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Peracarid monophyly and interordinal phylogeny inferred from nuclear small-subunit ribosomal DNA sequences (Crustacea: Malacostraca: Peracarida)

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Abstract.—Peracarids are a large group of malacostracan crustaceans whose systematics and phylogeny are uncertain. The present phylogenetic study of peracarids is, to our knowledge, the first that includes full-length nuclear (n) small-subunit (SSU) ribosomal DNA (rDNA) sequences for representatives of every peracarid order. Sequence length varied substantially (1807–2746 base pairs), and two variable regions (V4 and V7) contained long expansion segments. Variable regions also exhibited significantly greater heterogeneity in nucleotide frequencies than did core regions. Maximum-parsimony, maximum-likelihood, Bayesian, and distance phylogenetic estimates indicated a monophyletic Peracarida that excluded the Mysida and included the Thermosbaenacea. This peracarid clade received strong support under Bayesian, maximum-parsimony, and distance analyses, but not maximum likelihood. Further, the thermosbaenacean lineage does not occupy a basal position relative to other peracarids, as suggested by many morphology-based phylogenies. The phylogenetic position of the Mysida within the Malacostraca remains uncertain, but a sister-group relationship between Mysida and Lophogastrida (i.e., a monophyletic Mysidacea) was consistently rejected, a result that also differs from those of most morphology-based phylogenies. High Bayesian clade-credibility support was obtained for an (Isopoda + Tanaidacea + Cumacea) clade and a (Lophogastrida + [Spelaeogriphacea + Amphipoda]) clade. Within the peracarid clade, internal branches were considerably shorter than terminal ones, so relationships among some peracarid lineages were equivocal. Data partitions corresponding to stem and loop regions in a secondary-structure model for nSSU rRNA had congruent phylogenetic signal but differed in nucleotide composition and evolutionary model. Maximum parsimony and Bayesian phylogenetic estimates based on the loop partition generally shared more nodes in trees inferred from combined data than did those based on stems, even though the stem partition had roughly twice as many characters.

Peracarids comprise roughly one-third of all crustaceans. The well over 21,000 described species are free-living, symbiotic, or parasitic, and occur in marine, freshwater, and even terrestrial habitats. The current accounting of peracarid species diversity is probably a gross underestimation, as peracarids can be found in some of the most remote regions (e.g., marine caves, groundwater, springs, and the deep sea). Supporting this view is the observation that the number of described peracarid species has actually tripled in only the past 20 years.

Peracarids were first proposed as a su-

perorder within the class Malacostraca, subclass Eumalacostraca, by Calman (1904), who united five orders (Amphipoda, Cumacea, Isopoda, Mysidacea, and Tanaidacea) on the basis of several morphological features. Three new orders (Mictacea, Spelaeogriphacea, and Thermosbaenacea) have been discovered since the mid-1950's, and two orders (Mysida and Lophogastrida) are arguably suborders of the Mysidacea. Nine orders of peracarids, at most, are currently recognized (Martin & Davis 2001; Appendix 1).

A short list of so-called peracarid features proposed by Calman (1909) includes a mandible with an articulated accessory process (lacinia mobilis), a carapace (in most cases) that remains unfused with at least four of the posterior thoracic somites, and a first thoracic somite that is fused with the head. Peracarids also possess, uniquely among crustaceans, a brood pouch that is formed, in most cases, by oostegites derived from thoracic epipods. Peracarids lack a larval phase (e.g., nauplius or zoea), and the young emerge from the brood pouch as prejuveniles (i.e., mancas, in all but mysidaceans, thermosbaenaceans, and most amphipods) that resemble adults to varying degrees. Unfortunately, synapomorphies for the Peracarida are rare because even the relatively few features suggested by Calman are not applicable across all orders, and some of these features are found in nonperacarids as well. For example, thermosbaeanaceans brood their young in a dorsal region that is formed from the carapace rather than in a ventral pouch formed from oostegites as in most other peracarids. Further, not all peracarids possess a carapace, and the homology of this feature and many others remains undetermined (see, e.g., Watling 1999). For these reasons, considerable debate has surrounded the correct diagnosis of the superorder and its rightful constituents, particularly since the discovery of new orders.

Alternative taxonomic and phylogenetic scenarios.—Hessler & Watling (1999) recently reviewed the historical debate about peracarid taxonomy and phylogeny, and Richter & Scholtz (2001) also reviewed pertinent issues concerning relationships among peracarids and other malacostracan lineages. Monophyly for this superorder has been questioned as new orders have been discovered, and evolutionary relationships among the diverse peracarid lineages remain poorly understood. Various morphology-based phylogenetic schemes (Fig. 1) have been proposed, some of which are computer-assisted cladistic analyses of morphological characters (e.g., Pires 1987, Wagner 1994, Schram & Hof 1998, Wills 1998, Richter & Scholtz 2001), but little phylogenetic consensus has emerged from these efforts. Watling (1999) attributed this lack of agreement to the immense difficulty of correctly assessing the homology of morphological features in peracarids, and he questioned the efficacy of performing cladistic analyses on seemingly homoplasious characters. More recent morphology-based cladistic studies have focused within specific peracarid lineages (e.g., Amphipoda: Vonk & Schram 2003, Lörz & Held 2004; Isopoda: Brandt & Poore 2003).

A glance at some of the alternative phylogenetic schemes (Fig. 1) illustrates major points of contention. Of primary interest is whether the Peracarida is monophyletic and what orders are included within it. Some (e.g., Monod 1924; Fig. 1c–g) consider the Thermosbaenacea to be a peracarid lineage; others place this taxon in its own suborder, Pancarida (sensu Siewing 1958), that is sister to the Peracarida (Fig. 1a, b, h), because thermosbaenaceans have a different developmental origin and location of the brood chamber. Controversy also exists as to whether the Mysidacea is a natural taxon and a peracarid lineage that includes mysids and lophogastrids (Fig. 1a–f, h), or whether mysids and lophogastrids are nonperacarid lineages that each merit ordinal status and are more closely allied to the Eucarida (Fig. 1g).

Taxonomic and phylogenetic uncertainty

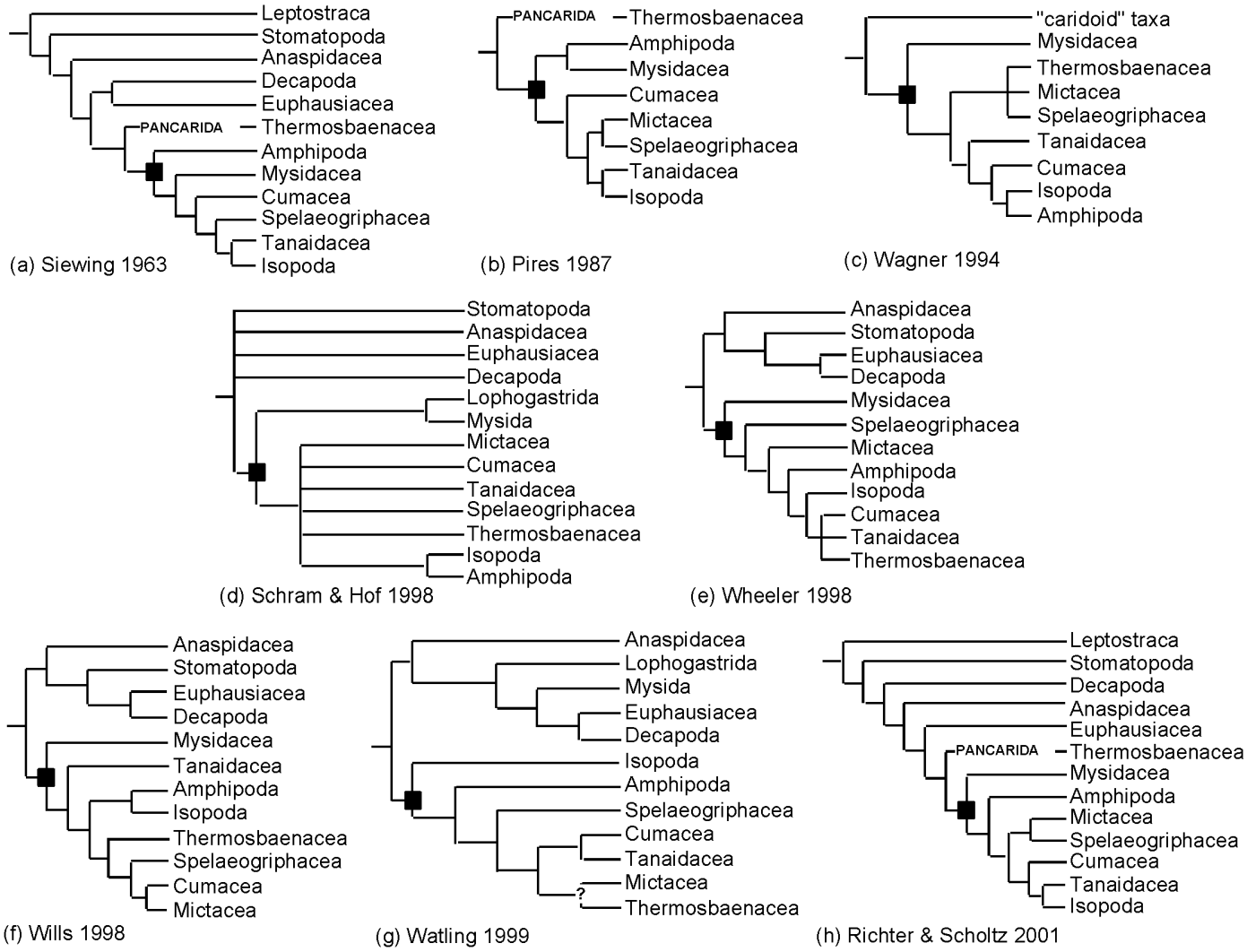

Fig. 1. Alternative phylogenetic hypotheses for selected extant malacostracan orders, with an emphasis on the Peracarida. Drawings modified from (a) Siewing (1963); (b) Pires (1987); (c) Wagner (1994); (d) Schram & Hof (1998); (e) Wheeler (1998); (f) Wills (1998); (g) Watling (1999); (h) Richter & Scholtz (2001).

also surrounds the enigmatic Spelaeogriphacea and Mictacea, two taxa that are often grouped together (Fig. 1b, c, h). A fairly recent cladistic analysis (Shen et al. 1998) that included Spelaeogriphacea-like fossils, however, found the order Spelaeogriphacea to be paraphyletic with respect to tanaids and cumaceans and not with mictaceans. Bowman et al. (1985), in their original diagnosis of the Mictacea, noted similarities of this order to mysidaceans, spelaeogriphaceans, and thermosbaenaceans, and they intentionally selected the name Mictacea, from the Greek ''miktos'' meaning mixed, to highlight their observation that several mictacean characters are found in other orders of peracarids. Gutu (1998) later removed *Mictocaris halope* Bowman & Iliffe, 1985, from the Mictacea and placed it with spelaeogriphaceans in a newly proposed peracarid order, Cosinzeneacea. That same year, Gutu & Iliffe (1998) erected the Bochusacea, another new order of peracarids, to accommodate the genus *Hirsutia* (which they removed from the Mictacea) and a newly described species *Thetispelecaris remix* Gutu & Iliffe, 1998. Shortly thereafter, Gutu (2001) proposed a sister-group relationship between the Bochusacea and Tanaidacea. Here, we follow the classification of Martin & Davis (2001), which does not recognize either the Cosinzeneacea or the Bochusacea.

Views also differ concerning the sister group of peracarids (e.g., Thermosbaenacea, Fig. 1a, b, h), the basal peracarid lineage (e.g., either Mysidacea, Fig. 1c–f, h; Amphipoda, Fig. 1a; or Isopoda, Fig. 1g), and the sister group of isopods (e.g., either Amphipoda, Fig. 1c, d, f, or Tanaidacaea, Fig. 1a, b, h). Some have even proposed (Nyland et al. 1987) that the Isopoda be removed from the Peracarida entirely.

New and extremely promising avenues of research are contributing to our understanding of evolution and underlying homologies both in peracarids and in other malacostracans. Among these are studies in comparative developmental genetics that investigate the control of body-segment organization by the expression of the *engrailed* segment-polarity gene (Scholtz et al. 1993, 1994) and homeotic, or Hox, gene expression (Panganiban et al. 1995, Averof & Patel 1997, and more generally, Schram & Koenemann 2004); studies of early embryonic cell lineages and cell fate (Dohle & Scholtz 1997; Gerberding & Scholtz 1999, 2001; Sholtz 2000); and comparative ultratructural studies on the alimentary canal of malacostracans, including peracarids (e.g., Storch 1987, 1989; Oshel & Steele 1988; Coleman 1990; Suh 1990; Ulrich et al. 1991; De Jong & Casanova 1997a, 1997b; Kobusch 1998; Wallis & Macmillan 1998; De Jong-Moreau et al. 2000; De Jong-Moreau & Casanova 2001).

More recently, various molecular approaches (primarily DNA sequencing) have been used to investigate questions about systematics and phylogenetics within peracarid orders (e.g., Amphipoda: France & Kocher 1996a, 1996b; Sherbakov et al. 1999; Englisch & Koenemann 2001; Englisch et al. 2003; Lörz & Held 2004; Cumacea: Haye et al. 2004; Isopoda: Held 2000; Michel-Salzat & Bouchon 2000; Dreyer & Wägele 2001, 2002; Mattern & Schlegel 2001; Wetzer 2002; Lophogastrida: Casanova et al. 1998; Mysida: Meland & Willassen 2004; Mysidacea: Meland 2003; Tanaidacea: Larsen 2001) and among selected peracarids and other eumalacostracans (Jarman et al. 2000). Lacking to date is a molecularly based study of higher-level malacostracan taxa that expressly focuses on peracarids and includes representatives of all nine putative peracarid orders.

The purpose of our study was to obtain nucleotide-sequence data for the nuclear (n) small-subunit (SSU; also called 18S) ribosomal RNA (rRNA) gene (i.e., nSSU rDNA) for representatives of all major malacostracan lineages and orders of peracarids (Appendix 1) and to use a variety of phylogenetic-inference methods on these data (1) to assess peracarid monophyly (i.e., to determine which orders comprise a mono-

phyletic peracarid clade, whether thermosbaenaceans are included, whether the Mysidacea are a monophyletic clade and whether they are included in the Peracarida or whether the Mysida and Lophogastrida are in separate orders, either or both of which may be in the Peracarida) and (2) to infer phylogenetic relationships among peracarid orders. The inference of relationships within orders of peracarids and among other malacostracan lineages was not a major focus of this study, although some preliminary findings are presented. We also compared phylogenetic estimates based separately on stem and loop regions of the nSSU rRNA molecule with those obtained for combined (i.e., stem + loop) data, and we explored, to some degree, the effect that stem and loop partitions with different characteristics have on phylogenetic estimation using rDNA data.

Materials and Methods

Selection of taxa.—Species included in the present study are presented in Appendix 1 according to the classification of Martin & Davis (2001). Both molecular (Spears & Abele 1999) and morphological (Richter & Scholtz 2001) studies support malacostracan monophyly, with the order Leptostraca as a basal lineage. We therefore designated the leptostracan *Nebalia* sp. as the outgroup in this study. At least one representative of every malacostracan and peracarid order was included (Appendix 1), except for the syncarid order Bathynellacea and the monotypic eucarid order Amphionidacea. Admittedly, this sampling scheme fails to represent the vast species diversity among malacostracans, but it was not our primary intent to discern relationships within this class of crustaceans, nor was it to infer relationships within the orders of peracarids. We therefore included relatively few nonperacarids and at least one representative from each of the nine putative orders of peracarids. A few additional species were included for the more speciose peracarid suborders (i.e., Amphipoda and Isopoda). We omited the many additional species from these suborders that are available in GenBank to keep the number of species to one that would permit computer-intensive maximum-likelihood analyses.

Specimens were preserved in 95–100% ethanol for subsequent DNA extraction and kept at −80°C when possible. Voucher specimens are maintained in the laboratory of L. G. Abele, Department of Biological Science, Florida State University, Tallahassee, Florida, with the exception of *Spelaeogriphus lepidops* (in the laboratory of Les Watling, University of Maine, Darling Marine Center, Walpole, Maine), and *Thetispelecaris remex* (in the laboratory of Thomas M. Iliffe, Texas A&M University at Galveston, Texas). The authors acknowledge the generosity of colleagues (Appendix 1) who donated specimens, some of which are extremely rare and/or which required extraordinary effort to collect.

Molecular protocols.—Genomic DNA was extracted from up to 0.5 g of muscle tissue from live or ethanol-preserved animals. For extremely small specimens (e.g., thermosbaenaceans and mictaceans), either an entire individual or a substantial portion of one was used in an extraction subsequent to rinsing and sonication of the tissue in sterile distilled water to remove debris. Extractions were performed according to a phenol/chloroform/isoamyl alcohol procedure outlined by Kocher et al. (1989) or with the G-NOME® DNA Kit (Q-BIOgene, Inc., Carlsbad, California) with a scaled-down protocol (i.e., 500-μl total volume). After precipitation in 100% ethanol, DNA pellets were washed with 70% ethanol, dried, suspended in 10–200 μl of sterile distilled water (depending on the amount of starting tissue and the size of the DNA pellet), and stored at −20°C prior to gene amplification.

Standard protocols (see, e.g., Spears & Abele 2000) were followed for (1) polymerase-chain-reaction (PCR) amplification (Saiki et al. 1988) of full-length nSSU

rDNA with a Perkin-Elmer DNA Thermal Cycler 480 (Perkin-Elmer, Foster City, California); (2) purification and cloning (when necessary) of PCR products; and (3) cycle sequencing of fluorescently labeled DNA templates with half reactions with the Applied Biosystems, Inc. (ABI; Foster City, California) PRISM®Big Dye™ Terminator Ready Reaction Kit and a room-temperature ethanol/EDTA cleanup. Most peracarids harbored endosymbionts that coextracted and coamplified when peracarid tissue and DNA were used, respectively; peracarid PCR products therefore required gel purification and/or cloning before sequencing (Spears & Abele 2000).

We performed automated DNA sequencing using an ABI Model 373A Automated Sequencing System (during the early phase of this study) and an ABI PRISM® 3100 Genetic Analyzer (during the later phase). Several brands of *Taq* polymerase (2.5 Units) were used with equal success in 100-μl PCR reactions to amplify both strands of the entire nSSU rRNA gene, either as a single PCR product or in overlapping fragments. Several DNA primers (available upon request) that are homologous to regions dispersed throughout the nSSU rRNA gene were used for both symmetric PCR amplification and bidirectional sequencing of double-stranded PCR products.

A consensus sequence was assembled for each species with Sequencher™ 4.1. Appendix 1 lists the GenBank accession numbers of the sequences.

Sequence alignment and analysis.—A multiple alignment of the nSSU rDNA sequences was generated by ClustalX 1.81 (Thompson et al. 1997) with a gap-opening penalty of 10.00, a gap-extension penalty of 0.20, a 30% identity for delaying divergent sequences, a transition weight of 1.00, and the ClustalW (Thompson et al. 1994) DNA-weight matrix. The complete alignment is available from the first author upon request. The secondary structure of a ribosomal RNA molecule comprises double-stranded (''stem'') and single-stranded (''loop'') regions, which were mapped onto the multiple alignment on the basis of Van de Peer et al.'s (1997) model of nSSU rRNA secondary structure, and any necessary adjustments in the alignment were made by eye. Portions of hypervariable and other ambiguous regions in the alignment were omitted from subsequent phylogenetic analyses. We identified the numbered nucleotide positions corresponding to stem and loop data partitions by executing the alignment file in the computer program PAUP* 4.0b10 (Swofford 2002) and obtaining a data matrix of numbered characters.

A partition-homogeneity test (also known as the incongruence-length-difference test; Farris et al. 1994, 1995) in PAUP* (with the heuristic search option for 1000 replications and 10 random-sequence-addition replicates) assessed whether stem and loop partitions of the data contained conflicting phylogenetic signal. PAUP* also yielded summary sequence statistics, such as uncorrected measures of sequence divergence, transition : transversion (Ti : Tv) ratios between pairs of taxa (for assessment of saturation of transition substitutions; see, e.g., Knight & Mindell 1993), nucleotide frequencies, and tests for bias in nucleotide frequencies across taxa. All partitions were examined for phylogenetic signal with the test for skewness in a tree-length distribution (Hillis & Huelsenbeck 1992) and the permutation-tail-probability test (Archie 1989, Faith & Cranston 1991), both implemented in PAUP*.

Phylogenetic analyses.—Maximum-parsimony (MP; Camin & Sokal 1965) analyses were performed on unordered and equally weighted parsimony-informative characters with the heuristic-search option in PAUP* for all three data partitions. Starting trees were obtained by random stepwise addition of sequences for 10,000 replicates and with tree-bisection-reconnection (TBR) branch swapping on best trees only. The option MULTREES was implemented; ''steepest descent'' was not implemented. Both delayed (DELTRAN) and accelerated

(ACCTRAN) methods of mapping character-state transformation on trees were used, and the phylogenetic trees in both cases were compared on the basis of topology, tree length, and consistency index (C.I.; Kluge & Farris 1969). MP estimates were similarly compared when alignment gaps were coded either as a fifth character state or as missing data.

We performed maximum-likelihood (ML; Felsenstein 1981) analyses on all unambiguously aligned characters using the heuristic-search option in PAUP* with TBR branch swapping and random stepwise addition of sequences to obtain starting trees for 10 replicates. Modeltest version 3.06 (Posada & Crandall 1998) with the Akaike Information Criterion (AIC) was used to select a model of nucleotide evolution (see, e.g., Yang et al. 1994) resulting from hierarchical log-likelihood-ratio tests for increasingly complex models as outlined by Huelsenbeck & Crandall (1997) and starting with a neighbor-joining (Saitou & Nei 1987) topology that was inferred with Jukes & Cantor (1969) corrected distances. We optimized model parameters for stem-, loop-, and combined-data partitions using a successive-approximations approach (see, e.g., Swofford et al. 1996) implemented with PAUP*. Heterogeneity in nucleotide-substitution rates among lineages was assessed by a likelihood-ratio test (Felsenstein 1981) comparing the best ML estimate of phylogeny (i.e., the one with the highest log likelihood, ln L) both with and without the assumption of a molecular clock.

Because the ML method of phylogenetic inference as implemented in PAUP* uniformly applies only a single model of nucleotide evolution (and its corresponding parameter values) to a set of data, the ML phylogeny estimated from the combined data partition according to one model of evolution may not be the most accurate reflection of phylogeny if stem and loop partitions are best described by different models. The partition-homogeneity test was used to determine whether stem and loop partitions were homogeneous and combinable, but this test is susceptible to incorrect rejection or acceptance of congruence among partitions under different conditions (see, e.g., Hipp et al. 2004, and references therein). The computer program MrBayes 3.0b4 (Huelsenbeck & Ronquist 2002), which permits a simultaneous ML search on multiple data partitions with their respective models of evolution, was therefore used to investigate the effect, if any, of data partitions and model selection on phylogenetic inference.

We conducted Bayesian analyses (see, e.g., Rannala & Yang 1996; Larget & Simon 1999; Huelsenbeck et al. 2001, 2002) using the Modeltest-generated model of evolution for (1) combined (‘‘linked’’) data, using both optimized and default initial settings for model parameters (to determine whether the specification of optimized parameter values improved Bayesian support for clades); (2) ‘‘unlinked’’ stem and loop partitions, again using both optimized and default initial settings for model parameters (for the same reason); (3) stem and loop data separately, each without specifying parameter values (to determine whether the Bayesian consensus topologies based on the two data partitions were congruent with each other and also with the Bayesian consensus topology for combined data and to compare the clade-credibility values of these topologies). MrBayes 3.0b4 (the most current version) does not permit the user to specify correctly the proportion of invariant sites for a given model of nucleotide substitution, so an unreleased beta version of MrBayes, compiled by Fredrik Ronquist (Florida State University, Tallahassee, Florida), was used to implement the cases above where parameter values had to be specified.

Negligible differences in clade support were observed when either specific model-parameter values or default settings were used. Bayesian analyses of separate stem and loop partitions were therefore conducted with default parameter settings only. In

all cases, four Markov-Monte-Carlo chains were run simultaneously for four million generations, and a sample tree was chosen every 100 generations. Trees that were chosen once likelihood scores converged on a stable value were used to create a 50%-majority-rule consensus tree in PAUP*. In this regard, a very conservative approach was taken; trees chosen from the first one million generations were discarded as ''burn-in.''

The minimum-evolution method using LogDet- (or ''paralinear'') transformed distances (Lake 1994, Lockhart et al. 1994) was implemented in PAUP* because this method is said to be less sensitive than MP and ML to heterogeneity among taxa in nucleotide composition (although, unlike ML, it presently cannot account for among-site heterogeneity in substitution rates). We chose the value for the proportion of invariant sites (*I*) that was optimized by ML using the successive approximations approach. LogDet analyses were run with this value of *I* under a variety of conditions (e.g., removing *I* in proportion to nucleotide frequencies estimated from either all sites or constant sites only and both with and without adjustment for the mean number of substitutions over all sites), after which topologies and tree scores were compared. These analyses were done for combined data (both with and without the inclusion of two long-branched taxa, *Thetispelecaris remex* and *Tethysbaena argentarii*), stem data, and loop data, so that comparisons could be made with other methods of phylogenetic estimation.

Nodal support in MP trees was determined from decay indices (i.e., the number of character-state changes required to dissolve a particular node; Bremer 1988, 1994) and nonparametric-bootstrap proportions (BP; Felsenstein 1985) with heuristic search options described above with 1000 pseudoreplications for both MP (with 100 random-sequence-addition replicates) and distance analyses. For ML, which is more computationally intensive, a sample of 200 bootstrapped pseudoreplicates (each with five random-sequence-addition replicates) was selected from the combined data and used in heuristic searches. Heuristic searches of bootstrapped pseudoreplicates were repeated under two different conditions so that nodal support could be compared: once with the optimized model-parameter values specified a priori and a second time with estimated values. No significant variation in clade support was found for these two cases; therefore, after stem and loop partitions were bootstrapped separately, ML analyses were conducted with only the optimized parameter values for each partition. Bayesian clade-credibility values (P) for nodes in 50%-majority-rule consensus trees provided an additional measure of support for phylogenetic relationships.

Comparisons were made among optimal MP, ML, and several alternative phylogenetic hypotheses suggested by the peracarid literature (Fig. 1); we obtained the latter by implementing the ''enforce topological constraints'' option for a heuristic search in PAUP*. More general topological constraints were used than the alternative phylogenetic hypotheses shown in Fig. 1 (e.g., isopods and tanaids would be constrained to be sister taxa, but the positions of other taxa were allowed to vary). For MP, we implemented Templeton's nonparametric test (Templeton 1983) and the Kishino-Hasegawa (K-H) parametric test (Kishino & Hasegawa 1989) using PAUP*; for ML, we implemented the one-tailed K-H test and the nonparametric Shimodaira-Hasegawa (S-H) test (Shimodaira & Hasegawa 1999) (using RELL optimization with 1000 bootstrap pseudoreplicates), also using PAUP*.

Results

Sequence and multiple-alignment analyses.—Length heterogeneity among the SSU sequences was substantial, ranging from 1807 base pairs (bp) for three crustaceans (the outgroup taxon *Nebalia* sp. and two peracarids, *Gnathophausia ingens* and *G.*

zoea) to 2746 bp for the valviferan isopod *Idotea metallica* (Table 1). For all peracarid crustaceans except the Mysida and Lophogastrida, nSSU rDNA sequences exceeded 1900 bp. All crustacean sequences were similar in length for so-called ''core'' regions of the SSU molecule (940–950 bp; Table 1), presumably because of strong functional constraints on evolution in this region (see, e.g., Gerbi 1985). In contrast, sequence length in ''variable'' regions of the nSSU rRNA molecule showed a much wider range (864–1803 bp; Table 1), and an unambiguous alignment of data was difficult and sometimes impossible for portions of these regions.

Variable regions exhibited significant heterogeneity in nucleotide frequencies ($P < 0.01$; Table 1) compared to more homogeneous core regions and contributed to the significant heterogeneity in nucleotide frequencies observed for the entire nSSU rRNA molecule ($P < 0.01$; Table 1). This bias in nucleotide composition was reflected in the guanidine and cytosine content (% G + C) in different regions of the gene, with variable regions having a narrower range in % G + C than core regions (Table 1). The % G + C content in variable regions was not consistently higher for all taxa, however. For example, % G + C in variable regions was the same or lower than that of core regions for the outgroup *Nebalia* sp., the three lophogastrid species, the Mysida, the mictacean *Thetispelecaris remex*, and two of the three tanaids included in this study.

Comparisons of the sequence lengths of the eight variable regions (V1–V5 and V7–V9) of the eukaryotic nSSU rRNA molecule revealed some interesting patterns (Appendix 2). The largest differences in sequence length were confined to two so-called ''hypervariable'' regions (see, e.g., Tautz et al. 1988; Hancock and Dover 1988, 1990), ranging from 226 to 879 bp and 86 to 333 bp in the V4 and V7 regions, respectively (Appendix 2). In general, nonperacarid crustaceans and the peracarid orders Mysida, Lophogastrida, and Mictacea had the shortest V4 regions, and nonperacarid crustaceans and the Mysida and Lophogastrida had the shortest V7 regions. Amphipods were unique in having DNA insertions in the V5 and V8 regions (Appendix 2).

The average uncorrected sequence divergence (calculated from data presented in Appendix 3) between orders of nonperacarid malacostracans (excluding the outgroup *Nebalia* sp.) was relatively small and ranged from 3 to 5%. In contrast, the range in average sequence divergence between orders of peracarids (including the Mysida) was much greater (9% between the Mysida and Isopoda and 30% between the spelaeogriphacean *Spelaeogriphus lepidops* and the thermosbaenacean *Tethysbaena argentarii*). The average sequence divergence between the Mysida and nonperacarid taxa (excluding the outgroup *Nebalia* sp.) was only 6% (range = 4–8%), whereas that between the Mysida and other peracarids was almost three times greater (17%). *Spelaeogriphus lepidops* was the most divergent taxon within the Peracarida and ranged from 27 to 30% in sequence divergence in pair-wise comparisons with other taxa (Appendix 3).

Summary sequence statistics are shown in Table 2 for stem, loop, and combined data used in ML and Bayesian analyses. Nucleotide frequencies among these data partitions were homogeneous, although just barely for the combined data ($P = 0.06$). In contrast, all data partitions showed highly significant heterogeneity in nucleotide frequencies when only parsimony-informative characters were considered ($P < 0.05$; Table 3). Nucleotide composition, reflected in % G + C content, was not uniform across data partitions and varied similarly whether all unambiguously aligned characters (Table 2) or only parsimony-informative characters (Table 3) were considered; loop regions had only 40% and 44% G + C, respectively.

Transition : transversion ratios for all pairwise comparisons based on stem, loop,

Table 1.—Length (in nucleotides, nt) of the nuclear small-subunit (SSU) ribosomal RNA (rRNA) gene for crustaceans used in this study. Variable regions were identified with the SSU secondary-structure model of Van de Peer et al. (1997). Classification is according to Martin and Davis (2001). Percent G + C content is given in parentheses (calculated with PAUP* [Swofford 2002] and with missing nucleotides deleted). [1] = length approximate because of an indeterminate number of missing nucleotides; χ^2 = chi-square test for homogeneity of nt frequencies; ** = significant *P* value at $\alpha = 0.01$ with 78 degrees of freedom.

Taxon	Entire SSU rRNA	Core regions	Variable regions
Leptostraca			
Nebalia sp.	1807 (51%)	943 (50%)	864 (48%)
Stomatopoda			
Gonodactylus sp.	1818 (51%)	943 (50%)	875 (54%)
Squilla empusa	1818 (51%)	943 (50%)	875 (54%)
Anaspidacea			
Anaspides tasmaniae	1831 (51%)	943 (50%)	888 (53%)
Spelaeogriphacea			
Spelaeogriphus lepidops	2046 (55%)	941 (53%)	1105 (58%)
Thermosbaenacea			
Tethysbaena argentarii	2262 (53%)	940 (51%)	1322 (53%)
Lophogastrida			
Gnathophausia ingens	1807 (46%)	941 (48%)	866 (43%)
Gnathophausia zoea	1807 (46%)	941 (48%)	866 (44%)
Eucopia sp.	1808 (46%)	941 (48%)	867 (44%)
Mysida			
Heteromysis formosa	1817 (50%)	943 (50%)	874 (50%)
Neomysis integer	1816 (50%)	943 (51%)	873 (49%)
Mictacea			
Thetispelecaris remex	1933 (50%)	941 (51%)	992 (49%)
Amphipoda			
Gammarus oceanicus	2261 (55%)	946 (53%)	1315 (56%)
Caprella geometrica	2177 (53%)	950 (52%)	1227 (55%)
Phronima sp.	2284 (51%)	948 (50%)	1336 (51%)
Isopoda			
Paramphisopus palustris	2363 (54%)	945 (49%)	1418 (57%)
Asellus racovitzai	2145 (52%)	944 (49%)	1201 (54%)
Idotea metallica	2746 (54%)	943 (49%)	1803 (57%)
Tanaidacea			
Tanais dulongii	2002 (47%)	943 (50%)	1059 (44%)
Paratanais malignus	2355 (47%)	942 (47%)	1413 (47%)
Kalliapseudes sp.	2537 (54%)	942 (50%)	1595 (56%)
Cumacea			
Diastylis sculpta	2233 (54%)	940 (53%)	1293 (56%)
Spilocuma salomani	2092[1] (52%)	940[1] (50%)	1152[1] (55%)
Euphausiacea			
Nyctiphanes simplex	1854 (51%)	943 (50%)	911 (53%)
Meganyctiphanes norvegica	1853 (51%)	943 (50%)	910 (54%)
Decapoda			
Panulirus argus	1872 (51%)	942 (50%)	930 (53%)
Callinectes sapidus	1870 (51%)	943 (50%)	924 (53%)
χ^2 (*P*)	0.00**	1.00	0.00**

Table 2.—Summary statistics (obtained from PAUP* 4.0b10; Swofford 2002) for three data partitions (stems, loops, and combined [i.e., stems + loops]) based on a multiple alignment of 27 crustacean nuclear small-subunit ribosomal DNA sequences after the removal of ambiguously aligned nucleotide (nt) positions. Characters were used in maximum-likelihood, Bayesian, and distance analyses. Ti = transitions; Tv = transversions; g_1 = test statistic for skewness of a tree-length frequency distribution (Hillis & Huelsenbeck 1992); *PTP* = permutation-tail-probability test (Archie 1989, Faith & Cranston 1991) for hierarchical structure with the heuristic-search option and 1000 replications, each with 10 random sequence additions; χ^2 = chi-square test for homogeneity of nt frequencies; ** = significant *P* value at $\alpha = 0.01$ with 78 degrees of freedom.

	Stems	Loops	Combined
No. characters (% of combined)	1099 (65%)	597 (35%)	1696
No. constant characters	453 (64%)	251 (36%)	704
No. parsimony-uninformative variable characters	213 (68%)	101 (32%)	314
No. parsimony-informative characters	433 (64%)	245 (36%)	678
Mean nt frequency:			
Adenine	0.24 (0.21–0.26)	0.25 (0.21–0.28)	0.24 (0.21–0.27)
Cytosine	0.31 (0.30–0.32)	0.21 (0.19–0.23)	0.28 (0.27–0.29)
Guanine	0.24 (0.30–0.32)	0.19 (0.14–0.23)	0.22 (0.19–0.25)
Thymine	0.21 (0.19–0.23)	0.35 (0.33–0.38)	0.26 (0.24–0.28)
χ^2 (*P*)	0.90	1.00	0.06
%GC	55	40	50
Mean Ti : Tv ratio (range)	1.59 (0.75–6.0)	0.99 (0.00–2.57)	1.26 (0.5–3.12)
g_1	−1.202141**	−0.950073**	−1.121712**
PTP (*P*)	0.001**	0.001**	0.01**

Table 3.—Summary statistics (obtained with PAUP* 4.0b10; Swofford 2002) for parsimony-informative (P.I.) characters for three data partitions (stems, loops, and combined [i.e., stems + loops]) based on a multiple alignment of 27 crustacean nuclear small-subunit ribosomal DNA sequences after the removal of ambiguously aligned nucleotide (nt) positions. Characters were used in maximum-parsimony analyses. Ti = transitions; Tv = transversions; g_1 = test statistic for skewness of a tree-length frequency distribution (Hillis & Huelsenbeck 1992); *PTP* = permutation-tail-probability test (Archie 1989, Faith & Cranston 1991) for hierarchical structure with the heuristic-search option and 1000 replications, each with 10 random sequence additions; χ^2 = chi-square test for homogeneity of nt frequencies; for chi-square test, * and ** = significant *P* value at $\alpha = 0.05$ and 0.01, respectively, with 78 degrees of freedom.

	Stems	Loops	Combined
# P.I. characters	433	245	678
Mean nt frequency			
Adenine	0.27 (0.21–0.34)	0.29 (0.20–0.35)	0.28 (0.22–0.30)
Cytosine	0.26 (0.24–0.29)	0.20 (0.17–0.24)	0.24 (0.22–0.27)
Guanine	0.26 (0.20–0.32)	0.24 (0.14–0.32)	0.25 (0.18–0.32)
Thymine	0.21 (0.17–0.25)	0.27 (0.22–0.32)	0.23 (0.19–0.28)
χ^2 (*P*)	0.0008**	0.02*	0.0000**
%GC	52	44	49
Mean Ti : Tv ratio (range)	1.60 (0.75–16.00)	0.98 (0.00–2.57)	1.25 (0.50–3.00)
g_1	−1.84065**	−0.944012**	−1.093913**
PTP (*P*)	0.001**	0.001**	0.001**

and combined data averaged about 1.00 or above (Tables 2, 3), suggesting that transition substitutions were not saturated and that transitions and transversions could be weighted equally. Skewness tests and permutation-tail probability tests indicated significant hierarchical structure for all three data partitions ($P < 0.01$; Tables 2, 3), and a partition-homogeneity test detected no significant phylogenetic incongruence between stems and loops ($P = 0.456$), suggesting that these two partitions could be combined for subsequent phylogenetic analysis. Unequal stem and loop nucleotide composition (e.g., %G + C, Table 2) and unequal nucleotide frequencies (e.g., Thymine, Table 2) suggested, however, that nucleotide substitution might proceed differently in these two partitions. For these reasons, and because the partition-homogeneity test is not always an accurate method of assessing congruence among partitions (see, e.g., Hipp et al. 2004), phylogenetic analyses were performed separately on stem and loop partitions as well as on the combined data.

Maximum-parsimony (MP).—The method of character mapping and the treatment of gaps as missing data or a fifth character state resulted in identical topologies; the consistency index and nodal support (i.e., bootstrap, or BP, values) were slightly higher when gaps were treated as a fifth character state. For simplicity, therefore, results are reported for phylogenetic analyses performed on 678 parsimony-informative characters (and stem and loop partitions of these) with the DELTRAN option for character mapping and alignment gaps coded as a fifth character state.

Combined-data partition: The best MP estimate (Fig. 2a) resulted in a single tree having 2796 steps, a C.I. of 0.4785, and a retention index of 0.5357. The topology shows two major clades: clade B is a monophyletic Peracarida that excludes Mysida; clade A places the Mysida at the base of a (Stomatopoda + [Syncarida + (Eucarida: Euphausiacea + Decapoda)]) clade. The Isopoda are at the base of the peracarid clade, followed by the Tanaidacea, Cumacea, and the thermosbaenacean *Tethysbaena argentarii.* A bifurcation follows next, resulting in a (Spelaeogriphacea + Amphipoda) clade and a (Mictacea + Lophogastrida) clade (Fig. 2a). Two trees one step longer

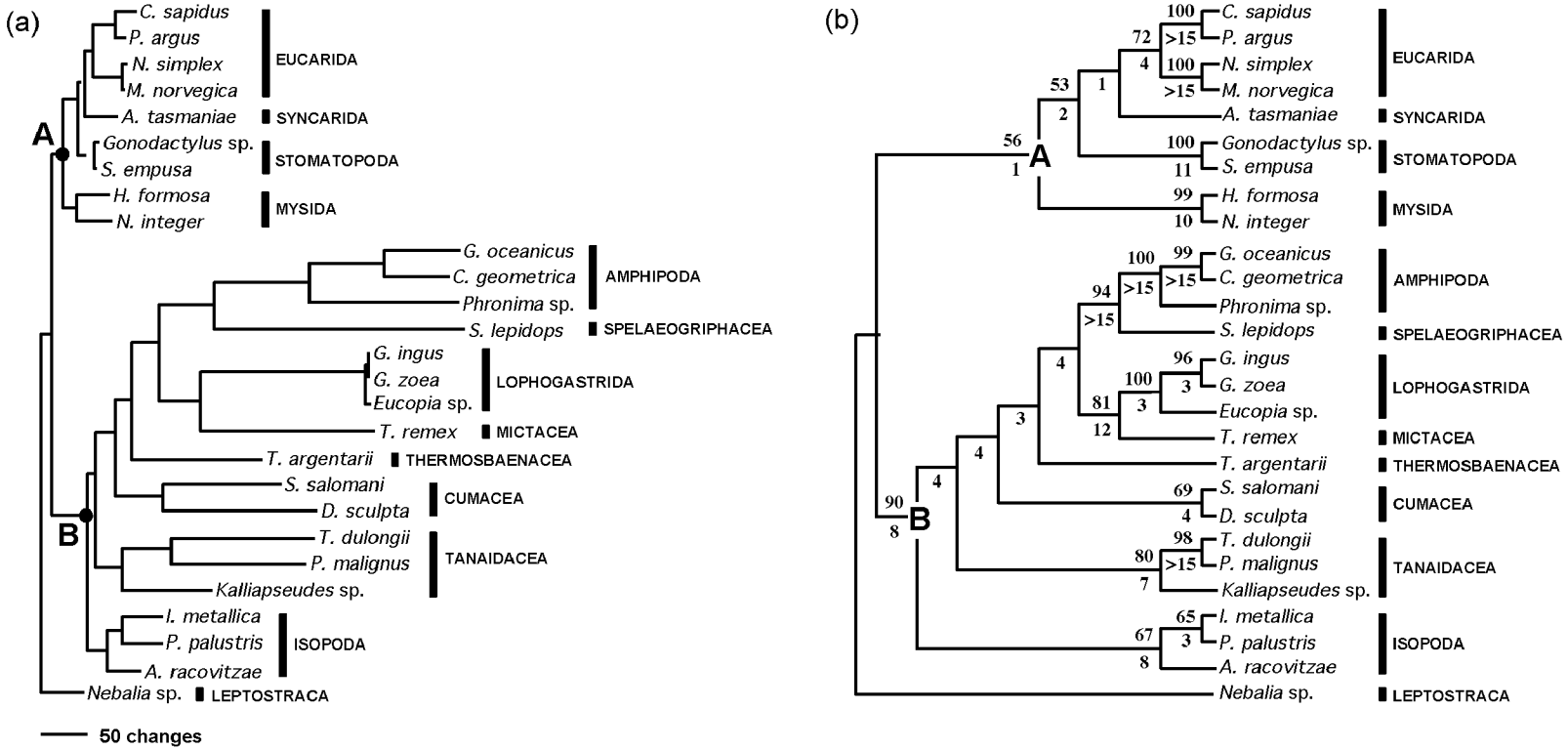

Fig. 2. Phylogram (a) and cladogram (b) of the most parsimonious estimate of phylogeny for 27 crustaceans based on 678 parsimony-informative and equally weighted characters of nuclear small-subunit ribosomal DNA by means of PAUP* 4.0b10 (Swofford 2002). The heuristic-search option was used with 10,000 random sequence-addition replicates. Stem and loop data were combined; gaps were coded as a fifth character state; tree length = 2796 steps; consistency index = 0.4785; retention index = 0.5357 (Farris 1989). Sidebars indicate orders of Crustacea. Node B indicates a monophyletic Peracarida clade as defined by the present study. In (b), numbers above branches indicate nonparametric-bootstrap (Felsenstein 1985) proportions ≥50 based on an MP heuristic search of 1000 pseudoreplicates, each with 100 random sequence additions; numbers below branches indicate decay indices (Bremer 1988, 1994).

than the MP result either switch the syncarid *Anaspides tasmaniae* with the stomatopod clade or place the Mysida as sister to the peracarid clade. These differences are indicated by a decay index of one for these nodes in Fig. 2b. Additional decay indices ≤15 steps are also shown in Fig. 2b. Support is strong (BP ≥ 90; decay index = 8; Fig. 2b) for a monophyletic Peracarida that excludes Mysida but includes the thermosbaenacean *T. argentarii,* so we reject the notion of a pancarid lineage apart from the Peracarida. Similarly strong support (BP ≥ 90; decay indices ≥ 10; Fig. 2b) is seen for individual stomatopod, decapod, euphausid, mysid, lophogastrid, and amphipod clades. An isopod clade receives minimal (BP = 68) and a tanaid clade slightly higher support (BP = 75), and yet both of these clades have relatively high decay indices, of eight and seven, respectively (Fig. 2b). A high bootstrap proportion (94; Fig. 2b) is also seen for the sister-group relationship between the spelaeogriphacean *Spelaeogriphus lepidops* and amphipods, but support is minimal for relationships among other peracarid orders and among nonperacarid malacostracans (e.g., BP ≤ 75; decay indices ≤ 4; Fig. 2b).

The branches are relatively long for tanaids, the mictacean *Thetispelecaris remex,* the basal amphipod *Phronima* sp., the spelaeogriphacean *Spelaeogriphus lepidops,* the thermosbaenacean *Tethysbaena argentarii,* both cumaceans (*Spilocuma salomani* and *Diastylus sculpta*), and the branch leading to the lophogastrid clade (Fig. 2a). In fact, most of the internal branches are short compared to the terminal ones. Phylogenetic estimates may therefore reflect some uncertainty (e.g., in the form of low nodal support) because synapomorphies for clades are relatively few.

Tests of alternative phylogenetic hypotheses suggested by Fig. 1 rejected ($P < 0.05$) a monophyletic Mysidacea (= Mysida + Lophogastrida, as in Fig. 1a, b, c, e, f, and h) but did not reject a sister-group relationship between Mysida and remaining peracarids. A sister-group relationship between the Isopoda and Amphipoda (as in Fig. 1c, d, and f) was found in two trees that were 49 steps longer than the best MP result (Figs. 2a, b), but these hypotheses were significantly longer ($P < 0.01$) and rejected. In contrast, an Isopoda + Tanaidacea clade (as in Fig. 1a, b, and h) was found in three trees that were only four steps longer than the best MP tree, and this hypothesis was not significantly worse ($P \geq 0.25$). A Spelaeogriphacea + Mictacea clade (Fig. 1b, h, and possibly c) was found in two trees that were 23 steps longer than the MP tree, one of which was significantly worse ($P = 0.04$). A ''Bochusacea'' clade (sensu Gutu & Iliffe 1998) comprising the mictacean *Thetispelecaris remex* + Tanaidacea occurred in a single tree that was also 23 steps longer than the best MP tree, but this alternative was rejected ($P = 0.05$). A clade comprising so-called ''relict'' cave-inhabiting taxa (i.e., *Spelaeogriphus lepidops* + *Thetispelecaris remex* + *Tethysbaena argentarii*) appeared in four trees that were 36 steps longer and significantly worse ($P \leq 0.01$) than the MP tree. Finally, an MP search constrained for the best ML topology shown in Fig. 3a produced a tree that was significantly longer (30 steps; $P = 0.02$) than the MP result.

Stem-data partition: The MP analysis of 433 P.I. characters produced nine equally most-parsimonious trees (tree length = 1647 steps; C.I. = 0.4888; R.I. = 0.2668). The strict-consensus topology (not shown) was similar to the best MP tree based on combined data (Fig. 2b); the two had 16 of 25 nodes in common (10 of these within the Peracarida, or clade B; Fig. 2b), but bootstrap support in the stem-based tree was lower for all but the Stomatopoda, Decapoda, Euphausiacea, Lophogastrida, and Amphipoda because of the fewer parsimony-informative characters in the stem partition. Other clades that were recovered included the Eucarida, Tanaidacea, Amphipoda, Isopoda, Lophogastrida, Spelaeogriphacea + Amphipoda, and *Thetispelecaris*

Table 4.—Models selected with Modeltest 3.1.0 (Posada and Crandall 1998) and optimized parameter values for stem, loop, and combined (stem + loop) nuclear small-subunit ribosomal RNA partitions that were used in ML and Bayesian phylogenetic analyses for 27 crustaceans (Appendix 1). n = number of unambiguously aligned nucleotide characters; GTR = general-time-reversible model; TIM = transition model; Γ = gamma-distributed rates; I = proportion of invariant characters; α = shape parameter for the gamma distribution of site-to-site variation; Π = nucleotide (A, C, G, T) frequency; R = relative nucleotide-substitution rate.

	Stems (n = 1099)	Loops (n = 597)	Combined (n = 1696)
Model	GTR + Γ + I	TIM + Γ + I	GTR + Γ + I
ln L	−9340.20815	−5584.26694	−15034.30047
Π_A	0.23940	0.23939	0.24171
Π_C	0.30776	0.21413	0.27633
Π_G	0.24086	0.20087	0.22049
Π_T	0.21198	0.34562	0.26147
I	0.17241	0.29308	0.18268
α	0.64814	0.81195	0.58821
R_{AC}	0.86089	1.00000	0.74052
R_{AG}	3.93819	3.48621	3.45876
R_{AT}	1.19328	0.71170	0.86035
R_{CG}	0.58694	0.71170	0.53632
R_{CT}	2.93350	1.54514	1.97347
R_{GT}	1.00000	1.00000	1.00000

remex (Mictacea) + Lophogastrida, (see, e.g., Fig. 2b).

Loop-data partition: The MP analysis of 245 parsimony-informative characters produced six equally most-parsimonious trees (tree length = 1137 steps; C.I. = 0.4688; R.I. = 0.2486). The strict-consensus topology (not shown) was similar to the best MP tree based on combined data (Fig. 2b); the two had 18 of 25 nodes in common (14 of these within the Peracarida; node B; Fig. 2b), but bootstrap support was lower in the loop-based tree for all but the Euphausiacea, Lophogastrida, and Amphipoda because of the relatively few parsimony-informative characters in the loop partition. The loop-based consensus topology had two more nodes in common with the MP topology for combined data than did the stem-based topology, and an MP analysis of bootstrapped loop data provided higher support (BP = 80) for a monophyletic Peracarida (excluding Mysida) than did the stem data (BP = 63). Further, the loop data recovered more nodes (14) within the Peracarida (i.e., node B; Fig. 2a) than did stem data (10 nodes), even though the loop partition had 43% fewer parsimony-informative characters (Table 3).

Maximum likelihood (ML).—Analyses using the ML method of phylogeny estimation were based on 1696 aligned characters (and stem and loop partitions of these) and used models of nucleotide substitution selected by Modeltest with optimized parameters (Table 4).

Combined-data partition: Fig. 3a shows the best ML estimate (ln L = −15034.30047) based on 1696 characters from combined stem and loop regions of the nSSU rRNA molecule with a general-time-reversal (GTR) model of nucleotide substitution (Lanave et al. 1984, Tavare 1986, Rodriguez et al. 1990) accommodating unequal base frequencies, six types of substitutions, gamma (Γ) distributed site-to-site variation in substitution rates, and a proportion of invariable (I) sites (i.e., GTR + Γ + I). Optimized parameter values are provided in Table 4. A likelihood-ratio test comparing identical ML topologies estimated with and without the assumption of a molecular clock did not find significant heterogeneity in nucleotide-substitution

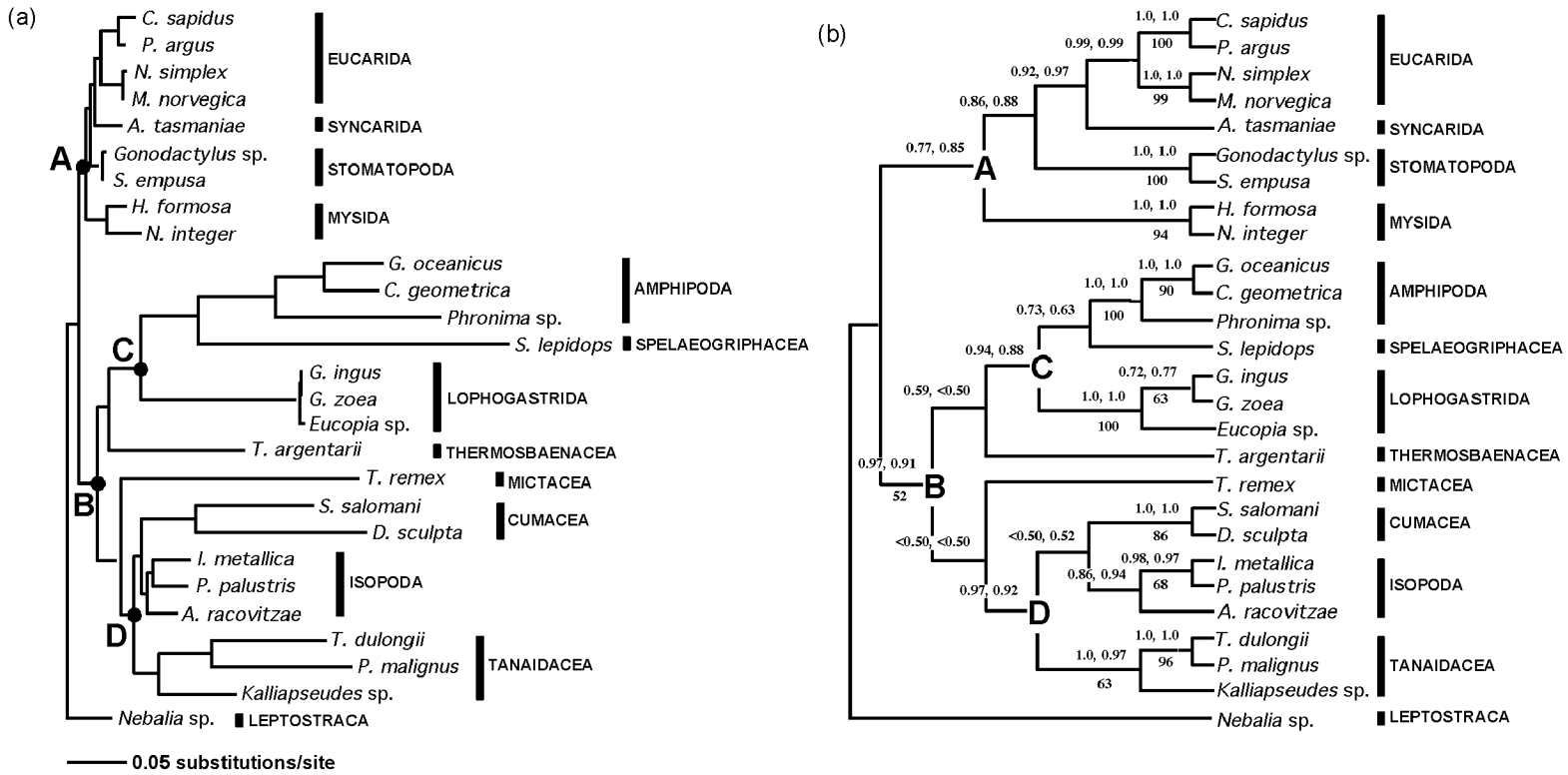

Fig. 3. Phylograms showing the best maximum-likelihood (ML; ln $L = -15034.30047$) estimate of phylogeny for 27 crustaceans based on 1696 equally weighted characters (stem and loop data combined) of nuclear small-subunit ribosomal DNA assuming a GTR + Γ + I model using PAUP* 4.0b10 (Swofford 2002). The heuristic-search option was used with 10 random sequence-addition replicates. Sidebars indicate orders of Crustacea. Node B indicates a monophyletic Peracarida clade as defined by the present study. In (b), the pair of numbers above each branch indicates Bayesian posterior probabilities for the congruent 50%-majority-rule consensus of 30,001 trees when default parameter settings were used with stem and loop partitions (first) linked and (second) unlinked and implemented in MrBayes 3.0b4 (Huelsenbeck & Ronquist 2002); numbers below branches indicate nonparametric bootstrap (Felsenstein 1985) proportions ≥50 based on an ML heuristic search of 200 pseudoreplicates, each with five random sequence additions.

rates among lineages [$\lambda = -2$ ($-15038.39952 + 15034.30047$); $\lambda = 8.1981$; $\alpha < 0.01$, 25 degrees of freedom). For simplicity, therefore, only the results obtained without assumption of a molecular clock are presented.

The best ML phylogeny (Fig. 3a) had 20/25 nodes in common with the MP tree, the most notable of which were (1) node B (indicating a monophyletic Peracarida that excludes the Mysida), (2) node A (placing the Mysida at the base of a [Stomatopoda + (Syncarida + [Euphausiacea + Decapoda])] clade), and (3) the (Spelaeogriphacea + Amphipoda) clade. Node B bifurcates into (1) a Lophogastrida + (Spelaeogriphacea + Amphipoda) clade (node C) having the thermosbaenacean *Tethysbaena argentarii* as its sister taxon, and (2) a Tanaidacea + (Isopoda + Cumacea) clade (node D) having the mictacean *Thetispelecaris remex* as its sister taxon. These relationships do not, however, receive strong bootstrap support (Fig. 3b), and none of the alternative hypotheses described above for MP was rejected ($P \geq 0.06$) by either the K-H or the S-H test. The MP topology (Figs. 2a, b), however, was significantly worse ($P = 0.02$) than the ML tree by the K-H test only. Because the uniform application of specific model-parameter values to bootstrapped pseudoreplicates may have contributed to the lower nodal support in ML than in MP trees, ML analyses were repeated on 100 bootstrapped pseudoreplicates with the GTR + Γ + I model and unspecified model-parameter values, but this approach did not improve BP values for any nodes.

Upon closer inspection, much of the discrepancy between the best MP and ML estimates of phylogeny (Figs. 2, 3) could be accounted for by different positions within the Peracarida of the mictacean *Thetispelecaris remex* and the thermosbaenacean *Tethysbaena argentarii,* two species with relatively long branches in both trees. Long-branched taxa may have a destabilizing effect on phylogenetic inference, particularly under MP (see, e.g., Felsenstein 1978), so the combined data from MP and ML were reanalyzed after the sequences for these two species were deleted. The resulting MP (length = 2359 steps; C.I = 0.5210; R.I. = 0.3053) and ML topologies (ln $L = -13464.64692$) (not shown) were more congruent with respect to peracarid relationships than were those in which the two taxa were included. In general, the Peracarida contained a (Lophogastrida + (Spelaeogriphacea + Amphipoda) clade and a less-resolved (Isopoda + Cumacea + Tanaidacea) clade (i.e., clades C and D, respectively; Fig. 3b), and bootstrap support for peracarid monophyly increased from 90 to 96 in the MP bootstrap-consensus tree once the two problematic taxa were deleted.

Stem-data partition: Stem data were best represented by a GTR + Γ + I model of nucleotide substitution (the same as for the combined partition) for 1099 unambiguously aligned characters with optimized parameter values shown in Table 4. The best ML estimate (ln $L = -9340.20895$; topology not shown) did not recover a monophyletic peracarid clade, and differed most notably from the best ML estimate for combined data in (1) removing the mictacean *Thetispelecaris remex* from the Peracarida and having it branch off the tree first, after the outgroup *Nebalia* sp., and (2) disrupting the Eucarida clade by placing the euphausids *Nyctiphanes simplex* and *Meganyctiphanes norvegica* as sister taxa to the Stomatopoda and not the Decapoda. Therefore, the only ''major'' node recovered from the smaller set of stem data was node D (Fig. 3b), and the stem-based ML tree had only nine of 16 nodes within the peracarid clade in common with the best ML estimate based on the combined partition. Among these, however, was the Spelaeogriphacea + Amphipoda clade.

Loop-data partition: Loop data were best represented by a transition (TIM) model of nucleotide substitution (a more restricted model than GTR having only four substitution types) with gamma distributed rates (Γ) and a proportion of invariant sites (I)

(i.e., TIM + Γ + *I*) for 597 unambiguously aligned characters with optimized parameter values shown in Table 4. In contrast to the ML tree based only on stem data, the best ML estimate (ln $L = -5584.26694$; topology not shown) for loop data did recover eucarid and peracarid clades, but the loop-based ML tree recovered fewer than half of the 16 peracarid nodes seen in the combined-data tree. The ML tree based on loop data alone also differed in two notable ways from those based on stem or combined data: (1) the sister-group relationship between the spelaeogriphacean *Spelaeogriphus lepidops* and the Amphipoda was disrupted (*S. lepidops* was placed within the Tanaidacea, making the latter paraphyletic) and (2) the cumacean clade was placed within the Isopoda, making isopods paraphyletic.

Bayesian analyses.—Bayesian analyses were performed on stem, loop, and combined partitions. Differences in clade credibility values (*P*) were negligible for nodes in 50%-majority-rule-consensus trees whether model-parameter values for a given data partition were specified or not. Results are therefore presented for Bayesian analyses that were conducted when the default settings were used.

Combined data, with stem and loop partitions linked: Figure 3b shows the clade-credibility values for nodes in the 50%-majority-rule consensus of 30,001 trees resulting from a Bayesian analysis and the GTR + Γ + *I* model (i.e., the same model used in the ML analysis of combined data) for 1696 nucleotide characters. The Bayesian consensus topology was similar to MP and ML trees in excluding the Mysida from a monophyletic Peracarida, which received significant support ($P = 0.97$; node B; Fig. 3b). The Bayesian and ML trees (Fig. 3b) were even more similar and had nodes C and D within the Peracarida in common. These nodes received very high *P* values (>0.90; Fig. 3b), and relationships within node D (Lophogastrida + [Spelaeogriphacea + Amphipoda]) were identical under these two methods of phylogeny estimation.

Combined data, with stem and loop partitions unlinked: The topology of the 50%-majority-rule consensus of 30,001 trees when stem and loop partitions were unlinked was highly congruent with that obtained when these partitions were linked (e.g., nodes A, B, C, and D appeared in both consensus topologies; Fig. 3b), except that, in the unlinked analysis, the *P* values for clade B (peracarid monophyly) and several nodes therein were slightly lower. In contrast, support for clade A (Mysida + Stomatopoda + Syncarida + Eucarida) and relationships therein were slightly higher.

A Bayesian analysis performed after the deletion of *Thetispelecaris remex* and *Tethysbaena argentarii*, as for MP and ML, yielded a consensus topology (not shown) similar to that in Fig. 3b; i.e., peracarids were monophyletic ($P = 1.00$) and contained clades C and D, each with $P = 0.71$. Eucarid monophyly was supported ($P = 0.92$), but relationships among the major malacostracan lineages were unresolved.

Stem-data partition: A Bayesian analysis of the 1099 characters in the stem partition was as uninformative as the ML analysis. None of the major peracarid nodes in the Bayesian consensus tree for combined data (Fig. 3b) was recovered with $P > 0.50$ when only stem data were used, nor was the eucarid clade recovered. Instead, it produced a major polytomy of crustacean orders, except for the improbable pairing of the Stomatopoda + Euphausiacea (seen also in the ML analysis based only on stem data), and this clade received surprising support ($P = 0.94$).

Loop-data partition: Similar to the ML estimate obtained with only loop data (597 characters), a Bayesian analysis (consensus topology not shown) supported peracarid monophyly, and the credibility value for this clade ($P = 0.92$) was comparable to that seen with the combined data (see, e.g., Fig. 3b, node B). Clade-credibility values were lower, however, for relationships within the peracarid clade and for those among nonperacarid orders.

LogDet/paralinear distance analyses; combined data.—Topologies were identical regardless of the set of conditions under which searches were conducted (i.e., whether the optimized proportion of invariant sites [$I = 0.18268$] was removed in proportion to nucleotide frequencies estimated from all sites or only from constant sites, both with and without adjustment for the mean number of substitutions over all sites). The resulting phylogram (minimum-evolution score = 1.91057; Fig. 4a) shared the following with topologies estimated under MP (Figs. 2a, b), ML (Figs. 3a), and Bayesian analyses (Fig. 3b): (1) a monophyletic peracarid clade that excluded mysids but not lophogastrids (node B; Fig 4a); (2) a clade comprising stomatopods, the syncarid, and eucarids, with mysids at the base (node A; Fig. 4a); (3) a (Lophogastrida + [Spelaeogriphacea + Amphipoda]) clade (node C; Fig. 4a); (4) a branching order that was identical within amphipod, isopod, and tanaid clades; and (5) long branches for the same peracarid taxa.

The distance-based phylogeny differed most from the MP, ML, and Bayesian estimates with respect to relationships within clade A; namely, the Eucarida clade was disrupted, and stomatopods and not decapods were the sister taxa to euphausids. This relationship did not receive strong bootstrap support (BP = 54; Fig. 4b), however. Peracarid monophyly (clade B) received higher support than under any other analytical method (BP = 100; Fig. 4b). Support for clade C (BP = 60; Fig. 4b) was higher than in MP (Fig. 2b) but lower than in Bayesian analyses (Fig. 3b), and support was lacking for a (Tanaidacea + [Isopoda + Cumacea]) clade that received high support under Bayesian analyses (node D, Fig. 3b). The topology obtained when the long-branched taxa *Thetispelecaris remex* and *Tethysbaena argentarii* were deleted was completely congruent with that from the original analysis when all taxa were included.

Stem-data partition: The phylogeny (not shown) inferred from 1099 stem characters had 20/25 nodes in common with the combined-data tree (Fig. 4a) and recovered nodes A and B. Relationships within clade A were identical in the two cases, whereas those within the peracarid clade differed slightly, primarily because a rearrangement of *Thetispelecaris remex* disrupted clade C. The only sister-group relationship recovered between peracarid orders was the spelaeogriphacean + amphipod clade.

Loop-data partition: The phylogeny (not shown) inferred from 597 loop characters had slightly fewer (17/25) nodes in common with the combined-data tree (Fig. 4a) than did the stem-based distance estimate, but it still recovered node B (indicative of peracarid monophyly). Node A (i.e., a clade comprising mysids and nonperacarid malacostracans) was absent, as were eucarid and tanaid clades, but the loop data did recover clade C (Lophogastrida + [Spelaeogriphacea + Amphipoda]), a peracarid clade that was absent in the stem-based data.

Discussion

Ours is the first phylogenetic study of peracarids, to our knowledge, that includes full-length nSSU rDNA sequences for at least one representative of every peracarid order, and from it we can conclude: (1) MP, ML, Bayesian, and distance analyses of the combined (stem + loop) data indicate that the superorder Peracarida is a monophyletic malacostracan lineage that includes the orders Amphipoda, Cumacea, Isopoda, Lophogastrida, Mictacea, Spelaeogriphacea, Tanaidacea, and Thermosbaenacea. (2) All phylogenetic estimates, regardless of method, refute monophyly of the Mysidacea; the order Mysida is excluded from the Peracarida, but its affinity with nonperacarid malacostracans is equivocal (except in Bayesian analyses). (3) A fully resolved Peracarida is lacking, in large part because of the presence of short internal branches between orders and much longer branches within the orders themselves. (4) ML and Bayesian

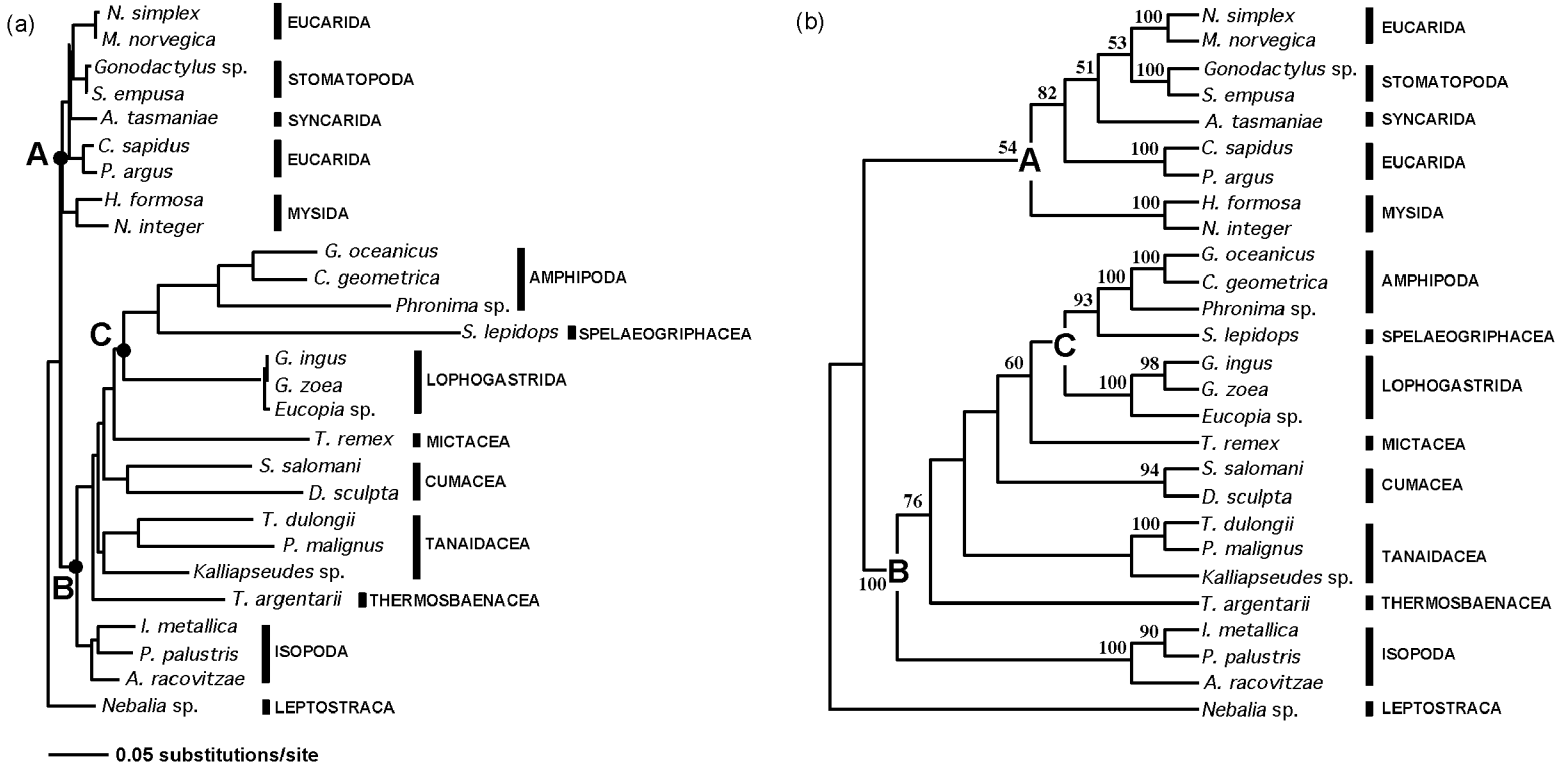

Fig. 4. Phylogram (a) and cladogram (b) showing the best estimate (minimum-evolution score = 1.91057) of phylogeny for 27 crustaceans based on 1696 equally weighted characters (stem and loop data combined) of nuclear small-subunit ribosomal DNA based on LogDet/paralinear-transformed distances (Lake 1994, Lockhart et al. 1994) when $I = 0.18268$ was removed in proportion to nucleotide frequencies estimated from constant sites while adjustment was made for the mean number of substitutions over all sites according to the heuristic-search option in PAUP* 4.0b10 (Swofford 2002). Sidebars indicate orders of Crustacea. Node B indicates a monophyletic Peracarida clade as defined by the present study. In (b), numbers above branches indicate nonparametric bootstrap (Felsenstein 1985) proportions ≥50 based on a heuristic search of 1000 pseudoreplicates.

analyses suggest two major clades within the Peracarida, one comprising (Lophogastrida + [Spelaeogriphacea + Amphipoda]) and another comprising a less resolved (Isopoda + Tanaidacea + Cumacea); the (Spelaeogriphacea + Amphipoda) clade is also supported by MP and distance analyses. (5) The phylogenetic affinities of the orders Thermosbaenacea and Mictacea with respect to other peracarids remain uncertain. (6) Eumalacostraca, traditionally comprising the suborders Eucarida, Syncarida, and Peracarida, is not monophyletic and does not include the Stomatopoda. Instead, the Eucarida and Syncarida are sister taxa, more related to the Mysida and Stomatopoda than to the Peracarida.

Molecules and morphology.—Richter & Scholtz (2001) provide a concise and thorough summary, which we draw upon here, of the issues relevant to malacostracan (including peracarid) evolution and of key morphological features associated with the constituent groups. A benefit of having a molecularly based phylogenetic hypothesis is the ability to reevaluate hypotheses of morphological evolution. We do so below for some of the more contentious hypotheses and ''problematic'' taxa, but our efforts are hampered somewhat in two respects: (1) the molecular estimate is not completely resolved (i.e., not *all* nodes receive strong support), and (2) malacostracan and peracarid phylogeny enjoy very little consensus despite the vast body of literature on this topic, because different authors use different approaches to phylogeny reconstruction and/or authors interpret characters differently. This latter point reflects concerns expressed by Watling (1999) that the assessment of character homology has not received sufficient attention and that cladistic analyses of phylogenetically uninformative (not to be confused with parsimony uninformative) characters serve only to confound our understanding of peracarid relationships.

Thermosbaenacea: Our findings based on nSSU rDNA clearly place thermosbaenaceans within the Peracarida and refute the notion that they merit a separate eumalacostracan superorder, Pancarida, as depicted in Fig. 1a, b, and h. The presence of expansion segments (Table 1; Appendix 2) in the nSSU rRNA gene of *Tethysbaena argentarii* can also be viewed as evidence that this species belongs in the Peracarida (see further discussion below), but we cannot resolve the phylogenetic position of *T. argentarii* within the Peracarida. Thermosbaenaceans can be distinguished from other peracarids by the lack of oostegites and by the presence of a dorsal region for brooding their developing young that is formed from the carapace. In other peracarids, the brood pouch, if present, is ventral and derived from oostegal plates, and our understanding of the evolution of this complex character and its phylogenetic significance is probably incomplete. For example, Fryer (1964) thought the absence of the ''typical'' ventral peracarid brood pouch among sphaeromatid and epicarid isopods to be significant, and Watling (1999) argued against the homology of oostegites and oostegal-plate formation in peracarids.

Richter & Scholtz (2001) performed a cladistic study of 93 characters for 19 malacostracans using parsimony and concluded that thermosbaenaceans are the sister-group of peracarids, citing embryological differences (e.g., the number and arrangement of ectoteloblasts). Some of their cladograms, however, position thermosbaenaceans within the Peracarida. Frankly, few morphological features ally thermosbaenaceans with peracarids. The most obvious is a lacinia mobilis in adults (see also Richter et al. 2002), but Richter & Scholtz (2001) also identified a maxillipedal epipod that functions in ventilation (although Watling [1999] challenges the synapomorphic status of this character, and this epipod is absent in amphipods and mictaceans), the absence of epipods on thoracopods 2–8 (although these are present in amphipods and lophogastrids), paired entodermal plates, direct

development, and the presence of yolk on the posterior portion of the embryo.

Mysida, Lophogastrida, and Eucarida: Questions concerning the phylogenetic affinities among these taxa and their position within the Malacostraca have persisted for years. The Mysida and Lophogastrida are considered most frequently to be members of a single order, the Mysidacea (Fig. 1 a–c, e, f, h), placed by Boas (1883) in the Schizopoda along with the Euphausiacea (shrimp-like ''krill''). Calman's (1904, 1909) revision of malacostracan classification transferred mysidaceans to the superorder Peracarida and allied euphausids with decapods in the superorder Eucarida. Mysidaceans as such have long been recognized as a ''connecting link'' between peracarids and other malacostracan groups (Siewing 1963: p. 99) or ''not too far removed from the syncarid/eucarid ancestor of the Peracarida'' (Dahl 1992: p. 344). Hence, this order often appears in a basal position in peracarid phylogenetic schemes (Fig. 1c, e, f, h). None of our best estimates of phylogeny (Figs. 2–4) supports mysidacean monophyly, however. Instead, all of our analyses definitively separate mysids and lophogastrids and include only the latter within the Peracarida. The Mysida, in contrast, are allied with eucarid, syncarid, and hoplocarid taxa, albeit equivocally.

Among the features supporting the traditional view of mysidacean monophyly are a nonrespiratory epipod on thoracopod 1 (Hessler 1983), the presence of lateral extensions associated with bipartite cones of the ommatidia (although bipartite cones are seen in other peracarids), and the presence of a posterior tooth on the labrum (Richter & Scholtz 2001), but these latter characters have been studied in relatively few peracarids.

Our finding against mysidacean monophyly is not entirely novel. Watling (1983) challenged the mysidacean affinity with peracarids and completely revised eumalacostracan classification by proposing a ''pen and paper'' phylogenetic scheme (i.e., one that is not inferred from any explicit criteria of phylogeny reconstruction) that abandoned all previous notions of peracarid monophyly. Watling (1983) proposed the removal of mysids and lophogastrids from peracarids and their alliance with syncarids and eucarids, on the basis, in part, of features of the carapace, compound eye, mandible, blood system, and maxilliped. Mysids and lophogastids were in turn differentiated by more subtle differences in these same characters. Calman (1904) also recognized similarities (e.g., the ''caridoid facies'') among euphausids, lower decapods, mysids, and lophogastrids, but he did not use these to justify a close relationship among these taxa.

Watling (1999) revised his earlier view of peracarid evolution upon a critical reassessment of carapace, foregut, and oostegite homology, and a synthesis of evidence from both morphology and developmental genetics. The resulting phylogenetic scheme retained the more inclusive Eucarida and placed mysids close to euphausids and decapods (a view supported by Jarman et al. [2000] on the basis of nuclear large-subunit [nLSU] rDNA but not by our analyses of nSSU rDNA, discussed further below). As shown in Fig. 1g, Watling (1999) presents two major divisions within the Eumalacostraca: a ''caridoid'' clade (Syncarida + Lophogastrida + Mysida + Euphausiacea + Decapoda), characterized by changes in the blood system and abdominal musculature, and a second clade comprising all remaining peracarids, characterized by features associated with the mandibles, cephalothoracic shield, and the manner by which Hox-gene expression controls thoracic-limb development. Our findings (Figs. 2–4) do not support either of these two major divisions, particularly with respect to the alliance between lophogastrids and eucarids, and our findings differ also with respect to relationships among the remaining peracarids in Watling's second clade.

We find this phylogenetic conflict puzzling, especially considering the host of fea-

tures cited by Watling in support of a close relationship between lophogastrids, mysids, and eucarids. Watling's summary of eumalacostracan carapace and foregut features (1999: tables I and II, respectively) does not unequivocally justify the removal of lophogastrids or mysids from the Peracarida, however. For example, a dorsal fold in the carapace is seen in lophogastrids, mysids, euphausiaceans, and dendrobranchiate shrimp, but it is also present in thermosbaenaceans (Watling 1999: table I). Another carapace feature, thoracic pleural folds, involves the first thoracic somite in lophogastrids and mysids but also in thermosbaenaceans and spelaeogriphaceans. Admittedly, Watling makes a strong case for moving away from assessing relationships solely on the basis of presence or absence of character states; he attempts to look for shared patterns of development for a given feature such as the carapace. Even the evolutionary pattern for carapace development proposed by Watling (1999: pp. 76–77) does not, however, preclude origin of two lines from a mysidacean-like ancestor, one leading to peracarids via lophogastrids and another leading to eucarids via mysids.

Watling (1999) also cites aspects of foregut morphology that distinguish mysids, euphausids, and decapods from thermosbaenaceans and other peracarids and place lophogastrids in an intermediate position, but his summary of foregut features (Watling 1999: table II) does not entirely substantiate this distinction. For example, euphausids (see, e.g., Suh 1990, Ullrich et al. 1991) lack an enlarged anterior foregut and calcified teeth along dorsolateral ridges of the gastric mill, but these two features are present in mysids and decapods. In contrast, De Jong & Casanova's (1997a, 1997b) comparative studies of the foregut suggest that lophogastrids, and not mysids, share more foregut features with decapods. A more recent foregut study (De Jong-Moreau & Casanova 2001) supports mysidacean monophyly, however, placing lophogastrids (specifically *Gnathophausia gracilis*) as the more primitive lineage. A preliminary study (De Jong-Moreau et al. 2000) of the midgut and hepatopancreas of mysidaceans also suggests monophyly for the group. Many of these early studies include relatively few taxa, so claims of mysidacean monophyly on the basis of foregut morphology may be premature. These studies may indeed prove to be useful in elucidating phylogenetic relationships among peracarids and other malacostracans, but additional species must be studied for a better assessment of the distribution of foregut and midgut character states within and among lineages. The same can be said for preliminary yet promising studies on Hox- and *engrailed*-gene expression, but the *engrailed* studies on crustaceans to date (Scholtz et al. 1993, 1994; Scholtz 1995) indicate that patterns of expression for this gene in mysids are more similar to patterns observed in decapods than to those in amphipods.

The summary phylogeny proposed by Richter & Scholtz (2001; Fig. 1h) and based on a cladistic analysis of morphological characters differs from Watling's (1999) and ours in (1) retaining mysidacean monophyly, (2) placing the Mysidacea within the Peracarida, and (3) abolishing the Eucarida sensu stricto. In their scheme, decapods are basal to a ''Xenommacarida'' clade comprising syncarids, euphausids, pancarids, and peracarids and characterized by apomorphies associated with the ommatidia and thoracopods 2–8, but their character matrix (Richter & Scholtz 2001: appendix) indicates that not all of these apomorphies are unambiguous, as the authors state.

Isopoda, Cumacea, and Tanaidacea: All of our phylogenetic analyses place isopods within the Peracarida, refuting Nyland et al.'s (1987) opinion, based on myocardial ultrastructure, that this order represents an independent eumalacostracan lineage. Nor did any of our phylogenetic estimates group isopods and amphipods as sister taxa (e.g., Schram's [1986] ''Edriophthalma''; see also

Schram & Hof 1998), although this relationship appears in many alternative hypotheses (Fig. 1c, d, and f). Instead, our findings invariably place isopods in a clade with both tanaids (a relationship advocated by several authorities; e.g., Fig. 1a, b, h) and cumaceans (similar to what was proposed by Siewing [1953, 1963] and similar to Watling's [1981] ''mancoid'' lineage, which also included spelaeogriphaceans). Although support for this clade is strong in MP (Fig. 2b) and Bayesian (Fig. 3b) analyses, relationships within it remain equivocal. Various characters have been observed to support the Isopoda + Tanaidacea + Cumacea relationship suggested by our molecularly based phylogenetic estimates: (1) a reduction of the oostegites upon molting after each brood (Siewing 1953); (2) a reduced carapace and absence of epipods on thoracopods 2–8 (although this feature is also seen in spelaeogriphaceans) (Hessler 1983); and (3) a variable number of ectoteloblasts (also seen in Mysida), a dorsally folded embryo, and similarities in midgut formation and in the manca (Richter & Scholtz 2001). A close relationship among these three taxa is also seen in one of Richter & Scholtz's (2001) most parsimonious cladograms.

Amphipoda: Consensus is good among systematists that amphipods are a monophyletic clade within the Peracarida, and our molecularly based phylogenetic analyses (Figs. 2–4), including those with additional amphipod species (not shown), substantiate this view. We will therefore not review the several synapomorphies that characterize this clade (see, e.g., Dahl 1977). Additional evidence that amphipods are peracarids is their having expanded nSSU rRNA genes (Table 1), and evidence of their monophyly is in the presence of expansion segments in the V5 and V8 regions of the nSSU rRNA molecule (Appendix 2), i.e., a molecular synapomorphy for this clade. Considerable debate, however, has surrounded the position of amphipods within the Peracarida (Fig. 1).

Our findings consistently place amphipods sister to spelaeogriphaceans (discussed below), and these taxa occur with lophogastrids in a clade. This view is not consistent with the more recent phylogenetic hypotheses set forth by Watling (1999; Fig. 1g) and Richter & Scholtz (2001; Fig. 1h). Nor is it consistent with that of Poore (in prep.), who confirmed Wagner's (1984) cladogram (Fig. 1c) on the basis of parsimony analyses of twice as many (93) morphological characters. Watling (1999) placed amphipods in a basal position with respect to other peracarids (excluding isopods) on the basis of the presence of a functional maxillipedal epipod (even though the epipod is lost in amphipods) and characters associated with the heart (which are not, however, unambiguously apomorphic). Richter & Scholtz (2001; Fig. 1h), in contrast, place amphipods in a more derived position with respect to other peracarids on the basis of two unambiguous apomorphies: (1) a continuous anteroposterior degree of differentiation in the development of the segments bearing the first and second antennae and the mandibles from the nauplius to the postnauplius larva, and (2) the absence of a palp on the second maxilla.

Spelaeogriphacea: We find no evidence cited in the morphological literature to support a Spelaeogriphacea + Amphipoda clade, yet this clade appeared consistently in all of our phylogenetic estimates based on the nSSU rRNA data (Figs. 2–4). Instead, the Mictacea are proposed most often as the sister group of spelaeogriphaceans (Fig. 1b, h, and possibly c). In support of this latter view, Richter & Scholtz (2001) identified two characters: the first maxilliped lacking a palp and the absence of compound eyes. These two characters are homoplasious, however, with respect to amphionids and bathynellaceans, respectively. Future studies should include sequence data from additional species of spelaeogriphaceans to reveal whether the relationship with amphipods persists, but we hope that these preliminary findings encourage fur-

ther investigation into a morphological explanation for this intriguing relationship. The most recent morphology-based cladistic study of peracarids (Poore, in prep.) reveals no support for a spelaeogriphacean + amphipod relationship. We note, however, that Richter & Scholtz (2001) identified some character states shared by spelaeogriphaceans and amphipods, although these are symplesiomorphies and not synapomorphies. They include a second maxilla with a vestigial or absent palp and the presence of yolk on the posterior portion of the embryo.

We can make some interesting observations, however, that taken together might suggest a possible evolutionary scenario linking amphipods and spelaeogriphaceans: (1) amphipods, although ecologically diverse, are known from the same habitats to which spelaeogriphaceans are restricted (groundwater and freshwater pools in caves); (2) spelaeogriphaceans have an ancient fossil record (discussed below), whereas amphipods do not; and (3) amphipods are considered a highly derived peracarid lineage with several autapomorphic features. However unlikely, it is perhaps possible that amphipods arose from a spelaeogriphacean-like ancestor that was once more widespread.

Mictacea, Bochuscaea, Cosinzeneacea: Unfortunately, we cannot assess the validity of these three taxonomic categories, the species they comprise, or their phylogenetic affinities without additional sequence data from species of *Mictocaris* and *Hirsutia. Thetispelecaris remex* has been classified, along with *Hirsutia*, as a member of the Bochusacea (sensu Gutu & Iliffe 1998), a novel taxonomic arrangement that has not received much published scrutiny by systematists. The more traditional view places *Hirsutia* and *Mictocaris* within the Mictacea. Because we follow the classification of Martin & Davis (2001) here, we place *T. remex* within the order Mictacea (Appendix 1), but we acknowledge that this species is not a ''typical'' mictacean (if such a thing exists!). Nevertheless, our preliminary results suggest that a second novel order, Cosinzeneacea, erected by Gutu (1998) to include the Spelaeogriphacea and Mictacea, may not be a natural taxon, because of the strong sister-group relationship between the spelaeogriphacean *Spelaeogriphus lepidops* and amphipods that appears in all of our phylogenetic estimates (Figs. 2–4).

Syncarida: Complete understanding of the relationship between peracarids and other malacostracans is somewhat hampered by the lack of specimens suitable for molecular study for rare but key taxa (e.g., the syncarid order Bathynellacea and the eucarid order Amphionidacea). Among our molecularly based phylogenetic estimates, only Bayesian analyses provide support for nodes delineating a mysid → hoplocarid → syncarid → eucarid branching order. Syncarids are an ancient lineage with a Permian fossil record (see, e.g., Schram 1982, Briggs et al. 1993) and they are considered most often to be a somewhat isolated (Calman 1904) or primitive eumalacostracan group. Dahl (1992), for example, cites the absence of a dorsal fold in the carapace and thoracic morphology (with respect to ventilation) in syncarids as plesiomorphic features, and Oshel & Steele (1988) view the syncarid foregut as plesiomorphic compared to that of eucarids and peracarids. Bayesian analyses of the molecular data support a close syncarid + eucarid relationship, and Dahl (1992) notes a few features shared by the Syncarida and Eucarida that support this result, including naupliar eyes, antennular statocysts, and a proximal articulation among thoracopods.

Hoplocarida: The relationship of the Hoplocarida (represented in the present study by the order Stomatopoda) with other malacostracans has fostered considerable debate as well (reviewed in Watling et al. 2000), but two alternative views have generally prevailed. One considers hoplocarids an independent offshoot that diverged early in malacostracan evolution (i.e., one that warrants its own subclass; see, e.g., Siewing

1963, Schram 1986, Martin & Davis 2001; Fig. 1a, d, h). Another view considers the Hoplocarida a superorder within the subclass Eumalacostraca, equivalent in rank to the Syncarida, Peracarida, and Eucarida. Richter & Scholtz (2001), for example, place stomatopods as the basal eumalacostracan lineage and not as an independent malacostracan subclass. In our study, only Bayesian analyses of the molecular data permit an unequivocal assignment of stomatopods within the Malacostraca, but none of our phylogenetic estimates, regardless of the method used to generate them, places stomatopods basally with respect to other ingroup malacostracans.

Long branches.—Long branches appear in all peracarid orders except the Mysida (which we exclude from the Peracarida), the Lophogastrida, and the Isopoda. Long branches are generally viewed as an indication that a lineage either has had an ancient divergence followed by an extended period of independent evolution or has an accelerated rate of evolution. We can rule out the latter possibility because a likelihood-ratio test did not find evidence of significant heterogeneity in nucleotide-substitution rates among lineages for a given topology. Ancient divergences, in contrast, remain a likely explanation. For example, the fossil record (Schram 1982, Briggs et al. 1993) reveals that spelaeogriphaceans and tanaids extend to the Carboniferous and that Permian cumaceans are not far behind, and all three of these lineages have relatively long branches in MP, ML, and distance phylograms (Figs. 2a, 3a, 4a, respectively). The correspondence between lineage longevity (as indicated by the fossil record) and branch length is not upheld in all cases, however. For example, fossil thermosbaenaceans are known only from the Quarternary, and amphipods only from the mid-Tertiary, yet species from both groups have relatively long branches in our trees. In contrast, the Isopoda, which are known from the Carboniferous, have relatively short branches. Further, the oldest isopod lineage, the Phreatoicidea, is not basal to the more recent isopod families represented in our study (see Figs. 2a, 3a, 4a). Lophogastrids, on the other hand, have a relatively ancient (Triassic) fossil record, and yet both lophogastrid families we included exhibit very short branches within their clade (Figs. 2a, 3a, 4a). Differences in evolutionary rates may therefore indeed exist among the taxa we used in this study, despite the likelihood-ratio test.

Highly divergent lineages may also be explained, in part, by a limited dispersal capability (e.g., as for tube-dwelling tanaids and other deep-sea species) and/or patchy distribution (e.g., the occurrence of spelaeogriphaceans in isolated habitats in South America, South Africa, and Australia). Three orders, represented by *Thetispelecaris remex, Tethysbaena argentarii,* and *Spelaeogriphus lepidops,* all exhibit long branches regardless of phylogenetic methodology (Figs. 2a, 3a, 4a), but these never ''attract'' and form a clade in any of our phylogenetic analyses, so they probably represent remnants of quite different and ancient peracarid lineages that were once more widespread. It has been suggested that thermosbaenaceans (Wagner 1994) and spelaeogriphaceans (Poore & Humphreys 2003) are Tethyan relicts that became stranded in groundwater as a result of marine regression. Sequence data from additional species in these three orders are needed to assess this claim further and to establish their affinities with other peracarids.

Long branches may be a source of systematic error in phylogeny estimation (Felsenstein 1978, Anderson & Swofford 2004), particularly when parsimony is used. For example, clades having a single long branch may be misplaced and/or placed more basally in a tree because synapomorphies with their true sister group are eroded. This effect probably explains the lack of nodal support observed in Figs. 2a, 3a, and 4a among many of the long-branched peracarid lineages. Not all of the long-branched peracarids are randomly attracting

each other to produce spurious clades, however; i.e., many branches that are long, and potentially *could* attract one another, do not (e.g., *Thetispelecaris remex*, *Tethysbaena argentarii*, and *Spelaeogriphus lepidops*, as discussed above). On the contrary, many cases in which long branches do attract in our phylograms (Figs. 2a, 3a, 4a) form quite ''sensible'' clades (e.g., the Amphipoda, Cumacea, and Tanaidacea clades). The presence of relatively short internal branches within the peracarid clade (node B; Figs. 2a, 3a, 4a) is also potentially problematic and can lower clade support. Our findings indicate, however, that certain clades remain stable despite the short branches connecting their component taxa (e.g., the Isopoda and the larger Isopoda + Tanaidacea + Cumacea clade; Figs. 2a, 3a, 4a).

Comparisons among molecular studies.—Jarman et al. (2000) conducted MP and ML analyses on partial nuclear large-subunit (nLSU) rDNA data to investigate eumalacostracan relationships, and their study included six of the nine putative peracarid orders. They found mysids to be the sister group of euphausids, and a syncarid was basal to this clade. This result contradicts ours, based on nSSU rDNA, that indicated strong support for a monophyletic Eucarida in which euphausids and decapods are sister taxa and mysids are more basal. The discrepancy between the two molecular studies is perplexing, because nuclear SSU- and LSU rRNA genes are not truly independent but are linked by the rRNA operon. One might therefore expect them to yield congruent gene trees, although the trees might differ in nodal support. Indeed, nuclear SSU and LSU rRNA partitions have been combined in numerous phylogenetic studies for diverse taxa, including crustaceans (e.g., Crandall et al. 2000, Oakley & Cunningham 2002).

The stem and loop partitions of the nLSU rDNA data used in the Jarman et al. (2000) study may have different characteristics (e.g., nucleotide composition or heterogeneity in substitution rates) that could confound phylogenetic estimation when a single model of evolution is used to describe the combined data, as was done by the authors. Another possible explanation for the discrepancy between the nuclear SSU and LSU rDNA results might be differences in taxonomic sampling. For example, the focus of Jarman et al.'s (2000) study was eumalacostracan taxa, so stomatopods were not included. The authors also included decapods from two families of dendrobranchiate shrimp, whereas our study used sequences from a more derived decapod suborder, the Pleocyemata. These differences aside, some significant phylogenetic similarity exists between the two studies, namely, that mysidacean monophyly is rejected and that lophogastrids, not mysids, appear within a peracarid clade. Jarman et al. (2000) also suggested, as do we, close relationships between amphipods and lophogastrids and among isopods, cumaceans, and tanaids.

Some of our results are also consistent with those of Meland's (2003) phylogenetic study of malacostracan lineages. Meland likewise used nSSU rDNA but only for the more variable V4–V7 regions of the SSU rRNA molecule. His study's primary focus was relationships within the Mysida, so he included only four of nine peracarid orders. Nonetheless, both the Meland (2003) study and ours support the removal of the Mysida from the Peracarida sensu stricto.

As stated above, our intent was not to infer relationships within peracarid orders, but our finding that an asellote was ancestral to a phreatoicid isopod, and that a valviferan was the most derived of the three, agrees with the results of Dreyer and Wägele (2002) and Meland (2003) for nSSU rRNA and also with those of Wetzer (2002) based on mitochondrial 12S rDNA (although this result changed when the author explored the effects of different character weighting and combining different gene partitions).

Length heterogeneity and expansion seg-

ments.—The presence of rapidly evolving expansion segments that contribute to extremely long nSSU rRNA sequences has been noted previously in peracarids but only for two of the nine putative orders (Isopoda: Choe et al. 1999; Held 2000; Dreyer & Wägele 2001, 2002; Mattern & Schlegel 2001; Meland 2003; Amphipoda: Meland 2003). The present study adds representatives from the Cumacea, Mictacea, Spelaeogriphacea, Tanaidacea, and Thermosbaenacea to the list of peracarid orders with expanded nSSU rRNA genes (Table 1; Appendix 2).

The reason why expansion segments are found in the nSSU rRNA genes of some taxa and not others is unknown, but our results (not shown) from PCR amplification and DNA sequencing in this study indicate a correspondence between peracarid specimens that have an expanded nSSU rRNA gene and the presence of an endosymbiont. A similar correspondence between expanded nSSU rRNA genes and endosymbionts has been observed for certain branchiopod lineages (e.g., cyclestherid conchostracans and cladocerans; Spears & Abele 2000), but the significance of this finding remains unknown.

Expansion segments can supplement phylogenetic information contained in the more conservative core regions of the gene. This effect has been demonstrated for serolid isopods (Held 2000) and some oniscid isopods (Mattern & Schlegel 2001). Dreyer and Wägele (2002) were also able to detect phylogenetic patterns in DNA insertions and deletions among several families of isopods. Different species of the valviferan isopod *Idotea* were sequenced in the present study (*I. metallica*) and in Meland (2003; *I. baltica*), yet both had longer nSSU rDNA sequences than any other taxa in the respective studies. Meland sequenced only from the V4 to the V7 regions of the gene, but the lengths of the expansion segments in this region were comparable for the two species of *Idotea,* further supporting the view that features of expansion segments in nSSU rRNA genes can be clade specific and phylogenetically informative. Expansions segments, when present, occur primarily in the V4 and V7 regions of the molecule (Appendix 2). The one exception we found was among amphipods, which possessed in addition expanded V5 and V8 regions and which we interpret as a synapomorphy for this group.

Jarman et al. (2000) mention that some of their partial nLSU rDNA eumalacostracan sequences had expansion segments, but they did not provide details of how these were distributed among the taxa they sequenced.

Our observation that mysids and lophogastrids lack expansion segments (Table 1) might be taken as evidence of evolutionary relatedness and viewed as justification for a monophyletic Mysidacea, but all of our phylogenetic analyses (Figs. 2–4) of malacostracan nSSU rDNA suggest otherwise by conclusively separating these two taxa and positioning the Lophogastrida, and not the Mysida, within the Peracarida sensu stricto. Clearly, the absence of expansion segments in the nSSU rRNA gene is a plesiomorphy among malacostracans, as this is the condition in the outgroup *Nebalia* sp. and all other nonperacarid species included in this study, except for the lophogastrids.

Two alternative evolutionary scenarios could explain the absence of expansion segments in lophogastrids: (1) an expanded nSSU rRNA gene is a synapomorphy for the Peracarida if this taxon excludes Mysida (i.e., an expanded nSSU rRNA gene was present in the common ancestor of peracarids) and this character state was subsequently lost in the lophogastrid lineage, and (2) lophogastrids represent the basal peracarid lineage (a view not inconsistent with the fossil record), and the expansion of nSSU rRNA genes occurred soon after in peracarid evolution. Our results strongly reject mysidacean monophyly, but scenario (2) above would be consistent with a paraphyletic Mysidacea that has Mysida followed by Lophogastrida at the base of the

Peracarida. A decay index of one for the Mysida node (Fig. 2b) indicates that this latter view is plausible.

MP analyses of the bootstrapped molecular data kept relationships among peracarid lineages equivocal, for the most part (Fig. 2b). Bayesian analyses (Fig. 3b) provided more resolution but still did not identify any particular group as being basal. Lophogastrids are basal, however, in clade C of Fig. 3. Adding taxa to phylogenetic analyses can often reorder branches, so the inclusion of nSSU rDNA data for additional peracarid species, particularly mictaceans and thermosbaenaceans, might result in a phylogeny that firmly establishes a basal peracarid lineage, possibly the Lophogastrida. This result would in turn reveal which of the two scenarios of expansion-segment evolution applies, although we favor the second hypothesis for two reasons: (1) ample morphological evidence supports the Lophogastrida as a somewhat primitive taxon (discussed above), and (2) we are aware of no examples of species that have lost expansion segments once their presence was established within a clade.

Data partitions.—The stem and loop partitions of the SSU molecule for peracarids studied herein have very different nucleotide frequencies, but combining these data obscures the difference and may adversely affect accurate phylogeny estimation. In general, LogDet/paralinear-distance analysis of the combined data did not improve phylogenetic resolution over that of MP and ML, possibly because this method does not account for among-site substitution-rate heterogeneity. We suspect that a combination of nucleotide compositional bias and substitution-rate heterogeneity throughout the nSSU rDNA gene contributes both to the differences we observed in the MP, ML, and distance estimates using combined data and to the lack of nodal support in the bootstrapped ML result. We found that the faster-evolving loop partition was generally more informative than its stem counterpart, and Bayesian consensus trees based on loop data were more congruent with those based on combined data, even though stem data included almost twice the number of characters. The presence of a monophyletic peracarid clade that excluded mysids remained a consistent feature, however, in all topologies whether they were inferred from only stem or loop data, despite the smaller number of nucleotide characters in each of these partitions. The same was true no matter what method of phylogenetic estimation was used.

We therefore recommend against the a priori use of a single model of nucleotide evolution (or of a single set of optimized parameter values, for that matter) for nSSU rDNA when phylogenies are inferred, as this practice can lead to the uniform application of inaccurate model-parameter values to stem and loop sequences. A more suitable approach would be to assess features relevant to nucleotide substitution (e.g., nucleotide frequencies, among-site heterogeneity in substitution rates) separately for stems and loops to determine whether they differ. Otherwise, ML analyses of combined data may lead to an inaccurate or poorly supported estimate of phylogeny. This same argument applies to the use of maximum parsimony to infer relationships (e.g., Conant & Lewis 2001, Buckley & Cunningham 2002), although we did not explore alternative character weighting for MP in the present study.

In contrast, Bayesian analyses are more likely to produce an accurate reflection of phylogeny when partitions differ in parameter values, because this method can simultaneously accommodate multiple models of nucleotide evolution. The present study confirms this point: the Bayesian consensus topology obtained by analysis of unlinked stem and loop partitions, each with its respective model, was congruent with the ML phylogeny, but many more clades were supported by high credibility values. Accuracy with the Bayesian method, however, still requires correct model specification, which is

no small matter (see, e.g., Buckley & Cunningham 2002 and references therein).

Several conclusions, as noted above, emerge from the present study, and we believe that the nSSU rRNA gene is a promising molecular marker for inferring phylogenetic relationships among peracarid lineages but less so for superorders of eumalacostracans. Additional nSSU rDNA evidence, particularly for species of mictaceans, spelaeogriphaceans, and thermosbaenaceans, will undoubtedly further clarify peracarid relationships, particularly if new sequence data can break up some of the long branches observed in the present study. Improvement in the phylogeny will in turn permit a more thorough assessment of morphological-character evolution in this group. Data would be useful, too, from an additional DNA marker that does not evolve too rapidly and that is not linked to the rRNA operon. Such data would permit a comparison of independent ''gene trees,'' and congruence among trees could be taken to reflect the ''true'' phylogeny.

Our molecularly based phylogenetic analyses suggest a scenario of peracarid (and eumalacostracan) evolution not unlike that proposed by Richter & Scholtz's (2001) parsimony analysis of malacostracan morphological characters or even Siewing's (1963) earlier assessment of morphology: ''The reconstruction of the natural system of the Peracarida by means of homologies shows that isopods and amphipods are end points of two divergent lines of evolution which arise near the mysids. One of these lines comprises the Cumacea, Tanaidacea, and Isopoda; the other line comprises only the Amphipoda'' (p. 98). This conclusion parallels our findings almost exactly, but whereas Richter & Scholtz (2001) and Siewing (1963) considered amphipods to be closer to a monophyletic Mysidacea, we find instead that amphipods (with spelaeogriphaceans) are closer only to lophogastrids. Mysids, according to our results, have an affinity with nonperacarid eumalacostracans, but this result, too, is not inconsistent with Siewing (1963), when he says, ''The Mysidacea, including the Lophogastrida, are in many respects original, representing the connecting link to other malacostracan groups, particularly to the Eucarida and Pancarida'' (p. 99). Although our findings indicate that the Mysidacea are not monophyletic, they do suggest that mysids, and probably lophogastrids, are *both* ''original'' (to use Siewing's terms); i.e., mysids represent the ancestral ''connection'' to nonperacarid crustaceans, whereas lophogastrids are closer to the ancestor of peracarids. The distribution of expansion segments in the nSSU rDNA gene in malacostracans supports this view as well. Our findings contrast with those of Siewing (1963), Richter & Scholtz (2001), and even Pires (1987) in showing the Thermosbaenacea, or Pancarida in these authors' views, firmly embedded within the Peracarida and not ancestral to this clade.

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Appendix 1

Classification (Martin and Davis 2001), GenBank accession numbers (GB), and collection data (site, date, and collector and/or provider) for crustacean species used in the present study.

Class Malacostraca Latreille, 1802
Subclass Phyllocarida Packard, 1879
Order Leptostraca Claus, 1880
Family Nebaliidae Samouelle, 1819
Nebalia sp. (GB L81945)
Subclass Hoplocarida Calman, 1904
Order Stomatopoda Latreille, 1817
Family Gonodactylidae Giesbrecht, 1910
Gonodactylus sp. (GB L81947)
Family Squillidae Latreille, 1802
Squilla empusa Say, 1818 (GB L81946)
Subclass Eumalacostraca Grobben, 1892
Superorder Syncarida Packard, 1885
Order Anaspidacea Calman, 2904
Family Anaspididae Thomson, 1893
Anaspides tasmaniae Thomson, 1893 (GB L81948)
Superorder Peracarida Calman, 1904
Order Spelaeogriphacea Gordon, 1957
Family Spelaeogriphidae Gordon, 1957
Spelaeogriphus lepidops Gordon, 1957 (Bat Cave, Table Mountain, South Africa; 23 Oct 1993; gift from L. Watling; GB AY781414)
Order Thermosbaenacea Monod, 1927
Family Thermosbaenidae Monod, 1927
Tethysbaena argentarii Stella, 1951 (Punta degli Stretti Cave, Monte Argentario, Italy; 28 Feb 1995; T. and F. Spears; GB AY781415)
Order Lophogastrida Sars, 1870
Family Lophogastridae Sars, 1870
Gnathophausia ingens Dohrn, 1870 (Gulf of Mexico, 3552 m depth, DGOMBII station 1 (JSSD1), #20861; 3 Aug 2002; E. Escobar-Briones, F. Alvarez, and M. Wicksten; GB AY781416)
Gnathophausia zoea Willemoes-Suhm, 1873 (Gulf of Mexico, 3410 m depth, DGOM station 4 (JSSD4), #20859; 9 Aug 2002; E. Escobar-Briones, F. Alvarez, and M. Wicksten; GB AY781417)
Family Eucopiidae Sars, 1885
Eucopia sp. (DeSoto Canyon, Gulf of Mexico, 1870 m, 28°54.49′N, 87°39.47′W, station S36 rep.3; 12 Aug 2002; M. Wicksten; GB AY781418)
Order Mysida Haworth, 1825
Family Mysidae Haworth, 1825
Heteromysis formosa S. I. Smith, 1871 (Woods Hole Marine Biological Laboratory, Department of Marine Resources, Woods Hole, Massachusetts; 1996; GB AY781419)
Neomysis integer Leach, 1814 (location and date unknown; gift from W. Kobusch; GB AY781420)
Order Mictacea Bowman, Garner, Hessler, Iliffe, & Sanders, 1985
Family Hirsutiidae Sanders, Hessler & Garner, 1985
Thetispelecaris remex Gutu & Iliffe, 1998 (Basil Minns Blue Hole, Great Exuma Island, Bahamas; 20 Mar 2000; T. Iliffe; GB AY781421)
Order Amphipoda Latreille, 1816
Suborder Gammaridea Latreille, 1802
Family Gammaridae Latreille, 1802
Gammarus oceanicus Segerstråle, 1947 (Woods Hole Marine Biological Laboratory, Department of Marine Resources, Woods Hole, Massachusetts; 1993; GB AY781422)
Suborder Caprellidea Leach, 1814
Family Caprellidae Leach, 1814
Caprella geometrica Say, 1818 (Woods Hole Marine Biological Laboratory, Department of Marine Resources, Woods Hole, Massachusetts; 1993; GB AY781423)
Suborder Hyperiidea Milne Edwards, 1830
Family Phronimidae Rafinesque, 1815
Phronima sp. (location unknown; 1995; gift from M. Grygier; GB AY781424)
Order Isopoda Latreille, 1817
Suborder Phreatoicidea Stebbing, 1893
Family Amphisopodidae Nicholls, 1943
Paramphisopus palustris (Glauert, 1924) (Lake Monger, Perth, W. Australia; 31°55′S 15°50′E; amongst reeds on shore; 22 Mar 1995; D. Jones and G. D. F. Wilson; gift from G. D. F. Wilson and the Australian Museum (P. 44487), Sydney; GB AY781425)
Suborder Asellota Latreille, 1902
Family Asellidae Latreille, 1802
Asellus racovitzai (Williams, 1970) (Newport Sulfur Spring, Wakulla Co., Florida; T. and F. Spears; 25 Mar 1995; GB AY781426)
Suborder Valvifera Sars, 1882
Family Idoteidae Samouelle, 1819
Idotea metallica Bosc, 1802 (Woods Hole Marine Biological Laboratory, Department of Marine Resources, Woods Hole, Massachusetts; 1990; GB AY781427)
Order Tanaidacea Dana, 1849
Suborder Tanaidomorpha Sieg, 1980
Family Tanaidae Dana, 1849
Tanais dulongii (Audouin, 1826) (Alvarado Lagoon, western Gulf of Mexico, 30 Oct 2000; gift from E. Escobar-Briones; GB AY781428)
Family Paratanaidae Lang, 1949
Paratanais malignus Larsen, 2001

(Frenchman's Bay, Botany Bay, New South Wales, Australia; date unknown; gift from K. Larsen and the Australian Museum, Sydney; GB AY781429)

Suborder Apseudomorpha Sieg, 1980

Family Kalliapseudidae Lang, 1956

Kalliapseudes sp. (from shallow sandy beach, northern Gulf of Mexico, Ocean Springs, Mississippi; 1993; S. LeCroy; GB AY781430)

Order Cumacea Kröyer, 1846

Family Diastylidae Bate, 1856

Diastylus sculpta G. O. Sars 1871 (location and date unknown; gift from L. Watling; GB AY781431)

Family Bodotriidae Scott, 1901

Spilocuma salomani Watling, 1977 (surf zone, northern Gulf of Mexico, St. George Island, Franklin Co., Florida; 1992; T. Spears and N. Seganish; GB AY781432)

Superorder Eucarida Calman, 1904

Order Euphausiacea Dana, 1852

Family Euphausiidae Dana, 1852

Nyctiphanes simplex Hansen, 1911 (32°53′N, 117°17′W; 0–90 m; La Jolla Canyon, California (eastern Pacific); date unknown; R. McConnaughey; GB AY781433)

Meganyctiphanes norvegica M. Sars, 1857 (location and date unknown; gift from W. Kobusch; GB AY781434)

Order Decapoda Latreille, 1802

Family Palinuridae Latreille, 1802

Panulirus argus Latreille, 1804 (Gulf of Mexico, Florida Keys; date unknown; gift from W. F. Herrnkind; GB AY781435)

Family Portunidae Rafinesque, 1815

Callinectes sapidus Rathbun, 1896 (northern Gulf of Mexico, off FSU Marine Lab, Florida; 21 Sep 1996; C. L. Morrison; GB AY781436)

Appendix 2.—Length (in nucleotides, nt) of the variable regions of the nuclear small-subunit (SSU) ribosomal RNA gene for crustaceans (Appendix 1) used in this study. Variable (V) regions V1–V9 (V6 is absent in eukaryotes) were identified with the SSU secondary-structure model of Van de Peer et al. (1997). Classification is according to Martin and Davis (2001). Percent G + C content is given in parentheses (calculated with PAUP* [Swofford 2002] and with missing nucleotides deleted); χ^2 = Chi-square test for homogeneity of nt frequencies; ** = significant *P* value at $\alpha = 0.01$ with 78 degrees of freedom.

Taxon	V1	V2	V3	V4	V5	V7	V8	V9
Leptostraca								
Nebalia sp.	33 (45%)	210 (46%)	68 (49%)	229 (50%)	72 (51%)	89 (48%)	59 (54%)	104 (45%)
Stomatopoda								
Gonodactylus sp.	33 (54%)	209 (51%)	68 (47%)	232 (54%)	72 (58%)	92 (52%)	50 (61%)	110 (53%)
S. empusa	32 (53%)	209 (52%)	68 (47%)	232 (55%)	72 (58%)	92 (52%)	59 (61%)	110 (54%)
Anaspidacea								
A. tasmaniae	33 (54%)	212 (51%)	68 (47%)	238 (53%)	72 (60%)	94 (56%)	59 (60%)	112 (51%)
Spelaeogriphacea								
S. lepidops	36 (64%)	224 (57%)	64 (59%)	360 (58%)	68 (63%)	180 (55%)	59 (51%)	114 (58%)
Thermosbaenacea								
T. argentarii	38 (50%)	327 (47%)	67 (51%)	298 (59%)	74 (59%)	332 (53%)	59 (58%)	127 (45%)
Lophogastrida								
G. ingens	32 (38%)	207 (36%)	69 (36%)	233 (40%)	72 (59%)	86 (49%)	58 (52%)	109 (54%)
G. zoea	32 (38%)	207 (37%)	69 (36%)	233 (40%)	72 (59%)	86 (53%)	58 (52%)	109 (54%)
Eucopia sp.	32 (38%)	208 (34%)	69 (36%)	233 (41%)	72 (59%)	86 (51%)	58 (52%)	109 (55%)
Mysida								
H. formosa	35 (49%)	211 (48%)	68 (48%)	228 (50%)	72 (51%)	90 (54%)	59 (54%)	111 (47%)
N. integer	35 (43%)	211 (46%)	68 (53%)	226 (47%)	72 (54%)	90 (49%)	59 (62%)	112 (48%)
Mictacea								
T. remex	35 (49%)	211 (47%)	69 (52%)	248 (47%)	72 (57%)	167 (50%)	61 (41%)	129 (51%)
Amphipoda								
G. oceanicus	36 (59%)	279 (52%)	70 (57%)	413 (55%)	84 (64%)	216 (56%)	103 (60%)	114 (54%)
C. geometrica	38 (58%)	242 (49%)	70 (53%)	332 (57%)	82 (59%)	236 (56%)	121 (56%)	106 (54%)
Phronima sp.	39 (62%)	277 (49%)	70 (41%)	382 (53%)	123 (52%)	234 (52%)	103 (52%)	108 (52%)
Isopoda								
P. palustris	33 (54%)	234 (48%)	68 (56%)	554 (57%)	72 (60%)	250 (64%)	59 (61%)	148 (53%)
A. racovitzai	33 (54%)	229 (46%)	68 (52%)	409 (56%)	72 (61%)	207 (62%)	59 (51%)	124 (50%)
I. metallica	33 (60%)	242 (47%)	68 (57%)	879 (58%)	72 (56%)	300 (64%)	59 (61%)	150 (57%)

Appendix 2.—Continued.

Taxon	V1	V2	V3	V4	V5	V7	V8	V9
Tanaidacea								
T. dulongii	34 (48%)	239 (44%)	69 (48%)	257 (38%)	69 (55%)	225 (48%)	57 (47%)	109 (42%)
P. malignus	35 (52%)	293 (47%)	68 (48%)	504 (47%)	73 (58%)	273 (47%)	59 (58%)	108 (43%)
Kalliapseudes sp.	33 (57%)	275 (53%)	68 (46%)	656 (59%)	72 (63%)	290 (54%)	60 (53%)	141 (55%)
Cumacea								
D. sculpta	35 (40%)	258 (55%)	70 (49%)	292 (54%)	72 (58%)	333 (57%)	110 (61%)	123 (57%)
S. salomani	33 (41%)	220 (55%)	67 (54%)	334 (51%)	72 (59%)	252 (59%)	58 (55%)	116 (54%)
Euphausiacea								
N. simplex	33 (54%)	226 (46%)	68 (49%)	237 (55%)	73 (61%)	106 (56%)	60 (60%)	108 (52%)
M. norvegica	33 (54%)	226 (49%)	68 (49%)	236 (56%)	73 (61%)	106 (55%)	60 (60%)	108 (52%)
D. sculpta	35 (40%)	258 (55%)	70 (49%)	292 (54%)	72 (58%)	333 (57%)	110 (61%)	123 (57%)
Decapoda								
P. argus	33 (54%)	216 (51%)	68 (49%)	240 (53%)	72 (60%)	103 (50%)	59 (59%)	138 (52%)
C. sapidus	33 (48%)	216 (50%)	68 (47%)	246 (53%)	73 (60%)	98 (51%)	59 (58%)	131 (56%)
χ^2 (P)	1.00	0.15	1.00	0.00**	1.00	0.08	1.00	1.00

Appendix 3.—Pairwise comparisons of sequence divergence (uncorrected and expressed as the proportional amount of change per nucleotide position) for 1696 aligned characters (after regions of ambiguous alignment were excluded) of nuclear small-subunit ribosomal DNA for 27 crustaceans used in this study. Calculations were obtained with PAUP* (Swofford 2002), and missing nucleotides were omitted.

Taxon	1	2	3	4	5	6	7
1 *Anaspides tasmaniae*	—						
2 *Kalliapseudes* sp.	0.11410	—					
3 *Thetispelecaris remex*	0.19022	0.21425	—				
4 *Asellus racovitzae*	0.06563	0.10433	0.18863	—			
5 *Nebalia* sp.	0.07238	0.13099	0.19373	0.08922	—		
6 *Gonodactylus* sp.	0.03855	0.10859	0.18714	0.06504	0.06260	—	
7 *Tethysbaena argentarii*	0.14075	0.16691	0.22115	0.13919	0.14917	0.13707	—
8 *Tanais dulongii*	0.16576	0.16312	0.23371	0.14289	0.17542	0.15963	0.20482
9 *Panulirus argus*	0.04284	0.10745	0.18976	0.06569	0.07304	0.03368	0.13900
10 *Paramphisopus palustris*	0.08151	0.10436	0.19516	0.05211	0.10202	0.07725	0.14528
11 *Gnathophausia ingus*	0.15736	0.18342	0.22604	0.16503	0.17484	0.15737	0.20497
12 *Eucopia* sp.	0.15843	0.18514	0.22676	0.16731	0.17629	0.15853	0.20814
13 *Gammarus oceanicus*	0.19919	0.21879	0.25759	0.19634	0.20836	0.19561	0.23146
14 *Spilocuma salomani*	0.15804	0.16691	0.22658	0.14837	0.16945	0.15624	0.19534
15 *Gnathophausia zoea*	0.15736	0.18342	0.22604	0.16503	0.17484	0.15737	0.20497
16 *Heteromysis formosa*	0.05574	0.12155	0.18664	0.07555	0.07792	0.04411	0.14824
17 *Paratanais malignus*	0.17797	0.17566	0.23891	0.16411	0.18764	0.17441	0.21878
18 *Spelaeogriphus lepidops*	0.27066	0.27203	0.30074	0.26643	0.27951	0.26624	0.28361
19 *Neomysis integer*	0.06183	0.12213	0.18850	0.07919	0.08041	0.05267	0.15064
20 *Caprella geometrica*	0.19283	0.21119	0.25678	0.18632	0.20013	0.18988	0.21466
21 *Diastylis sculpta*	0.19227	0.19497	0.26632	0.17890	0.20268	0.18870	0.21215
22 *Callinectes sapidus*	0.04917	0.10790	0.18871	0.07088	0.07751	0.04243	0.14393
23 *Nyctiphanes simplex*	0.04465	0.11347	0.18780	0.07421	0.07116	0.03672	0.14255
24 *Meganyctiphanes norvegica*	0.04223	0.11170	0.18791	0.06994	0.07121	0.03307	0.14140
25 *Phronima* sp.	0.23549	0.25198	0.27467	0.23454	0.24049	0.22941	0.24945
26 *Idotea metallica*	0.08477	0.10583	0.19555	0.05717	0.10526	0.07988	0.14319
27 *Squilla empusa*	0.03429	0.10557	0.18428	0.06323	0.05957	0.00490	0.13602

Taxon	8	9	10	11	12	13	14
8 *Tanais dulongii*	—						
9 *Panulirus argus*	0.15724	—					
10 *Paramphisopus palustris*	0.15462	0.07970	—				
11 *Gnathophausia ingus*	0.22127	0.15992	0.17766	—			
12 *Eucopia* sp.	0.22249	0.16098	0.18061	0.00614	—		
13 *Gammarus oceanicus*	0.23104	0.19995	0.19946	0.22262	0.22452	—	
14 *Spilocuma salomani*	0.19861	0.15497	0.14262	0.20832	0.20754	0.23207	—
15 *Gnathophausia zoea*	0.22127	0.15992	0.17766	0.00000	0.00614	0.22262	0.20832
16 *Heteromysis formosa*	0.16709	0.05700	0.08896	0.16358	0.16463	0.19818	0.16529
17 *Paratanais malignus*	0.17302	0.17564	0.17086	0.22083	0.22509	0.23301	0.21503
18 *Spelaeogriphus lepidops*	0.27165	0.26832	0.27497	0.28893	0.29115	0.28447	0.29404
19 *Neomysis integer*	0.17637	0.06983	0.09259	0.16352	0.16337	0.20303	0.16136
20 *Caprella geometrica*	0.23490	0.19299	0.19317	0.21367	0.21551	0.08804	0.22604
21 *Diastylis sculpta*	0.21473	0.18872	0.17510	0.23781	0.23785	0.24438	0.19616
22 *Callinectes sapidus*	0.15794	0.02030	0.08008	0.15930	0.15985	0.19585	0.15642
23 *Nyctiphanes simplex*	0.16199	0.03979	0.08576	0.16350	0.16397	0.19643	0.15806
24 *Meganyctiphanes norvegica*	0.15958	0.03674	0.08275	0.16307	0.16353	0.19349	0.15688
25 *Phronima* sp.	0.26785	0.23509	0.23499	0.25251	0.25383	0.18084	0.26542
26 *Idotea metallica*	0.15243	0.07991	0.05713	0.18297	0.18530	0.19996	0.14420
27 *Squilla empusa*	0.15723	0.03185	0.07666	0.15574	0.15681	0.19449	0.15441

Appendix 3.—Continued.

Taxon	15	16	17	18	19	20	21
15 *Gnathophausia zoea*	—						
16 *Heteromysis formosa*	0.16358	—					
17 *Paratanais malignus*	0.22083	0.18736	—				
18 *Spelaeogriphus lepidops*	0.28893	0.27384	0.28990	—			
19 *Neomysis integer*	0.16352	0.04352	0.18727	0.27080	—		
20 *Caprella geometrica*	0.21367	0.19422	0.23669	0.27361	0.19726	—	
21 *Diastylis sculpta*	0.23781	0.19069	0.24581	0.28939	0.19002	0.23312	—
22 *Callinectes sapidus*	0.15930	0.06209	0.17392	0.26900	0.07566	0.18765	0.18841
23 *Nyctiphanes simplex*	0.16350	0.05270	0.17867	0.27617	0.06552	0.19257	0.19226
24 *Meganyctiphanes norvegica*	0.16307	0.05029	0.17570	0.27205	0.06433	0.18962	0.19052
25 *Phronima* sp.	0.25251	0.23083	0.27584	0.29487	0.23628	0.18293	0.27570
26 *Idotea metallica*	0.18297	0.08978	0.16940	0.27071	0.09774	0.19800	0.17741
27 *Squilla empusa*	0.15574	0.04171	0.17261	0.26476	0.05394	0.18996	0.18512

Taxon	22	23	24	25	26	27
22 *Callinectes sapidus*	—					
23 *Nyctiphanes simplex*	0.04733	—				
24 *Meganyctiphanes norvegica*	0.04428	0.00367	—			
25 *Phronima* sp.	0.23304	0.23387	0.23279	—		
26 *Idotea metallica*	0.08149	0.08781	0.08478	0.23809	—	—
27 *Squilla empusa*	0.04123	0.03368	0.03003	0.22838	0.07745	