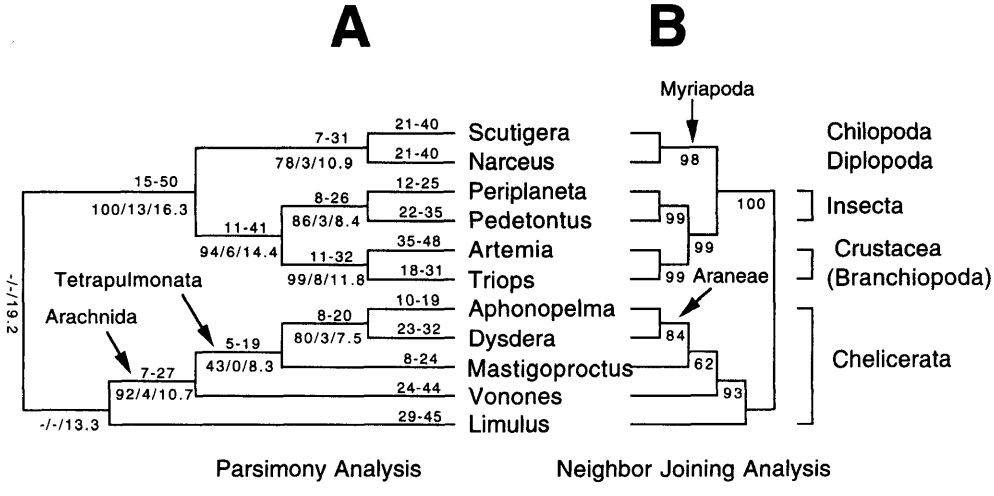

FIG. 4.—Phylogenetic relationships of arthropod taxa based on maximum-parsimony and neighbor-joining analysis of EF-1α + POLII combined amino acid sequences. *A*, One of two most-parsimonious trees (consistency index = 0.7733, retention index = 0.6667, tree length = 472, total number of characters = 558, number of parsimony-informative characters = 131). The other maximum-parsimony tree (not shown) placed *Mastigoproctus* and *Vonones* as sister taxa. The labeling scheme for branches is as in figure 2. *B*, The neighbor-joining tree with bootstrap values placed below branches. Chelicerata are used as outgroup for the other arthropod groups.

Table 5
Maximum-Parsimony (MP) Tree Lengths for Alternative Phylogenetic Hypotheses

Reference	Tree Length	Major Differences from Current Study (fig. 2*A*)
Molecular hypotheses		
Current study—MP result (fig. 2*A*)	537	
Current study—ML result (table 3)	539	*Vonones* and *Dinothrombium* reversed
Current study—NJ result (fig. 2*B*)	540	*Vonones* and *Dinothrombium* reversed; Branchiopoda sister to Hexapoda
Friedrich and Tautz (1995) (18S + 28S ML tree)	548	Crustacea sister to Hexapoda; Myriapoda sister to Chelicerata
Boore et al. (1995) (mitochondrial gene order)	543	Chelicerata sister to other Arthropoda
Wheeler, Cartwright, and Hayashi (1993) (18S + ubiquitin MP tree)	548	Crustacea split Hexapoda; Myriapoda sister to Hexapoda + Crustacea
Ballard et al. (1992) (12S MP tree)	551	Malacostraca sister to Hexapoda; Myriapoda sister to other Arthropoda
Turbeville et al. (1991) (18S NJ tree)	554	Malacostraca sister to Hexapoda; Diplopoda sister to Hexapoda + Crustacea; Acari sister to other Chelicerata
Turbeville et al. (1991) (18S MP tree)	555	Malacostraca sister to Hexapoda; Diplopoda sister to Chelicerata; Acari sister to other Chelicerata
Morphological hypotheses		
Wills, Briggs, and Fortey (1994)	548	Crustacea sister to Chelicerata
Boudreaux (1979)	548	Crustacea sister to Atelocerata
Manton (1973)	549	Crustacea sister to Annelida + Mollusca; Chelicerata sister to Crustacea + Annelida + Mollusca
Snodgrass (1938)	560	Myriapoda paraphyletic; Crustacea sister to Atelocerata

NOTE.—The 21-taxon, EF-1α data set was analyzed by maximum-parsimony after constraining the topology to published, alternative hypotheses. Groups not included in other studies, for example, the symphylan *Hanseniella* and all outgroups, were left unresolved. Unless otherwise stated, Crustacea were assumed to be monophyletic. Differences in tree length reflect both goodness of fit of the EF-1α data set to the altered topology and its degree of resolution. For example, the Boore et al. (1995) topology is minimally resolved. ML, maximum likelihood; NJ, neighbor-joining.

strained. Most neontologists recognize two principal arthropod clades—Mandibulata and Chelicerata—with the mandibulates including the sister clades Crustacea and Atelocerata. According to this scheme, mandibulates are united by having heads composed of five appendage-bearing somites, including mandibles associated with the second embryologically postoral somite (Snodgrass 1938; Boudreaux 1979; Weygoldt 1986; Wheeler, Cartwright, and Hayashi 1993). In contrast, some paleontologists recognize Schizoramia and Atelocerata (or Uniramia), with Schizoramia encompassing chelicerates and a monophyletic Crustacea. The schizoramians are characterized by multiramous rather than uniramous appendages and by chewing mouthparts presumably derived from the bases rather than tips of the head appendages (Cisne 1974; Wills, Briggs, and Fortey 1994). However, both schemes are questionable given comparative morphological, paleontological, and developmental evidence for common primitive patterns of head segmentation, gnathobasic mouthparts, and multiramous appendages in all extant arthropods, with modifications such as the chelicerate prosoma being derived from this crustacean-like ground plan (Weygoldt 1979; Shultz 1990; Kukalová-Peck 1992; Wägele 1993; Averof and Cohen 1997). Furthermore, recent cladistic analyses of morphological characters have revealed a lack of compelling synapomorphies for Crustacea, such that several workers have acknowledged the possibility that crustaceans are a para- or polyphyletic grade of primitively aquatic arthropods (Wägele 1993; Averof and Akam 1995). Indeed, characters typically used to diagnose Crustacea—two pairs of antennae, planktonic nauplius larva, epipodial gills—may be primitive features that are absent or highly modified in the largely terrestrial chelicerates, myriapods, and hexapods. Thus, given the paucity of morphological synapomorphies for Crustacea, along with the possibility that the crustacean *Bauplan* represents a grade of organization primitive for all extant arthropods, crustacean para- or polyphyly has emerged as a reasonable hypothesis. Indeed, our data are in complete accord with the hypothesis of crustacean polyphyly and are inconsistent with crustacean monophyly as well as the mandibulate and schizoramian concepts (tables 3 and 5).

Placement of crustaceans near hexapods is inconsistent with traditional classifications but is not unprecedented in molecular systematics studies. One analysis of 12S ribosomal DNA grouped hexapods and malacostracans, and placed myriapods as sister to all other arthropods plus onychophorans (Ballard et al. 1992). Nuclear ribosomal sequences have also grouped hexapods and crustaceans, including both malacostracans and branchiopods, with myriapods either sister to these or to chelicerates (Field et al. 1988; Turbeville et al. 1991; Friedrich and Tautz 1995). Similarly, several workers have cited developmental genetic and neuroanatomical evidence in proposing a closer relationship between hexapods and crustaceans, including both malacostracans and branchiopods, than between hexapods and myriapods (Averof and Akam 1995; Osorio, Averof, and Ba-

con 1995), although this information has yet to be interpreted in a strictly phylogenetic context. In contrast, Atelocerata have been recovered as a monophyletic clade by most morphology-based studies (Snodgrass 1938; Boudreaux 1979; Weygoldt 1986). Our analyses are in partial agreement with studies based on 18S ribosomal nucleotides in that EF-1α + POLII strongly groups hexapods and a subset of traditionally defined crustaceans, the branchiopods. However, our studies are in conflict with those that posit a close relationship between hexapods and all crustaceans.

The unexpected placement of branchiopods by this analysis suggests that hexapods may have originated from freshwater "crustaceans" rather than from terrestrial lobopods or some unknown marine lineage (Manton 1977; Little 1990; Meglitsch and Schram 1991; Averof and Cohen 1997). Throughout their known history, the branchiopods have been limited almost exclusively to freshwater habitats (Schram 1982), with extant anostracans, conchostracans, and notostracans tending to inhabit temporary pools and cladocerans occupying a range of lentic environments (Meglitsch and Schram 1991). When considered in the context of our phylogenetic results, the ancient association of branchiopods with freshwater suggests that ancestral hexapods also occurred there. Although no extant apterygote hexapod can be regarded as primitively aquatic, the life cycle of most "primitive" pterygote hexapods—ephemeropterans, odonates, plecopterans—is intimately associated with freshwater. The immatures are almost always gilled benthic larvae, and this appears to have been the case for many Paleozoic paleopterans and perhaps some apterygotes as well (Shear and Kukalová-Peck 1990). It is interesting to note in this context that the earliest recorded anostracan was eventually identified as an aquatic insect larva (Schram 1982). Unfortunately, the hypothesis of a freshwater ancestry for hexapods will be difficult to assess, as key events in the evolution of arthropod terrestriality apparently occurred during the Lower Silurian and Ordovician, and very few fossil-bearing freshwater and terrestrial deposits are known from these strata (Bergström 1979; Jeram, Selden, and Edwards 1990; Shear and Kukalová-Peck 1990). Still, absence of data has significance in this case, as existence of hexapod ancestors in the poorly recorded Ordovician and Silurian freshwater systems would explain the long-pondered absence of such fossils from the well-studied synchronous marine deposits from which most other major arthropod groups have been recorded (Bergström 1979).

Conclusions

Our analysis indicates that EF-1α and POLII contain information useful for resolving the phylogeny of the major arthropod lineages, and most relationships proposed here are well supported by this evidence. Still, we do not regard the apparent polyphyly of crustaceans or monophyly of Branchiopoda and Hexapoda as conclusive results and suggest that final resolution of these issues will depend on progress in three areas. First, the arthropod tree must be rooted unambiguously to ensure that a conclusion of polyphyly is not caused by inaccurate positioning of outgroups, although changes in outgroup position alone cannot alter our observation of crustacean polyphyly (fig. 2). This requires a more thorough sampling of nonarthropods, including more annelids and mollusks as well as onychophorans and tardigrades. Second, future analyses must be based on a much broader sample of crustaceans. All molecule-based analyses of arthropod relationships conducted thus far, including our own, have used representatives from only 1 or 2 of the 10 or so extant crustacean classes. Indeed, none have sampled the phylogenetically controversial Cephalocarida and Remipedia, each of which has been claimed as the most morphologically primitive of living crustaceans (Schram 1983, 1986; Hessler 1992). Furthermore, additional sampling of crustaceans may minimize concern about long-branch attraction that can complicate placement of highly divergent taxa (Felsenstein 1988) such as the malacostracans sampled here (fig. 2). In this regard, inclusion of partial EF-1α sequences from two additional malacostracans (a stomatopod and an amphipod) did not alter the topology shown in figure 2 (unpublished observation). Finally, more data are needed from other highly conserved nuclear protein-coding genes. We advocate the use of such genes for resolving ancient phylogenetic events because they have a distinct reading frame that minimizes ambiguities in base alignment and because translation to amino acids can minimize negative analytical effects caused by rapid evolution at functionally neutral base positions. However, a shortcoming of conserved genes is that potentially informative changes are rare by definition, thus requiring long sequences to gain adequate resolving power for large-scale phylogenetic problems. We regard development of additional nuclear genes as essential for further progress in resolving deep relationships in Arthropoda and other ancient metazoan lineages.

Acknowledgments

We thank Janet L. Easly for *Polyxenus fasciculatus,* Livy Williams III for *Mastigoproctus giganteus,* Suwei Zhao and Shoba Halan for technical support during the early phase of the research, Dave Swofford for providing test versions of PAUP*, Charles Mitter for encouragement and helpful discussions, Marcus Friedrich and Diethard Tautz for providing us their rDNA data set, and Doug Eernisse for thoughtful comments on the manuscript. This work was supported by the Center for Agricultural Biotechnology (J.C.R.), the General Research Board of the University of Maryland, the Maryland Agricultural Experiment Station (J.W.S.), and the National Science Foundation.

LITERATURE CITED

ADACHI, J., and M. HASEGAWA. 1994. Programs for molecular phylogenetics. Version 2.2. Institute of Statistical Mathematics, Tokyo.

ANDERSON, D. T. 1979. Embryos, fate maps, and the phylogeny of arthropods, Pp. 59–105 *in* A. P. GUPTA, ed. Arthropod phylogeny. Van Nostrand Reinhold, New York, N.Y.

AVEROF, M., and M. AKAM. 1995. Insect-crustacean relationships: insights from comparative developmental and molecular studies. Philos. Trans. R. Soc. Lond. B **347**:293–303.

AVEROF, M., and S. M. COHEN. 1997. Evolutionary origin of insect wings from ancestral gills. Nature **385**:627–630.

BALLARD, J. W. O., G. J. OLSEN, D. P. FAITH, W. A. ODGERS, D. M. ROWELL, and P. W. ATKINSON. 1992. Evidence from 12S ribosomal RNA sequences that onychophorans are modified arthropods. Science **258**:1345–1348.

BERGSTRÖM, J. 1979. Morphology of fossil arthropods as a guide to phylogenetic relationships. Pp. 3–56 *in* A. P. GUPTA, ed. Arthropod phylogeny. Van Nostrand Reinhold, New York, N.Y.

BOORE, J. L., T. M. COLLINS, D. STANTON, L. L. DAEHLER, and W. M. BROWN. 1995. Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. Nature **376**:163–165.

BOUDREAUX, H. B. 1979. Arthropod phylogeny with special reference to insects. John Wiley and Sons, New York, N.Y.

BREMER, K. 1988. The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42**: 795–803.

BUDD, G. 1993. A Cambrian gilled lobopod from Greenland. Nature **364**:709–711.

CAMMARANO, P., P. PALM, R. CRETI, E. CECCARELLI, A. M. SANANGELANTONI, and O. TIBONI. 1992. Early evolutionary relationships among known life forms inferred from elongation factor EF-2/EF-G sequences: phylogenetic coherence and structure of the archaeal domain. J. Mol. Evol. **34**:396–405.

CARPENTER, J. M. 1992. Random cladistics. Cladistics **8**: 147–153.

CHO, S., A. MITCHELL, J. C. REGIER, C. MITTER, R. W. POOLE, T. P. FRIEDLANDER, and S. ZHAO. 1994. A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1α recovers morphology-based tree for heliothine moths. Mol. Biol. Evol. **12**:650–656.

CISNE, J. L. 1974. Trilobites and the origin of arthropods. Science **186**:13–18.

DAYHOFF, M. O., R. M. SCHWARTZ, and B. C. ORCUTT. 1978. Atlas of protein sequence and structure. Vol. 5, Suppl. 3. National Biomedical Research Foundation, Washington, D.C.

DEAR, S., and R. STADEN. 1991. A sequence assembly and editing program for efficient management of large projects. Nucleic Acids Res. **19**:3907–3911.

DONOGHUE, M. J., R. G. OLMSTEAD, J. F. SMITH, and J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on rbcL sequences. Ann. Mo. Bot. Gard. **79**:333–345.

FAITH, D. P. 1991. Cladistic permutation tests for monophyly and nonmonophyly. Syst. Zool. **40**:366–375.

FAITH, D. P., and P. S. CRANSTON. 1991. Could a cladogram this short have arisen by chance alone?: On permutation tests for cladistic structure. Cladistics **7**:1–28.

FELSENSTEIN, J. 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. **22**:521–565.

FIELD, K. G., G. J. OLSEN, D. J. LANE, S. J. GIOVANNONI, M. T. GHISELIN, E. C. RAFF, N. R. PACE, and R. A. RAFF. 1988. Molecular phylogeny of the animal kingdom. Science **239**:748–753.

FRIEDLANDER, T. P., J. C. REGIER, and C. MITTER. 1992. Nuclear gene sequences for higher level phylogenetic analysis: 14 promising candidates. Syst. Biol. **41**:483–490.

———. 1994. Phylogenetic information content of five nuclear gene sequences in animals: initial assessment of character sets from concordance and divergence studies. Syst. Biol. **43**:511–525.

FRIEDRICH, M., and D. TAUTZ. 1995. Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods. Nature **376**:165–167.

GOULD, S. J. 1989. Wonderful life. W. W. Norton, New York, N.Y.

GIRIBET, G., S. CARRANZA, J. BAGUÑÀ, M. RUITORT, and C. RIBERA. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Mol. Biol. Evol. **13**: 76–84.

HASEGAWA, M., T. HASHIMOTO, J. ADACHI, N. IWAGE, and T. MIYATA. 1993. Early branchings in the evolution of eukaryotes: ancient divergence of *Entamoeba* that lacks mitochondria revealed by protein sequence data. J. Mol. Evol. **36**:380–388.

HASEGAWA, M., and H. KISHINO. 1994. Accuracies of the simple methods for estimating the bootstrap probability of a maximum-likelihood tree. Mol. Biol. Evol. **11**:142–145.

HECKER, K. H., and K. H. ROUX. 1996. High and low annealing temperatures increase both specificity and yield in touchdown and stepdown PCR. BioTechniques **20**:478–485.

HESSLER, R. R. 1992. Reflections on the phylogenetic position of the Cephalocarida. Acta Zool. **73**:315–316.

HOU, X.-G., L. RAMSKÖLD, and J. BERGSTRÖM. 1991. Composition and preservation of the Chengjiang fauna—a Lower Cambrian soft-bodied biota. Zool. Scripta **20**:395–411.

JERAM, A. J., P. A. SELDEN, and D. EDWARDS. 1990. Land animals in the Silurian: arachnids and myriapods from Shropshire, England. Science **250**:658–661.

JONES, D. T., W. R. TAYLOR, and J. M. THORNTON. 1992. The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci. **8**:275–282.

KÄLLERSJÖ, M., J. S. FARRIS, A. G. KLUGE, and C. BULT. 1992. Skewness and permutation. Cladistics **8**:275–287.

KLUGE, A. 1989. A concern for evidence and a phylogenetic hypothesis for relationships among *Epicrates* (Boidae, Serpentes). Syst. Zool. **38**:1–25.

KOJIMA, S. T. HASHIMOTO, M. HASEGAWA, S. MURATA, S. OHTA, H. SEKI, and N. OKADA. 1993. Close phylogenetic relationship between Vestimentifera (tube worm) and Annelida revealed by amino acid sequence of elongation factor-1α. J. Mol. Evol. **376**:165–167.

KRISTENSEN, N. P. 1981. Phylogeny of the insect orders. Annu. Rev. Ecol. Syst. **26**:135–157.

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.

LAKE, J. A. 1990. Origin of the Metazoa. Proc. Natl. Acad. Sci. USA **87**:763–766.

LITTLE, C. 1990. The terrestrial invasion: an ecophysiological approach to the origins of land animals. Cambridge University Press, Cambridge.

MANTON, S. M. 1973. Arthropod phylogeny—a modern synthesis. J. Zool. (Lond.) **171**:111–130.

———. 1977. The Arthropoda: habits, functional morphology and evolution. Clarendon Press, London.

MEGLITSCH, P. A., and F. R. SCHRAM. 1991. Invertebrate zoology. 3rd edition. Oxford University Press, New York, N.Y.

MINDELL, D. P., and R. L. HONEYCUTT. 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic applications. Annu. Rev. Ecol. Syst. **21**:541–566.

OSORIO, D., M. AVEROF, and J. P. BACON. 1995. Arthropod evolution: great brains, beautiful bodies. Trends Ecol. Evol. **10**:449–454.

PANGANIBAN, G., A. SEBRING, L. NAGY, and S. CARROLL. 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.

PATTERSON, C. 1989. Phylogenetic relations of major groups: conclusions and prospects. Pp. 471–488 *in* B. FERNHOLM, K. BREMER, and H. JÖRNVALL, eds. The hierarchy of life. Elsevier Science Publishers, Amsterdam.

PLATNICK, N. I. 1978. Review: the Arthropoda: habits, functional morphology and evolution by S. M. Manton. Syst. Zool. **27**:252–255.

RAMSKÖLD, L. 1992. Homologies in Cambrian Onychophora. Lethaia **25**:443–460.

SAKAMOTO, Y., M. ISHIGURO, and G. KITAGAWA. 1986. Akaike information criterion statistics. D. Reidel, Dordrecht.

SCHRAM, F. R. 1982. The fossil record and evolution of Crustacea. Pp. 93–147 *in* L. G. ABELE, ed. The biology of Crustacea. Vol. 1. Academic Press, New York, N.Y.

———. 1983. Remipedia and crustacean phylogeny. Pp. 23–29 *in* F. R. SCHRAM, ed. Crustacean issues, Vol. 1. Crustacean phylogeny. A. A. Balkema, Rotterdam.

———. 1986. Crustacea. Oxford University Press, New York, N.Y.

SHARP, P. M., and W.-H. LI. 1987. Ubiquitin genes as a paradigm of concerted evolution of tandem repeats. J. Mol. Evol. **25**:58–64.

SHEAR, W. A. 1992. End of the 'Uniramia' taxon. Science **359**:477–478.

SHEAR, W. A., and J. KUKALOVÁ-PECK. 1990. The ecology of Paleozoic terrestrial arthropods: the fossil evidence. Can. J. Zool. **68**:1807–1834.

SHULTZ, J. W. 1990. Evolutionary morphology and phylogeny of Arachnida. Cladistics **6**:1–38.

SMITH, S. W., R. OVERBEEK, C. R. WOESE, W. GILBERT, and P. M. GILLEVET. 1994. The genetic data environment and expandable GUI for multiple sequence analysis. Comput. Appl. Biosci. **10**:671–675.

SNODGRASS, R. E. 1938. Evolution of the Annelida, Onychophora and Arthropoda. Smithson. Misc. Collect. **97**:1–159.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony. Version 3.2.0d2(PTP). Smithsonian Institution, Washington, D.C.

TAN, Y., S. T. BISHOFF, and M. A. RILEY. 1993. Ubiquitins revisited: further examples of within- and between-locus concerted evolution. Mol. Phylogenet. Evol. **2**:351–360.

TELFORD, M. J., and R. H. THOMAS. 1995. Demise of the Atelocerata? Nature **376**:123–124.

TIEGS, O. W., and S. M. MANTON. 1958. The evolution of the Arthropoda. Biol. Rev. **33**:255–337.

TURBEVILLE, J. M., D. M. PFEIFER, K. G. FIELD, and R. A. RAFF. 1991. The phylogenetic status of arthropods, as inferred from 18S rRNA sequences. Mol. Biol. Evol. **8**: 669–686.

WÄGELE, J. W. 1993. Rejection of the "Uniramia" hypothesis and implications of the Mandibulata concept. Zool. Jahrb. Syst. **120**:253–288.

WÄGELE, J. W., and G. STANJEK. 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. Pp. 107–135 *in* A. P. GUPTA, ed. Arthropod phylogeny. Van Nostrand Reinhold, New York, N.Y.

———. 1986. Arthropod interrelationships—the phylogenetic-systematic approach. Z. Zool. Syst. Evolutionsforsch. **24**: 19–35.

WHEELER, W. C., P. CARTWRIGHT, and C. Y. HAYASHI. 1993. Arthropod phylogeny: a combined approach. Cladistics **9**: 1–39.

WHITTINGTON, H. B. 1985. The Burgess shale. Yale University Press, New Haven, Conn.

WILLMER, P. 1990. Invertebrate relationships: patterns in animal evolution. Cambridge University Press, Cambridge.

WILLS, M. A., D. E. G. BRIGGS, and R. A. FORTEY. 1994. Disparity as an evolutionary index: a comparison of Cambrian and Recent arthropods. Paleobiology **20**:93–130.

RICHARD G. HARRISON, reviewing editor

Accepted May 16, 1997