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REVIEW

Parasites of crustaceans (Isopoda: Bopyridae) evolved from fish parasites: Molecular and morphological evidence*

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Summary

The phylogenetic origin of isopods that live as parasites of crustaceans (Bopyridae) is examined using ssu rDNA sequences and morphological characters in separate and combined analyses. A study of split-supporting positions proves that information content of the ssu-rDNA alignment is not high, but the signals are sufficient to study relationships of the Bopyridae. The sequences most similar to those of bopyrids are the ones of the Cymothoidae, isopods that are specialized fish parasites. Topologies obtained with different tree reconstructing methods support a sister-group relationship between Bopyridae and Cymothoidae. This finding is in accordance with morphological characters, for which a cladistic analysis was prepared, and a combination of morphological and molecular data gives the same result. As a consequence, we propose to give up the classification of these parasites as a separate suborder "Epicaridea", to name the taxon "Bopyridae", which should have the same rank as the sistertaxon, the family Cymothoidae . The biology of related taxa is discussed to reconstruct an evolutionary scenario. We assume that evolution started with necrophagous and predatory cirolanid-like isopods specialized to feed on fishes, from which step by step temporary and then permanent fish parasites evolved. The latter are represented in the present fauna by the Cymothoidae. A change of hosts must have occurred in cymothoid-like ancestors, who moved from fishes to crustaceans and developed a special life cycle and morphology, giving rise to modern Bopyridae. This host change triggered a new phase of radiation of parasitic isopods.

Introduction

The taxon Isopoda contains a large number of species (>8400) with a remarkable diversity of ways of life. Isopods occur in marine pelagic and benthic habitats, in epigean and hypogean fresh water, a large lineage evolved on land (Oniscidea) and some species even adapted to life in deserts (e.g., species of *Hemilepistus*: e.g., Linsenmair 1975). Some marine isopods are passive filter feeders (Arcturidae: Moreira 1973; Wägele 1987), others feed on macroalgae (e.g., Idoteidae: Naylor 1955; Tuomi et al 1988). Some deep-sea species depend on detritus or plant remains (Menzies 1956, 1962; Wolff 1979), others are specialized on Foraminifera (Svavarsson et al 1993). An interesting question is the evolution of parasitism. Parasites with a highly modified morphology occur in the taxa Cymothoidae, Epicaridea (= Bopyridae of the present

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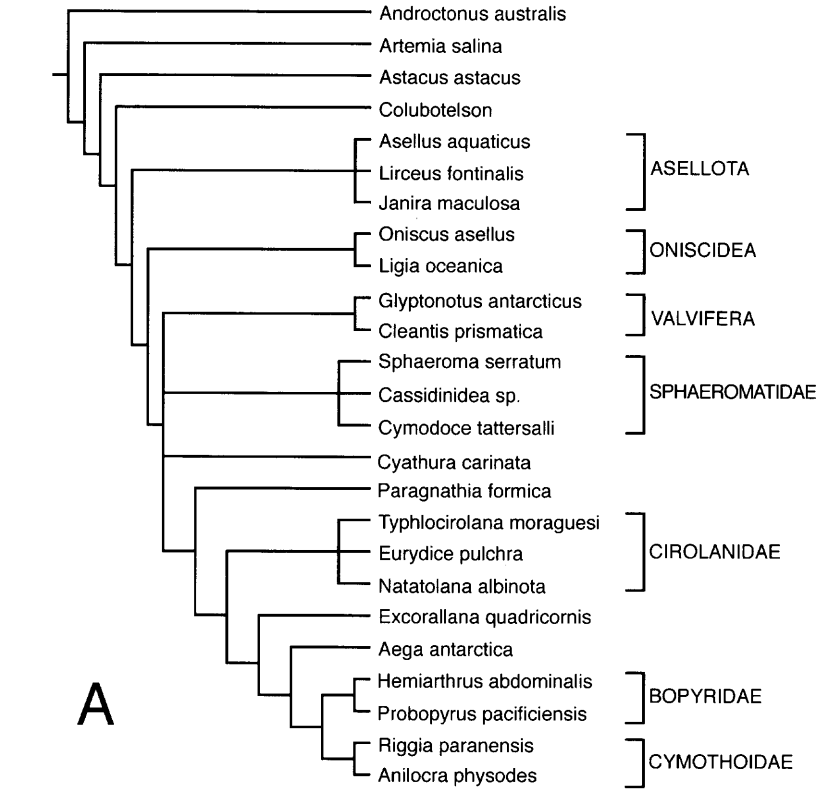

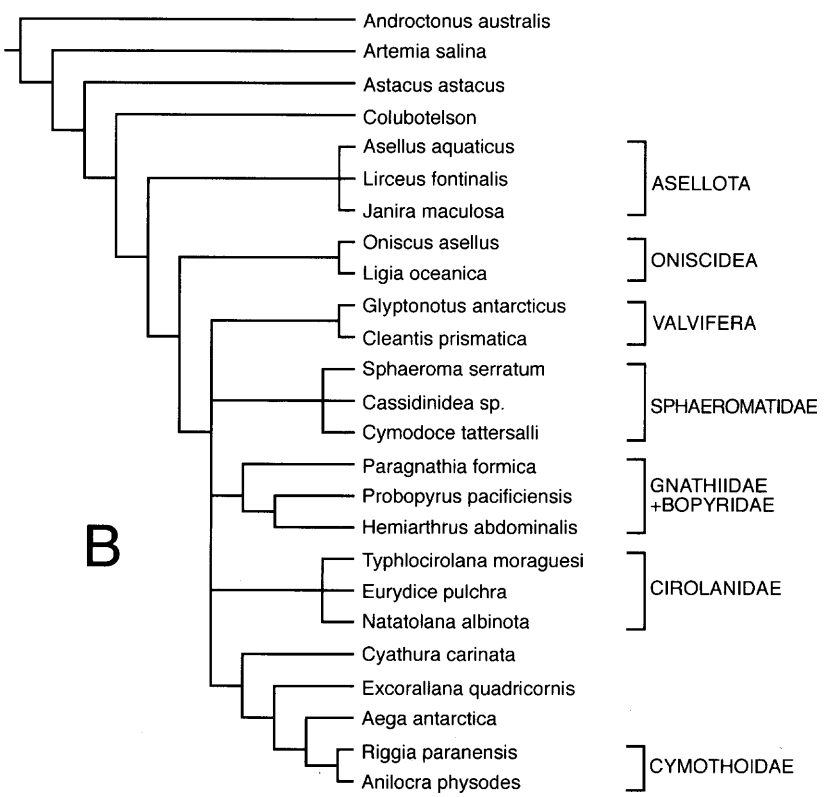

Fig. 1. A: Topology equivalent to that proposed by Wägele (1989), used to compare tree lengths when the data matrix is the ssu-rDNA alignment. Tree length estimated with MacClade is 1980. A sister-group relationship between bopyrids and cymothoids is assumed. B: As in Fig. 1A, but with a topology as proposed by Brusca and Wilson (1991). Tree length is 1993. Note sistergroup-relationship between gnathiids and bopyrids.

paper) and Gnathiidae, while the ectoparasitic Aegidae, Tridentellidae and Corallanidae at first sight resemble the predatory, non-parasitic Cirolanidae (Fig. 12). The Cymothoidae are parasites of fishes, while the Epicaridea (= Bopyridae) are found on crustaceans.

In this contribution we discuss the possible phylogenetic origin of the more derived groups of parasitic species, the Cymothoidae Leach, 1818 (parasites of fishes, about 400 known species) and the Epicaridea Latreille, 1831 (parasites of crustaceans, about 700 known species) and the evolution of parasitism in the clade of isopods to which these taxa belong. In the three hitherto published more comprehensive phylogenetic analyses of the order Isopoda the Epicaridea are placed in different sistergroup-relationships (Fig. 1): according to Wägele (1989) the Epicaridea (named therein "Bopyridae") are the sistertaxon of the family Cymothoidae, while they are placed close to the Gnathiidea in the cladistic analysis of Brusca and Wilson (1991). Tabacaru and Danielopol (1999) code a terminal taxon "Flabellifera" (a group of the traditional typological classification that contains the families Cymothoidae, Aegidae and other taxa) separately from the Epicaridea, wherefore they could not discover that the Epicaridea have to be placed *within* their taxon "Flabellifera" (a mistake coined "failure to recognize the encaptic order" in Wägele 1994). They place the Epicaridea in a clade comprising also the "Flabellifera", Anthuridea and Gnathiidea.

These three phylogenetic hypotheses, all based on morphological characters, imply different scenarios for the evolution of the parasites of crustaceans: Wägele (1989) assumes that step by step carnivorous isopods (similar to extant Cirolanidae) that prefer fish meat evolved to temporary ectoparasites of fishes (represented in the recent fauna by Corallanidae, Tridentellidae and Aegidae). An ancestor with this way of life gave rise to species that became highly specialized protandrous hermaphrodites with dwarfish males and large females that live permanently on their host. The descendants of these species are the modern Cymothoidae, a taxon that is believed to be monophyletic (e.g., Bruce 1981; Trilles 1991; Roman and Dalens 1999). According to Wägele (1989), the Epicaridea must have had a cymothoid-like ancestor that started to suck body fluids on crustaceans, their life cycles and also their morphology can be derived from cymothoid-like ancestors (Figs. 10–12). This host change triggered a new phase of adaptive radiation of the parasitic Isopoda.

In contrast to this scenario, based on the phylogeny estimated by Brusca and Wilson (1991) the Epicaridea are not related to cymothoids but share a common ancestor with the Gnathiidae (a critique ot the methodology of this analysis is found in Wägele 1994). The Gnathiidae live as adults in crevices, sponges or burrows, do not feed and have a unique sexual dimorphism (males with huge frontally directed mandibles), while the small larvae are active swimmers and suck blood on fishes (e.g., Stoll 1962; Upton 1987, Wägele 1988). The epicarids then could have evolved either from a non-parasitic ancestor or from a species with parasitic larvae. A transformation series for the epicarid morphological characters is not represented in the extant fauna when this phylogeny is assumed. According to the phylogeny reconstructed by Tabacaru and Danielopol (1999), epicarids arose directly from a non-parasitic ancestor, namely the common ancestor shared with the "Flabellifera", Anthuridea and Gnathiidea.

In the present study we present a first molecular analysis of the phylogeny of parasitic isopods based on a larger set of hitherto unpublished 18SrDNA sequences, to analyze the possible origin and evolution of the Bopyridae.

Material and Methods

Specimens

Sequences were obtained from the following species (in parenthesis: locality and collector, if other than the authors, and Genbank accession number): Suborder Phreatoicidea: *Colubotelson thomsoni* Nicholls, 1994 (Tasmania, Hytten Creek on campus of University of Tasmania, leg. Prof. A. Richardson, Acc. No. AF 255703); Suborder Asellota: *Asellus aquaticus* (Linnaeus, 1759) (Bielefeld/Germany, Acc. No. AF 255701); *Lirceus fontinalis* Rafinesque-Schmaltz, 1820 (Creek Island/USA, leg. Dr. T.C. Sparkes, Acc. No. AF 255702); *Janira maculosa* (Leach, 1814) (Bretagne/France, Acc. No. AF 255700); Suborder Oniscidea: *Ligia oceanica* (Linnaeus, 1767) (Galicia/Spain, Acc. No. AF 255698); *Oniscus asellus* Linnaeus, 1758 (Bielefeld/Germany, Acc. No. AF 255699); Suborder Valvifera: *Glyptonotus antarcticus* Eights, 1853 (Weddell Sea/Antarctica, Dr. C. Held, Acc. No. AF 255696); *Cleantis prismatica* (Risso, 1862) (Galicia/Spain, Acc. No. AF255697); Suborder Anthuridea: *Cyathura carinata* (Krøyer, 1847) (Flemhuder See/Germany, Acc. No. AF 332146); Suborder Sphaeromatidea: Sphaeromatidae: *Sphaeroma serratum* (Fabricius, 1787) (Galicia/Spain, Acc. No. AF 255694); *Cassidinidea* sp. (Venezuela, Acc. No. AF 255693), *Cymodoce tattersalli* Torelli, 1929 (Galicia/Spain, Acc. No. AF 255695); Suborder Cymothoida: Bopyridae: *Probopyrus pacificiensis* Roman-Contreras, 1993 (Guerrero /Mexico, leg. Dr. Roman-Contreras, Acc. No. AF 255683); *Hemiarthrus abdominalis* (Krøyer, 1841) (Baltic Sea, Dr. W. Kobusch, Acc. No. AF 255684); Cymothoidae: *Riggia paranensis* (Saidat, 1948) (Brazil, leg. Dr. V. Thatcher, Acc. No. AF 255685); *Anilocra physodes* (Linnaeus, 1758) (Bretagne/France, Acc. No. AF 255686); Aegidae: *Aega antarctica* (Hodgson 1910) (Weddell Sea/Antarctica, Dr. C. Held, Acc. No. AF 255689); Corallanidae: *Excorallana quadricornis* (Hansen, 1890) (Venezuela, Acc. No. AF

255688); Cirolanidae: *Natatolana albinota* (Vanhöffen, 1914) (Weddell Sea/Antarctica, Dr. C. Held, Acc. No. AF 255691); *Typhlocirolana moraguesi* Racovitza, 1905 (Mallorca/Spain, leg. Dr. Damian Jaume, Acc. No. AF 255692); *Eurydice pulchra* Leach, 1815 (Galicia/Spain, Acc. No. AF 255690); Gnathiidae: *Paragnathia formica* (Hesse, 1864) (Galicia/Spain, Acc. No. AF 255687). Sequences selected from Genbank: Decapoda: *Astacus astacus* Acc. No. U 33181; Anostraca: *Artemia salina* Acc. No. X 01723; Scorpiones: *Androctonus australis* Acc. No. X 77908.

Fixation and DNA extraction

Most living specimens were fixed in ice-cold ethanol (80%) and kept at < 4°C whenever possible, specimens fixed during field trips in warm ethanol yielded less DNA of high quality. It was usually not possible to get suitable DNA samples from specimens fixed in formalin. DNA was isolated from complete specimens or from tissue samples with phenol-chloroform as described by Maniatis (1982), or with the "blood and tissue-kit" of Quiagen, or with DTAB (see Gustincich et al 1991). The quality of the extracted DNA was controlled by electrophoresis.

PCR, cloning and sequencing

The 18SrRNA gene was amplified from whole genomic DNA using primers that proved to be successful in crustaceans (personal communaction of Dr. T. Spears) (primer 18a1: 5´- CCTA(CT)CTGGTTGATCCTGCCAGT - 3´, primer 1800: 5´- TAATGATCCTTCCGCAGGTT - 3´) and with the following cycle: 5 min. 94°C, 36 times 30 sec. 94°C, 50 sec. 52.5°C, 2.3 min. 72°C, then 10 min. 72°C and storage at < 4°C. PCR products were treated with a purification kit of Quiagen and, wherever necessary, separated into fragments by electrophoresis. When the quantity of the product was insufficient the complete gene was cloned in *E. coli* using the "TOPO-TA cloning kit" of Invitrogen. The purified DNA fragments were sequenced with fluorescent labelled primers in a Licor automatic sequencer. With exception of the universal primers "universal" and "reverse" (see Messing et al. 1981) all other primers were designed for this project (read from 5' to 3'):

Universal:	GCCCAGGGTTTTCCCAGTCACGAC;
reverse	TCACACAGGAAACAGCTATGAC;
100F seq	CCGCGAATGGCTCATTAAATCAG;
400F seq	ACGGGTAACGGGGAATCAGGG;
600F seq	CGTATATTAAAGTTG(CT)TGC;
700F seq	GTCTGGTGCCAGCC GCG;
1000F seq	CGATCAGATACCGCCCTAGTTC;
1155F seq	GTGAAACTTAAAGGAATTGACGG;
1250F seq	CCGTTCTTAGTTGGTGGAGCG;
1600F seq	GGTGTGTACAAAGGGCAGGGACG;
100R seq	GTGATTTAATGAGCCATTCGCGG;
400R seq	CCCTGATTCCCCGTTACCCGT;
600R seq	GCA(AG)CAACTTTAATATACG;
700R seq	CGC GGCTGCTGGCACCAGCAC;
1000R seq	GAACTAGGGCGGTATCTGATCG;
1155R seq	CCGTCAATTCCTTTAAGTTTCAG;
1250R seq	CGCTCCACCAACTAAGAACGGCC;
1500R seq	CATCTACGGCATCACAGA;
1600R seq	GGTGTGTACAAAGGGCAGGGACG.

The high number of primers was necessary because of the presence of long hypervariable insertions. Both strands of the gene were sequenced completely. The sequenced fragments were up to 800 bp long, a consensus sequence was constructed using DNASIS 2.1.

Alignment

Initially sequences were aligned using CLUSTALX (Thompson 1997), the result was controlled by eye with GeneDoc (Nicholas and Nicholas 1998) and corrected considering secondary structure information to reduce the positional variability. The secondary structures of variable regions were folded using mfold (Zucker 1991). Even in regions where positions occurred in paired strands, large areas were little conserved at the nucleotide level and alignment was ambiguous. Isopod seqences vary greatly in length (see Table 2) and many gaps had to be introduced in the alignment, the complete alignment was 4263 bp long. The following regions that were not alignable had to be cut out for the phylogenetic analyses. They were selected by eye and with the help of a variability analysis performed with TREECON (Van de Peer and De Wachter 1997) (numbers refer to the sequence of *Artemia salina*): 73-77, 124-137, 189-245, 646-710, 786-857, 1364-1386, 1699-1724. The second, shorter alignment used for this study had 1736 bp.

Phylogenetic analyses

Analyses were performed with the complete alignment and with the shortened alignment that contains more conserved positions and less ambiguous areas. The phylogenetic information content of the alignments was visualized constructing a spectrum of split-supporting positions with PHYSID (for details see Wägele and Rödding 1998a, b; Wägele 2000). This method allows the comparison of raw untransformed split-supporting patterns that are present in an alignment and does not require assumptions about models of sequence evolution or tree-constructing algorithms. The intention of this analysis is the same as in spectral analysis with Hadamard conjugation (see e.g. Hendy 1993; Lento et al. 1995), namely to identify the amount of support (in PHYSID: the number of putative synapomorphic nucleotides) for the splits present in the alignment without having to construct tree topologies. This allows to visualize the putative phylogenetic signal in comparison with the background noise composed of chance similarities that support nonsense-splits. Since PHYSID still does not estimate statistically the optimal noise level in supporting positions we used the default parameters (maximum noise allowed: 25% deviations in columns and rows of the alignment).

Saturation was studied comparing the transition: transversion rate with estimated pairwise maximum-likelihood distances (not shown). Most of the data points with high distance and low Ts:Tv-rate (around 1.0) belong to sequence pairs with *Anilocra*. Cymothoids are obviously fast evolving species where saturation of variable positions has to be considered. High Ts:Tv ratios >2 were found not only for closely related species (e.g., *Asellus* and *Lirceus*), but also for distantly related species pairs (e.g., species of Valvifera/Sphaeromatidae, Valvifera/

Table 1. Identification of long-branch sequences. The Z-scores shown here are based on pairs of sequences with *Colubotelson thomsoni* and a *Gammarus* sequence as outgroup.

Name	d-distances to Colubotelson	patristic distances to Colubotelson in ML-tree	rel.-rate test: Z-score	AT-content
Probopyrus pacificiensis	**0.13610**	**0.265**	**4.38**	0.497
Hemiarthrus abdominalis	**0.12923**	**0.202**	**4.24**	0.495
Riggia paranensis	**0.22694**	**0.381**	**7.42**	0.528
Anilocra physodes-	**0.30118**	**0.498**	**8.58**	0.522
Paragnathia formica	**0.18890**	**0.309**	**7.57**	0.518
Excorallana quadricornis	0.08050	0.111	**3.67**	0.483
Aega antarctica	0.07178	0.103	2.10	0.489
Eurydice pulchra	**0.10269**	**0.148**	2.68	0.487
Natatolana albinota	0.07397	0.110	**3.69**	0.487
Typhlocirolana moraguesi	0.09520	0.139	2.89	0.493
Cyathura carinata	0.06353	0.076	2.67	0.489
Cassidinidea sp	**0.13325**	**0.192**	**4.49**	0.487
Sphaeroma serratum	**0.13521**	**0.204**	**5.11**	0.506
Cymodoce tattersalli	0.07478	0.124	2.64	0.496
Glyptonotus antarcticus	0.05754	0.083	1.60	0.492
Cleantis prismatica	0.06528	0.089	1.23	0.495
Ligia oceanica	0.06570	0.102	2.37	0.485
Oniscus asellus	0.05594	0.088	1.31	0.490
Janira maculosa	**0.10185**	**0.151**	**3.48**	0.509
Asellus aquaticus	0.05808	0.075	2.422	0.495
Lirceus fontinalis	0.05219	0.061	0.97	0.497
Colubotelson	0	0	0	0.492

Table 2. Length variation of the 18SrRNA gene in isopods.

Species	V4 region	V 7 region	total length
Colubotelson thomsoni	551	271	2532
Asellus aquaticus	297	155	2129
Lirceus fontinalis	298	156	2138
Janira maculosa	272	93	2098
Ligia oceanica	355	361	2505
Oniscus asellus	885	288	2924
Glyptonotus antarcticus	562	195	2469
Cleantis prismatica	613	273	2646
Cyathura carinata	659	256	2659
Probopyrus pacificiensis	267	242	2286
Hemiarthrus abdominalis	330	214	2416
Riggia paranensis	280	194	2152
Anilocra physodes	266	203	2160
Aega antarctica	706	455	2910
Excorallana quadricornis	594	294	2607
Natatolana albinota	940	520	3269
Typhlocirolana moraguesi	734	374	2950
Eurydice pulchra	866	381	2993
Paragnathia formica	212	221	2116
Sphaeroma serratum	454	277	2413
Cassidinidea sp	470	336	2743
Cymodoce tattersalli	336	172	2264

Asellota, *Excorallana*/Asellota, etc.), wherefore we assume that not all transitional substitutions are saturated with multiple hits. Fast evolving sequences were excluded in most of the analyses to avoid long-branch problems. To identify these sequences we estimated evolutionary distances to the most basal taxon of isopods in our dataset, the phreatoicid *Colubotelson thomsoni* (in Table 1: Jin-Nei distance estimation as implemented in TREECON and Kimura two-parameter substitution model), and we calculated patristic distances in a maximum-likelihood topology between all species and the phreatoicid. Furthermore a relative rate test (Sarich and Wilson 1973; Wu and Li 1985) was carried out with the complete alignment using a sequence of the amphipod *Gammarus salinus* as outgroup, an example of Z-scores is presented in Table 1. The results (Table 1) agree well with the long branches observed in topologies estimated with distance, parsimony, and maximum likelihood methods. Base frequency homogeneity was tested with PAUP 4*. Base frequencies are quite similar in all sequences, with only little deviations from the mean (mean: A: 0.260; C: 0.231; G: 0.272; T: 0.236). The AT-content (Table 1) is always close to 0.5. Artifacts caused by a bias in base composition are not to be expected.

For tree construction with distance methods we used TREECON 1.3b (Van de Peeer and De Wachter 1997), and PAUP 4* (Swofford 1998) for maximum likelihood and parsimony analyses. Some parameters for the maxi-

Table 3. Data matrix for morphological characters (see Table 4).

Probopyrus pacificiensis	0110001111	1111110010	0001001???	1?11111?11	11?1111000
Hemiarthrus abdominalis	0110001111	1111110010	0001001???	1?11111?11	11?1111000
Riggia paranensis	0110001111	1111110010	0001001111	1111110000	1011111000
Anilocra physodes	0110001111	1111110010	0001001111	1111110000	1011111000
Paragnathia formica	0110001111	1111110001	0001001110	0010000010	01?0111100
Excorallana quadricornis	0110001111	1111110000	0001001110	0000000000	0001111000
Aega antarctica	0110001111	1111110000	0001001111	0001000000	0010111000
Eurydice pulchra	0110001111	1111110000	0001001100	0000000000	0000111000
Natatolana albionta	0110001111	1111110000	0001001100	0000000000	0000111000
Typhlocirolana moraguesi	0110001111	1111110000	0001001100	0000000000	0000111000
Cyathura carinata	0110001111	1111110010	1001111100	0000000000	01?1111000
Cassidinidea sp.	0110001111	1111111000	1010000000	0000000000	0000111000
Sphaeroma serratum	0110001111	1111111000	1010000000	0000000000	0000111000
Cymodoce tattersalli	0110001111	1111111000	1010001000	0000000000	0000111000
Glyptonotus antarcticus	0110001111	1111110000	0000000000	0000000010	0000111011
Cleantis prismatica	0110001111	1111110000	0000000000	0000000010	0000111011
Ligia oceanica	0110001111	1111110000	0000000000	0000000?10	0000111010
Oniscus asellus	0110001111	1111110010	0001000000	0000010?10	0000111010
Janira maculosa	0110001111	1111110100	0100000000	0000000100	0000011000
Asellus aquaticus	0110001111	1111110000	0100000000	0000000100	0000011000
Lirceus fontinalis	0110001111	111111?000	0100000000	0000000100	0000011000
Colubotelson	0110001111	1111110000	0000000100	0000000000	0000011000
Astacus astacus	0110001111	0010000010	0000000000	0000000000	0000000000
Artemia salina	0111110000	0000000000	0000000000	0000000000	0000000000
Androctonus australis	1000000000	0000000000	0000000000	0000000000	0000000000

Probopyrus pacificiensis	1?00000000	?01?010???	0
Hemiarthrus abdominalis	1?00000000	?01?010???	0
Riggia paranensis	0100000000	?000000???	0
Anilocra physodes	0100000000	?000000???	0
Paragnathia formica	0100000000	1000000???	0
Excorallana quadricornis	0100000000	1000000???	0
Aega antarctica	0100000000	1000000???	0
Eurydice pulchra	0100000000	1000000111	0
Natatolana albionta	0100000000	1000000111	0
Typhlocirolana moraguesi	0100000000	1000000111	0
Cyathura carinata	0100000000	?0000001??	0
Cassidinidea sp	0100000000	1000001000	1
Sphaeroma serratum	0100000000	1000001000	1
Cymodoce tattersalli	0100000000	1000001000	1
Glyptonotus antarcticus	0100000000	1000010000	0
Cleantis prismatica	0100000000	1000010000	0
Ligia oceanica	1100000000	1111110000	0
Oniscus asellus	1100000000	1111110000	0
Janira maculosa	1111111110	0000000000	0
Asellus aquaticus	1111110101	0000000000	0
Lirceus fontinalis	1111110101	0000000000	0
Colubotelson	0000000000	0000000000	0
Astacus astacus	0100000000	0000000000	0
Artemia salina	0000000000	0000000000	0
Androctonus australis	0000000000	0000000000	0

mum likelihood analyses could be estimated from the data (empirical base frequencies, gamma distribution), the transition-transversion ratio was set to 2 and the Hasegawa-Kishino-Yano model (1985) was selected. Bootstrapping was not applied for maximum-likelihood analyses due to prohibitive long computation times. The program MacClade (Maddison and Maddison 1992) was used to check changes of tree lengths under the parsimony criterion when alternative hypotheses were tested. Trees were rooted with the most basal species in our alignment, the scorpion *Androctonus australis*. We used for the purpose of this study (evolution of parasitic isopods) sequences of the more ancient isopod taxa as outgroups (Phreatoicidea and Asellota).

Table 4. List of morphological apomorphic character states (plesiomorphic states in parentheses).

1 – autapomorphic characters of arachnids: tagmata: prosoma/opisthosoma; 4 pairs of ventral lungs in opithosoma; etc.)
2 – ommatidia with cristalline cone (cone absent)
3 – at least first thoracopod with epipod (epipods absent)
4 – male antenna 2 modified to a large clasper (antenna not modified in male)
5 – phyllopodous thoracopods (articles of thoracopods well sclerotized, not flattened)
6 – epicontinental animals producing drought resistant eggs (eggs do not survive dessication)
7 – tagmosis: 8 thoracomeres and 6-7 pleomeres, the latter with appendages. No appendage-free abdomen. (tagmosis different)
8 – female gonopore on thoracomer 6 (not on thoracomer 6)
9 – male gonopore on thoracomer 8 or secondarily moved to margins between thoracomer 8 and pleomer 1 (not on thoracomer 8)
10 – stomach with ventral filter channel system, the filtrate being lead into digestive glands ; filter setae may be reduced secondarily (filter channel system lacking)
11 – female with marsupium formed by thoracopodal oostegites (marsupium absent)
12 – sixth pleonite fused with telson (not fused)
13 – first thoracopod modified to form a maxilliped (normal thoracopod, not modified)
14 – maxilliped (T1) without exopod (exopod present)
15 – pleopods with respiratory exopods and osmoregulatory endopods (pleopods used for swimming or pleopods absent)
16 – heart situated in posterior thorax and pleon (heart more anteriorly)
17 – eyes dorsal near caudal margin, cephalothorax in region of eyes partly immersed in pereonite 1, (not immersed, eyes lateral)
18 – pereopod 1 carposubchelate (carpus of pereopod 1 not enlarged)
19 – maxilliped with maximally 4 palpal articles (5 palpal articles or species without maxilliped)
20 – pereonite 7 reduced, without appendage (pereonite 7 present, pereopod 7 present)
21 – all pleonites fused dorsally in both sexes (not fused)
22 – only maximally 2 pleonites discernible in both sexes (more than 2 pleonites visible)
23 – sympod and endopod of uropods fused (not fused)
24 – grinding surface of mandibular molar reduced (present)
25 – body worm-like, elongate (body not elongate)
26 – uropod exopod folded over pleotelson (exopod laterally of endopod)
27 – mandible: setae below pars incisiva reduced (setae present)
28 – mandible: lacinia mobilis reduced (lacinia present)
29 – medial endite of maxilla 1 very small or absent (medial endite not reduced)
30 – maxilliped palp distally with laterally curved spines (spines or setae straight or bent medially)
31 – life cycle with small or dwarfish males and large females (males not distinctly smaller than females or dwarfish)
32 – protandric hermaphroditism (no hermaphroditism or protogynous hermaphorditism)
33 – young immatures able to swim, pleopods setose, adult females not able to swim, setae of pleopods reduced (pleopods in females as in young animals)
34 – perepods 1-3 predatory, subchelate, with elongated curved dactyli and acute claws (pereopods 1-3 not predatory or only pereopod 1 subchelate)
35 – all pereopods predatory, subchelate, with elongated curved dactyli and acute claws (pereopods not predatory or only pereopod 1 subchelate)
36 – palp of maxilliped much shorter than basipodite, maximally 3 palpal articles (palp larger, more than 3 articles)
37 – life cycle with 3 larval stages (epicaridium, micronisicium, cryptoniscium) (no larval stages or only 1 type of larva)
38 – appendix masculina on pleopod 2 reduced (appendix present)
39 – mandibular palp reduced (palp present)
40 – both pairs of maxillae absent or rudimentary (maxillae present)
41 – protopod of maxilliped in females broadened, leaflike, palp short, not more than 3 articles
42 – maxilla 2 absent (maxilla present)
43 – maxilla 2 with only 2 endites, endites bearing distally few curved spines (maxilla with 3 endites, endites distally with setae)
44 – endite of maxilliped absent (endite present)
45 – coxal articles transformed into lateral tergal plates (coxal articles cylindrical)
46 – biphasic moulting (monophasic moult)
47 – gut tube entirely of ectodermal origin (gut tube with entodermal midgut)
48 – second thoracopod transformed into maxilliped with broadened endopod articles (= pylopod) (second thoracopod is an ambulatory appendage)
49 – male genital papillae on articulation between pereonite 7 and pleonite 1 or on pleonite 1 (papillae ventrally on pereonite 7 or on coxae of pereopod 7)
50 – uropod sympod enlarged and folded ventrally to cover respiratory chamber (uropods with short sympod, inserting anterolaterally on pleostelson, not covering pleopods)
51 – telsonic region of pleotelson greatly shortened, therefore anus terminal and uropods inserting terminally or subterminally on telsonic apex (telson not shortened, anus proximoventrally, uropods laterally in area of pleonite 6)
52 – medial margin of maxilla 2 without row of filter setae (filter setae present)
53 – female pleopod 2 uniramous and no suture between sympod and ramus (sympod separated from rami, two rami present)
54 – male pleopod 2 exopod shortened, sympod enlarged, longer and wider than exopod (sympod shorter than rami, exopod broad, leaf-like)
55 – male pleopod 2 endopod transformed into copulatory organ with sperm pocket or sperm tube (male pleopod 2 similar to other pleopods but medially with appendix masculina)
56 – male pleopod 1 uniramous (biramous)

Table 4. (Continued).

57 – pair of male pleopod 1 medially fused (not fused)
58 – female pleopod 1 absent (present)
59 – female second pleopod operculiform (second pleopod not enlarged)
60 – third pleopod operculiform (third pleopod not enlarged)
61 – stomach: anterior filter channel oriented transversely (channel arranged longitudinally)
62 – between insertion of pereopods ventrally a water-conducting system composed of cuticular scales (rows of scales absent)
63 – antenna 1 very small, of 3 articles only (antenna 1 larger, more than 3 articles)
64 – maxilla 2 of oniscid-type, without strong spine-like setae, only 2 endites (with spines and setae, 3 endites)
65 – male pleopod 2 without leaf-like endopod, instead copulatory stylet present (oniscid-type) (endopod normally developed, leaf like, medially with styliform appendix masculina)
66 – antenna 1 with not more than 2 flagellar articles (more than 2 flagellar articles)
67 – exopod of pleopod 5 distally with rough tubercles (tubercles absent)
68 – mandible: area of setal row transformed into cutting edge
69 – medial endite of maxilla 2 strongly reduced, only proximal medial lobe with setae present (medial endite forming lobe as long as other 2 endites)
70 – mandible: molar process transformed into cutting edge
71 – pleopods 4 and 5 in comparison with 1-3 with weaker cuticle and pleopod 3 not similar in shape to 4 and 5 (all pleopods similar, with marginal swimming setae)

Morphological characters

The data matrix with morphological characters (Table 3, Table 4) has been compiled using characters described in Wägele (1989) and Brusca and Wilson (1991) and some additional features. Most characters have been discussed in Wägele (1989), wherefore a detailed character analysis is not presented here. Autapomorphies of single terminal taxa have largely been ommitted since they do not support partitions in trees. Characters of doubtful homology also were ommitted. Uropod characters, for example, were ignored because it is not clear which types of uropod variations are homologous (there are several different forms of styliform uropods). Where characters of the genera discussed herein are not illustrated or described in published literature, we examined specimens from the second author's collection. Parsimony analyses were carried out with PAUP4*.

Results

Note that in the following we use the family name "Bopyridae" as synonym for the suborder name "Epicaridea". The reason has been explained in Wägele (1989) and our new data confirm the earlier arguments (see below). If not stated otherwise, the results are based on the shorter alignment.

Sequences

The length of the complete individual sequences varies greatly, mainly due to the variability of the V4 and the V7 regions (Table 2). The shortest sequences were found in asellotes (represented by *Asellus*, *Lirceus*, *Janira*), which belong together with the phreatoicids to the oldest phylogenetic lineages of isopods (Wägele 1989; Brusca and Wilson 1991; Tabacaru and Danielopol 1999), but the phreatoicids (see *Colubotelson* in Table 2) have a longer sequence comparable to that of several other isopods. Short sequences are also present in the parasitic bopyrids and cymothoids (*Probopyrus*, *Riggia*, *Anilocra*), while other species considered to be related to cymothoids have very long genes (e.g., in *Aega*, *Natatolana*). It seems that elongation and shortening of the variable regions can occur convergently, there is no evidence that a short sequence is a primitive characer state, even though usually metazoan ssuRNAs are shorter than 2000 bp. Since large parts of the variable areas were not alignable we can not study the evolution of elongations for the time being.

Split-supporting positions

The spectra of split-supporting positions show that also the short alignment (1736 bp) is quite noisy, meaning that a large number of chance similarities occurs (Fig. 2). This fact already shows that an analysis of this alignment can be difficult, we must expect low resolution and groupings supported by analogies. The slope of the spectrum shows no marked difference between the high signals on the left part of the spectrum and the many nonsense-groupings following on the right part of the spectrum. Only the 49 best of 1328 splits of this alignment are shown (Fig. 2). The strongest signal separates the Cymothoidae from the rest of the sequences, meaning that these parasites have a high number of autapomorphies in the ssu rRNA gene. This is in accordance with the occurrence of long branches in the estimated phylogenetic trees (e.g. Figs. 4, 8). Split number 3 separates the Cymothoidae + Bopyridae from the remaining sequences. Many positions that are little variable in most

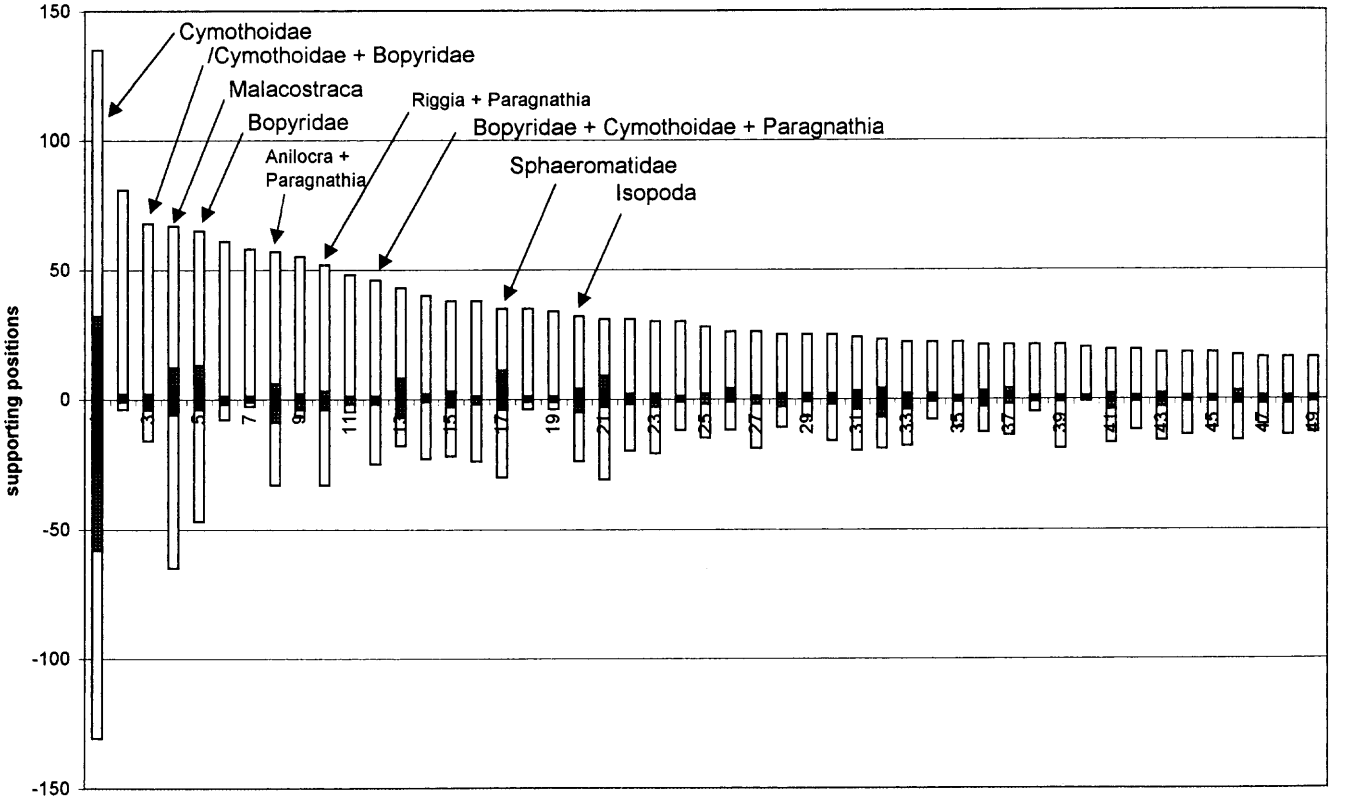

Fig. 2. Spectrum of split-supporting positions estimated with PHYSID. Black part of columns indicate how many split-supporting poisitions are highly conserved (binary positions). Highest signals in favor of a group are shown above the x-axis, below is seen the signal of the complementary group. "/" indicates that a named group is found below the x-axis

isopods show subtitutions in Cymothoidae or in Bopyridae, and many positions have putative synapomorphies of the clade Cymothoidae + Bopyridae. Since the signal for split 3 is one of the highest in our data set, the probability that this is a phylogenetic signal (and not the result of chance similarities) is higher than for all the following splits in the spectrum.

Other important splits seen in Fig. 2 separate the Malacostraca (split 4), the group Bopyridae + Cymothoidae + Paragnathia (split 12), the Sphaeromatidae (split 17) and the Isopoda (split 20). However, information conserved in favour of these splits is relatively poor in comparison with the first splits (left part of the spectrum).

We can use the patterns of split-supporting positions to compare alternative phylogenetic hypotheses. A major question discussed herein is the alternative between the shared ancestry of Bopyridae and Cymothoidae and the possible common origin of Bopyridae and Gnathiidae. Fig. 3 shows these alternative patterns: the sequences of Bopyridae and *Paragnathia* share 9 conserved positions, bopyrids and cymothoids share 16 positions. Furhermore, of the 9 positions conserved in *Paragnathia* + bopyrids, two also show the same character state in cymothoids, wherefore only 7 are unique for this split in comparison to 14 for the alternative hypothesis. Two highly conserved binary positions support the clade Cymothoidae + Bopyridae (513 and 673 in Fig. 3), zero binary positions fit to the alternative. So, the probability that one of these groups is monophyletic is higher for the group Cymothoidae + Bopyridae. We must test this hypothesis with other methods, and in future with more genes.

Distance trees

Neighbor-joining topologies estimated with different models of sequence evolution did "shake the tree" in the sense that species belonging to different suborders were mixed in clades, the topologies varying with the selected model. Nevertheless, the clade Bopyridae + Cymothoidae appeared in all topologies (example in Fig. 4). As already indicated by the spectrum (Fig. 2) and other analyses (Table 1) the branch lengths of cymothoids are exceptionally long, especially that of *Anilocra physodes,* in accordance with the findings obtained when Ts:Tv-rates were studied in relation to distances.

species	supporting positions	species	supporting positions
	0000000000011111		000011111
	2344567777802346		267912446
	4012170018560379		758560384
	7237334886832430		426552094
Probopyrus pacificiensis	**AAGATTTGCACGATTG**	***Probopyrus pacificiensis***	**CGAGTACTT**
Hemiarthrus abdominalis	**AAGATTTGCACGATTG**	***Hemiarthrus abdominalis***	**CGAGTACTT**
Riggia paranensis	**AAGATTTGCACGATTG**	***Paragnathia formica***	**CGAGTACTT**
Anilocra physodes	**AAGATTTGCACGATTG**	*Riggia paranensis*	TG**A**CC**A**TTT
Paragnathia formica	CGAC-G---**AAA**CGA	*Anilocra physodes*	C-**AA**C**A**TGA
Excorallana quadricornis	CGAC-GC---CAGTGA	*Excorallana quadricornis*	T--GCGTTC
Aega antarctica	CCAC-GC---AAGCGA	*Aega antarctica*	T--CCGTCC
Eurydice pulchra	CGAT-GCG--CAGCGG	*Eurydice pulchra*	T--ATGTCC
Natatolana albinota	CGAC-GC---AAGCCA	*Natatolana albinota*	T--CCGTCC
Typhlocirolana moraguesi	CGAC-GC--GAAGCGA	*Typhlocirolana moraguesi*	T-GCCGTCG
Cyathura carinata	GGAC-GC---AAGCGA	*Cyathura carinata*	T--CCGTCC
Cassidinidea sp.	CGAC-GC-A-CTGTGA	*Cassidinidea sp.*	TT-TCGTCC
Sphaeroma serratum	CGAA-GCG--CAGCTA	*Sphaeroma serratum*	T--TCGTCC
Cymodoce tattersalli	TAAC-GCG--AGGTTG	*Cymodoce tattersalli*	T--GCGTCC
Glyptonotus antarcticus	CGAC-GC---AAGCGA	*Glyptonotus antarcticus*	T--ACGTCC
Cleantis prismatica	CGAC-GC---AAGCGA	*Cleantis prismatica*	T--ACGTCC
Ligia oceanica	AGAC-GC---AAGCAA	*Ligia oceanica*	T--GCGCCC
Oniscus asellus	AGAA-GC---AAGCGA	*Oniscus asellus*	T--ACGTTC
Janira maculosa	CGAC-GCG--CAGTTA	*Janira maculosa*	T--CTGTAC
Asellus aquaticus	CGAC-GC---AAGCGA	*Asellus aquaticus*	T--ACGTCC
Lirceus fontinalis	AGAC-GC---AAGCGA	*Lirceus fontinalis*	T--ACGTCC
Colubotelson thomsoni	AAAC-GC---AAGCTA	*Colubotelson thomsoni*	T--ACGCCC
Astacus astacus	CGAC-GA---AAGCGA	*Astacus astacus*	T--ACGTAC
Artemia salina	CGGC-GG---AAGCGA	*Artemia salina*	T--ACGCGC
Androctonus australis	AGGC-GG---AAGCGA	*Androctonus australis*	T--ATGTGC

Fig. 3. Split-supporting patterns based on a ssu-rRNA alignment as identified with PHYSID, supporting alternative hypotheses of monophyly: Cymothoidae + Bopyridae (left pattern) vs. Cymothoidae + Gnathiidae (right pattern). Tolerated noise level: <25%. Numbers refer to positions of the alignment.

Maximum parsimony trees

Analyses using all taxa and the complete sequences (including highly variable regions: 4263 bp, 1840 parsimony-informative positions) yielded topologies with partly good resolution. The 50% majority-rule boostrap-consensus tree contains monophyletic isopods (100% support), monophyletic Cymothoida (87%) and the clade Bopyridae + Cymothoidae (100%) (Fig. 5).

We analyzed in greater detail the shorter alignment (1736 bp, 431 parsimony-informative charac-

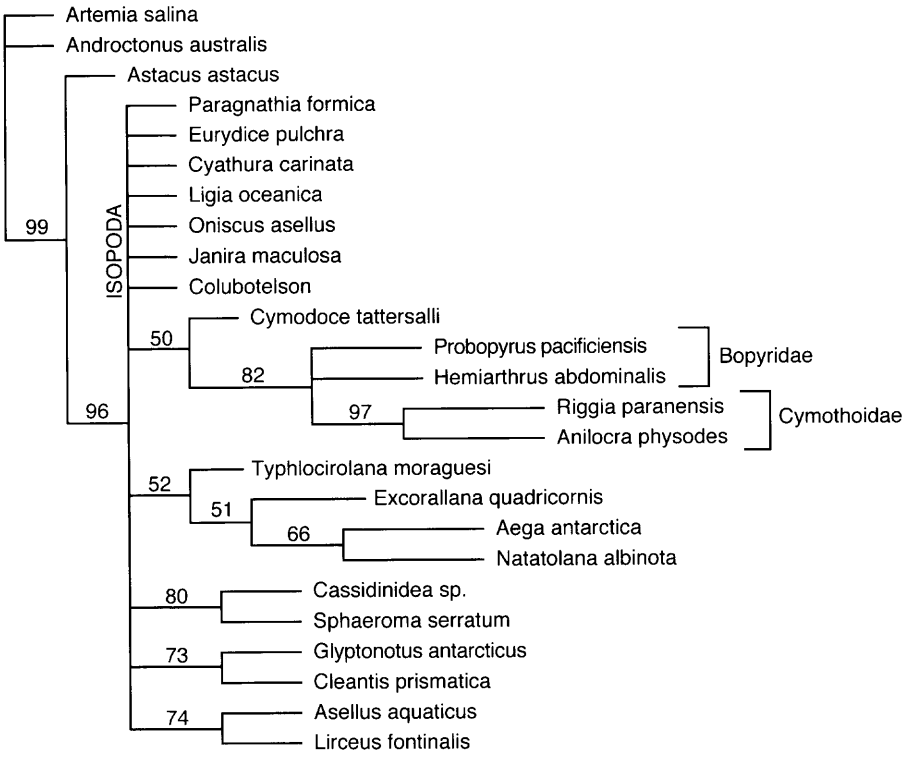

Fig. 4. Bootstrap 50% majority-rule consensus neighbor-joining tree (500 replications) estimated with a LogDet/paralinear model as implemented in PAUP4*, the proportion of invariable sites estimated from the data, rooted with *Androctonus australis*.

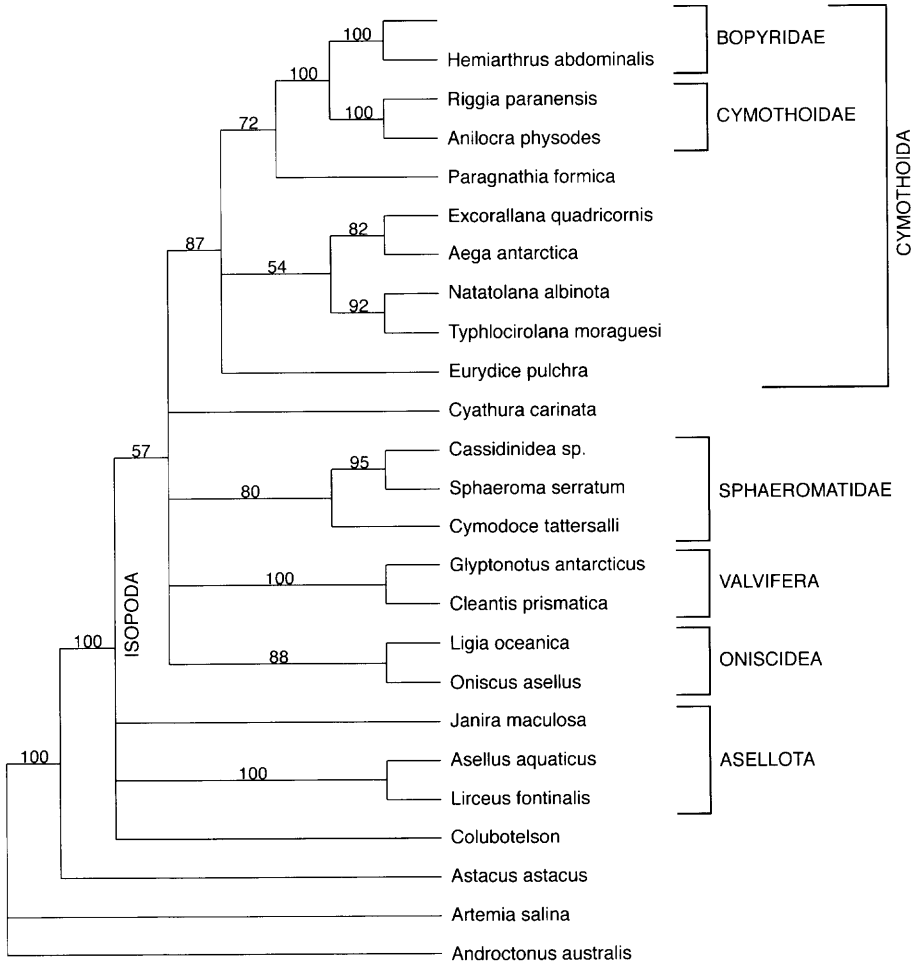

Fig. 5. Maximum parsimony 50% majority-rule bootstrap consensus tree (500 replications) estimated with all ssu-rRNA sequences and the complete alignment (4263 bp, 1840 MP-informative positions)

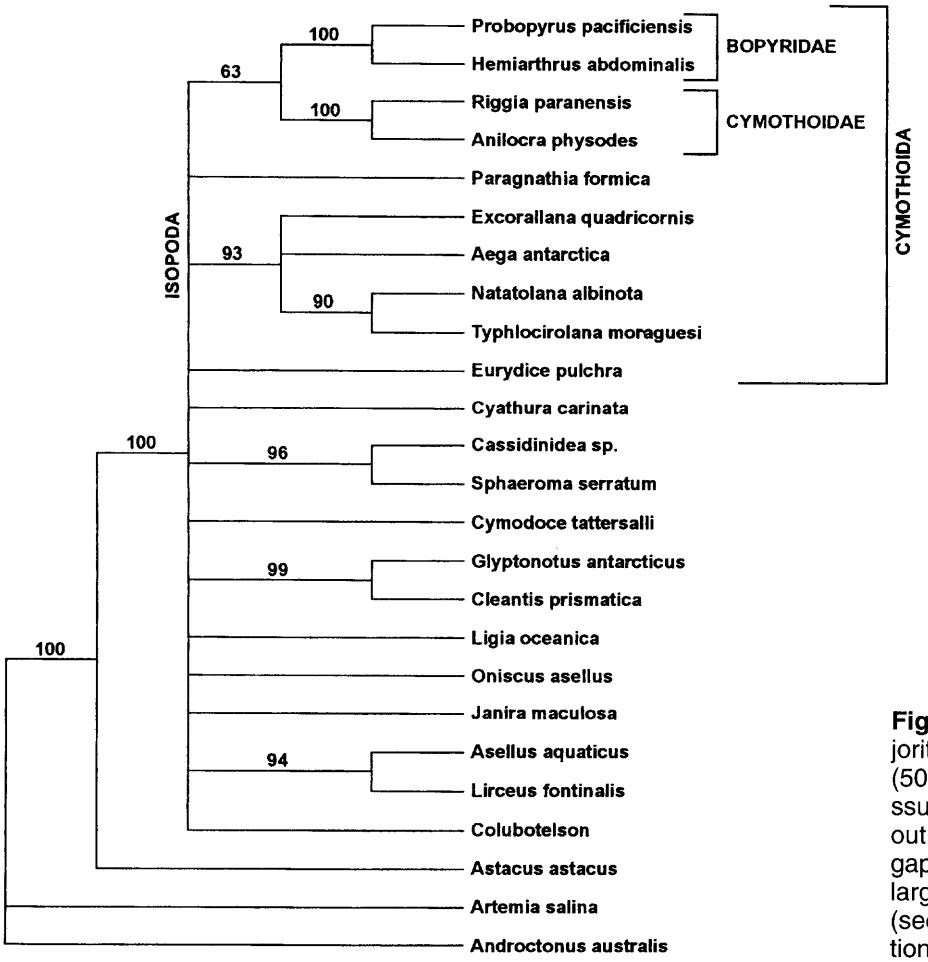

Fig. 6. Maximum parsimony 50% majority-rule bootstrap consensus tree (500 replications) estimated with all ssu-rRNA sequences, alignment without variable regions (1736 characters), gaps coded as "fifth nucleotide". The large number of chance similarities (see also Fig. 2) causes a low resolution of the topology.

ters) and excluded long-branch sequences to reduce the noise in the data. The sequences of the taxa of interest (first four sequences in Table 1) also are fast-evolving, wherefore there exists the danger of long-branch attraction. However, the distinct signal seen in the spectrum (Fig. 4) is better explained by true homology of shared character states.

Using all 25 sequences and coding gaps as "missing", resolution is much lower than with the complete alignment. Neither the Oniscidea nor the Cymothoida or the clade Bopyridae + Cymothoidae was recovered. Since the evolution of the 18SrDNA gene is characterized by insertions, elongations and deletions in variable regions we also coded gaps as "fifth nucleotide" to use additional information. In this case the clade Bopyridae + Cymothoidae is supported (63%, Fig. 6). The resolution is low due to the incompatibility of splits already noted in the spectral analyses. Interestingly, excluding the long-branch sequences identified in Table 1 (sequences of *Paragnathia*, *Eurydice*, *Cassidinidea*, *Sphaeroma*, *Janira*) the support for the clade Bopyridae + Cymothoidae increases to 97% even when gaps are treated as "missing" (Fig. 7A). Nevertheless, the resolution of the tree is still low for most major divergence events within the Isopoda. Obviously, the 18SrDNA gene is not very informative for early divergences of the Isopoda and more genes and species have to be considered. If the sequence of *Paragnathia formica* is also included (Fig. 7B) it appears as sistergroup of the Cymothoidae, but the reduced bootstrap support clearly indicates that there is more noise in the alignment than in the previos analysis. Inclusion of other long-branch sequences also reduces the support for most branches.

Maximum likelihood

ML topologies estimated with all 25 sequences, using different models of sequence evolution (the gamma distribution of rates and other parameters being estimated via maximum likelihood) supported the clade Bopyridae + Cymothoidae, but showed some peculiar combinations of genera (the anthurid *Cyathura* among oniscids, sphaeromatids and asellotes polyphyletic). Testing different models implented in PAUP 4* did not improve the situation. So again we reduced the number of species excluding

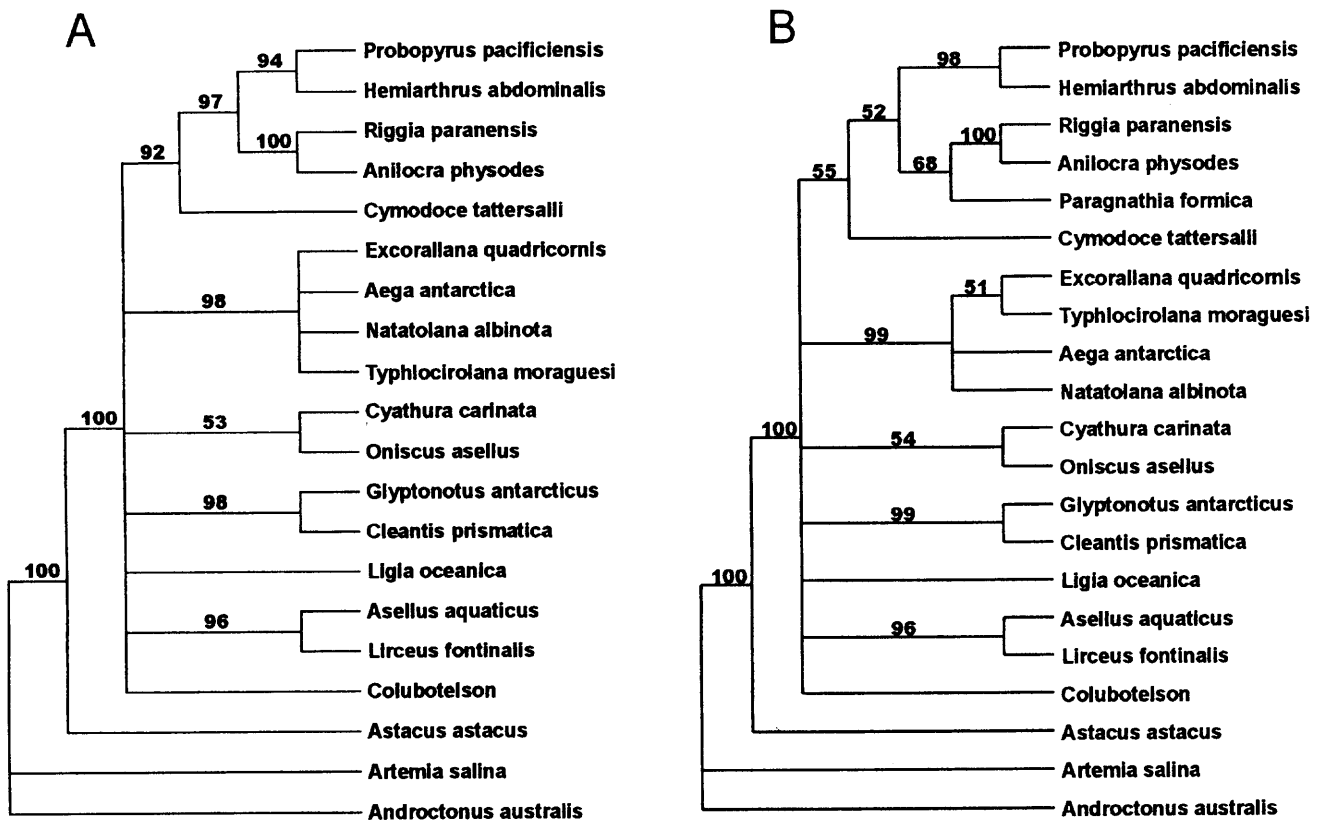

Fig. 7. A: Analysis as in Fig. 6, but excluding 5 long-branch taxa (*Paragnathia*, *Eurydice*, *Cassidinidea*, *Sphaeroma*, *Janira*). B: As in Fig. 7A, but including *Paragnathia formica.*

long-branch sequences and concentrated on the question of the relationship between bopyrids, cymothoids and gnathiids. An example is shown in Fig. 8: the clade Bopyridae + Cymothoidae is again recovered, the sequence of the gnathiid *Paragnathia formica* fits to a larger clade together with the other parasitic and predatory isopods and, in this example, with valviferans, a sistergroup-relationship between bopyrids and the gnathiid sequence is not supported. Repeated runs resulted in variations of this topology, with onsicids and valviferans in different places of the trees, but the group Bopyridae + Cymtohoidae was always monophyletic. Due to extreme long computation times a boostrap analyses was not possible.

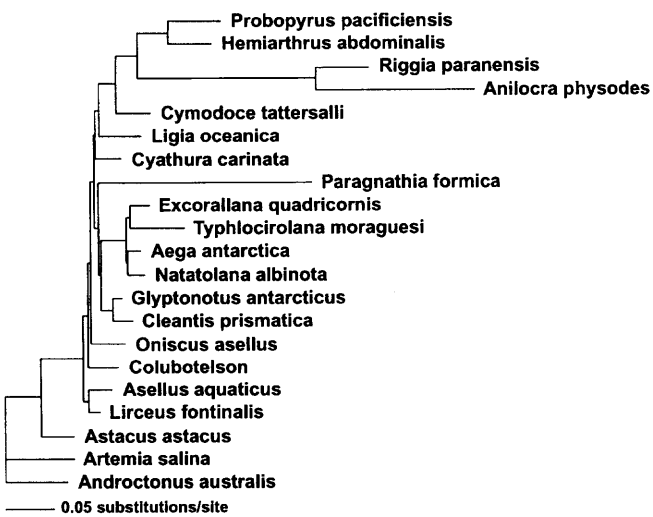

Fig. 8. ML topology for 21 sequences (long-branch taxa excluded), using the general-time reversibel model with rate heterogeneity, substitution rate matrix parameters and gamma distribution shape parameter estimated via maximum lieklihood, molecular clock not enforced, proportions of sites assuming to be invariable estimated from the data.

Tree-lengths of constrained topologies

Using the alignment of ssu-rDNA sequences as data matrix we constructed with the program MacClade topologies equivalent to the isopod phylogeny obtained from morphological characters by Wägele (1989) and by Brusca and Wilson (1991) (Fig. 1). Tree length is shorter for the first topology

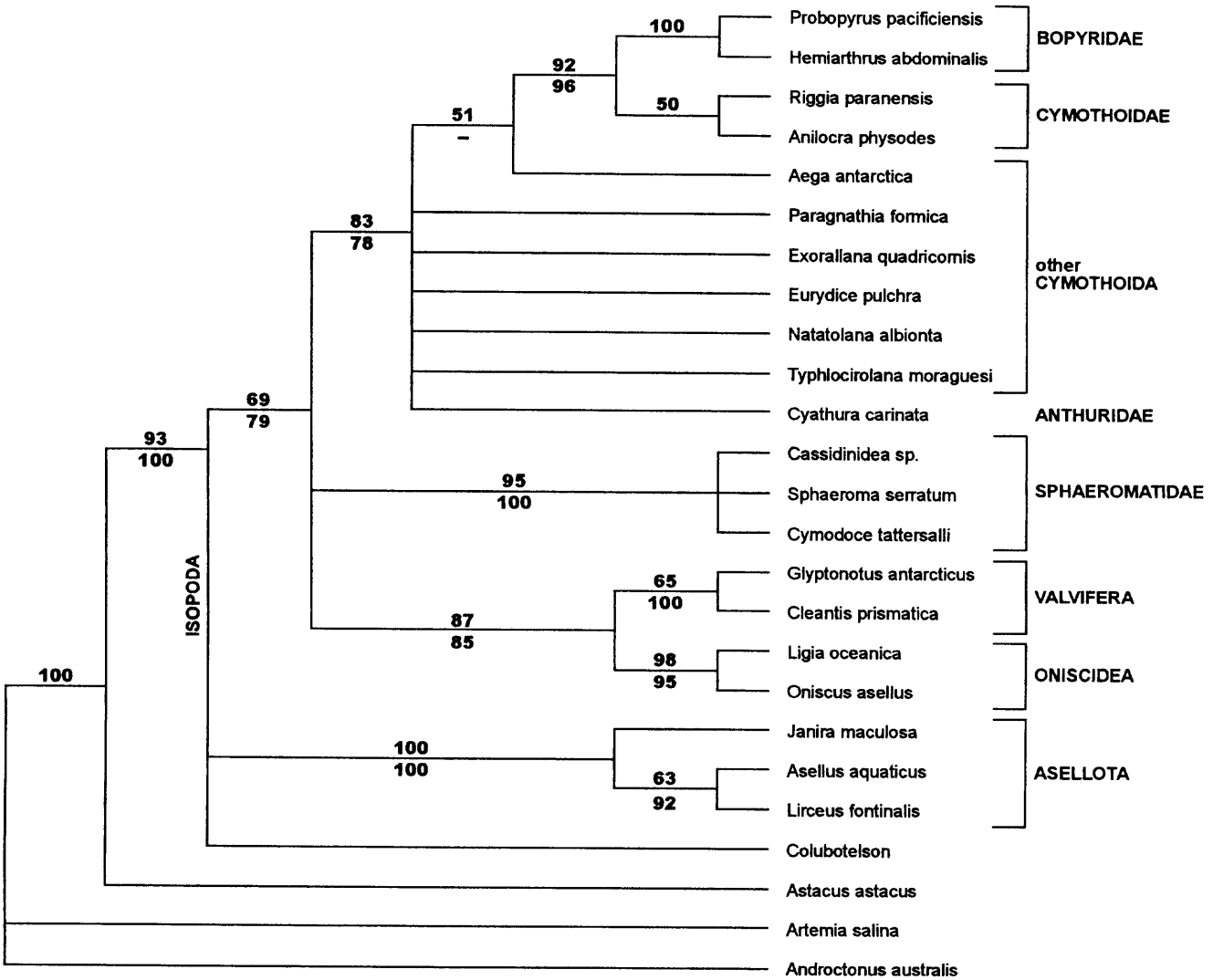

Fig. 9. Bootstrap 50% majority-rule consensus tree (500 replications), with values for morphological characters above the lines (see Tables 2 and 3) and values for combined molecular and morphological characters (weighing morphological characters by factor 10) below the lines.

(1980 instead of 1993 steps). If in Fig. 1A the bopyrids are transferred to the line leading to *Paragnathia,* tree length is 1987, i.e. 7 steps longer. The sequence data fit better to the topology of Fig. 1A.

Morphological characters

Fig. 9 shows the topology estimated with the morphological characters of tables 3 and 4 (support values above the lines). The monophyly of the following groups is confirmed: Isopoda, Asellota, Aselloidea (= *Asellus* + *Lirceus*), a clade comprising the Oniscidea (*Oniscus* + *Ligia*) and Valvifera (*Glyptonotus* + *Cleantis*), the Sphaeromatidae, and a group equivalent to the Cymothoida of Wägele (1989), which however also includes the anthurid *Cyathura carinata*. The clade Bopyridae + Cymothoidae is recovered with a bootstrap support of 92. The basal polytomy in the Cymothoida results from the difficulty to code transformation series (e.g. appearance of blunt spines on the P1 merus in cirolanids or aegids, and their assumed later reduction in cymothoids and bopyrids). (During the search for characters in published descriptions we noted again that taxonomic redescriptions are badly needed to understand the evolution of morphology within the families of the Cymothoida).

Combined data

Adding the morphological data to the ssu rRNA-alignment the topology calculated with maximum parsimony is very similar to the one based on morphology alone (Fig. 9), however the monophyly of

the Bopyridae + Cymothoidae is supported with a bootstrap value of 69. Giving morphological characters a higher weight, a procedure justified by the fact that morphological character state changes probably are more complex and not caused only by a single substitution, the dendrograms get a higher resolution for basal splits. Fig. 9 (support values below the lines) shows the result when morphological characters are given 10 times higher weight. The topology resembles more the pure morphological analysis, with a boostrap support of 96 for the clade Bopyridae + Cymothoidae.

Discussion

Information content of 18SrDNA sequences

The ssu rRNA gene might not be the best one for the study of early isopod phylogeny, even though this gene has often been used for the study of paleozoic radiations of other taxa (e.g., Friedrich and Tautz 1995; Carranza et al 1996; Cavalier Smith et al 1996; Spears and Abele 1999, etc.). The oldest known isopod fossil, a phreatoicid morphologically similar to extant species (Schram 1970), is of carboniferous origin, wherefore the first divergence events must be older than 300 million years. Sometimes the ssu-rRNA conserves even older phylogenetic signals, e.g. for the split between cnidarians and the Bilateria (Philippe et al. 1994; Wägele and Rödding 1998), but in most cases Cambrian or Precambrian radiations left little signal in this gene and multiple hits produced noise and false signals (see e.g.; Philippe et al. 1994; Abouheif et al. 1998, discussion of the Ecdysozoa-hypothesis in Wägele et al 1999).

The information content of our molecular data is not sufficient to recover the complete phylogeny of isopods, but at least for the origin of the Bopyridae there is a distinct signal in the alignments we used. The spectrum of supporting positions (Fig. 2) has shown that erosion of phylogenetic signal is a problem when isopod sequences are being compared, and this explains the low resolution of the estimated phylogenetic trees (Figs. 4–8). However, some useful information seems to be present in our ssu rRNA alignment, as indicated by the good agreement between molecular phylogenies, the stronger signals in the spectrum (Fig. 2), aspects of earlier morphological analyses (Fig. 1A, see Wägele 1989) and similarities with the cladistic analyses of morphological data presented herein (Fig. 9). In most topologies we find the clade Bopyridae + Cymothoidae. We will discuss the phylogeny of basal isopod groups elsewhere and concentrate here on the question of evolution of the parasitic species within the Cymothoida.

Evolution of the Bopyridae

All available evidence is compatible with the hypothesis that bopyrids evolved from a cymothoid-like ancestor. The sistergroup-relationship Cymothoidae + Bopyridae is supported by the ssu rDNA – data in distance-trees (Fig. 4), in analyses using MP- and ML-methods (Figs. 5, 6, 7) and when the total evidence (including morphological characters) is considered, and we find patterns of supporting positions (Figs. 2 and 3) for this clade that have a better support than most of the other splits of the alignment. These taxa share most similar ssu rDNA sequences, which also are shorter in comparison with cirolanids and aegids, similar mouthparts and hook-like claws (Fig. 11), the same type of life-cycle (Fig. 10). The tree lengths of constrained topologies equivalent to previous morphological analyses (Fig. 1) show that the bopyrid-cymothoid grouping is a more parsimonious solution than the gnathiid-bopyrid grouping when the molecular data are considered. The cladistic analysis presented herein (Fig. 9) does not include all isopod taxa, because we had to restrict the data matrix to selected species for which sequences were available, wherefore the cladistic reconstruction is incomplete and shows a polytomy including the Cymothoida and Anthuridea.

A dendrogram is just a skeletton around which an evoutionary scenario should fit as perfectly as possible. If available, evidence originating from data not used for phylogeny reconstruction can be used to test the plausibility of the phylogeny (Wägele 2000). Thus an evolutionary scenario can be used to falsify a phylogenetic hypothesis. In the case of the Cymothoida the scenario has partly been developed by earlier authors, who described the evolution from necrophagous and predatory cirolanid-like ancestors to cymothoid-like fish parasites (e.g., Menzies et al 1955; Brusca 1981; Delaney 1989; Wägele 1989). Cirolanids, corallanids, tridentellids, aegids and the less derived cymothoids all have a similar shape of the body (Fig. 12): head, thorax and pleon are not separated by deeper sutures and the oval body has a continuous streamlined outline. Tail fan and pleopods are well developed, the animals can swim efficiently, except in adult cymothoids. They can move in strong surf (e.g., species of *Eurydice*) or swim fast to attach to fishes (many aegids and young cymothoids). Most of these species eat fish (many Cirolanidae) or suck fish blood. It seems that the evolution of the whole group depended on the presence of fishes as food supply. Some cirolanids can feed on other items (e.g., species of *Eurydice* catching planctonic crustaceans or eating insects: own observations, Holdich 1981; *Anuropus* on jellyfishes: Barham and Pickwell 1969) but usually reports on the Cirolanidae mention that they have been found on dead fishes, in baited traps, some-

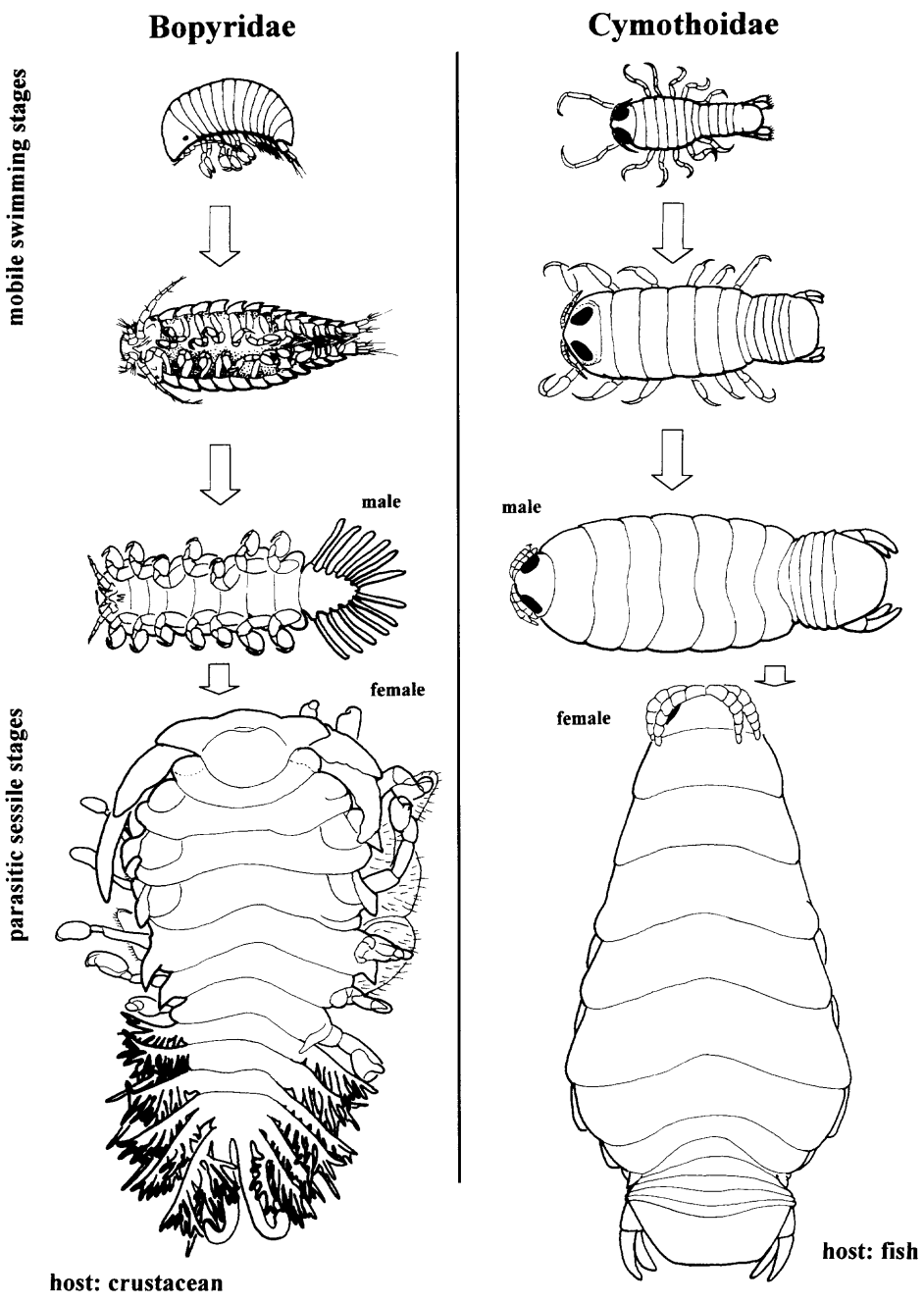

Fig. 10. Comparison of life-cycles of bopyrids and cymothoids.The bopyrids are represented by *Ione thoracica* (after drawings by Bourdon 1968; length of adult males: 3.8 - 4.4 mm; length of adult females: 6.5 - 8 mm), except the second stage, a microniscium of *Cepon elegans* (after Giard and Bonnier 1887). The cymothoids are stages of *Emetha audouini* (after Montalenti 1948 and Trilles 1962; length of adult males: 4 - 6.5 mm; length of adult females: 20 mm). The second cymothoid stage shown is a transitional one between the swimming young and a small male, just settling on a fish.

times on living fishes (e.g., Gruner 1965; Johnson 1976a, b; Bird 1981; Stepien and Brusca 1985; Bruce 1986; Wägele and Bruce 1989). Mouthparts of the carnivorous cirolanids are modified in comparison with omnivorous isopods. In the ectoparasitic tridentellids and corallanids these mouthparts are even more specialized (unfortunately the way of life of the Tridentellidae and Corallanidae is little known). We found *Excorallana quadricornis* on fishes caught with fishing-lines by Venezuelan fishermen, Williams and Williams (1985) reported to have seen corallanids on living fishes. The anterior hindgut of cirolanids, corallanids, aegids, cymothoids, and also in gnathiids, is highly dilatable, the

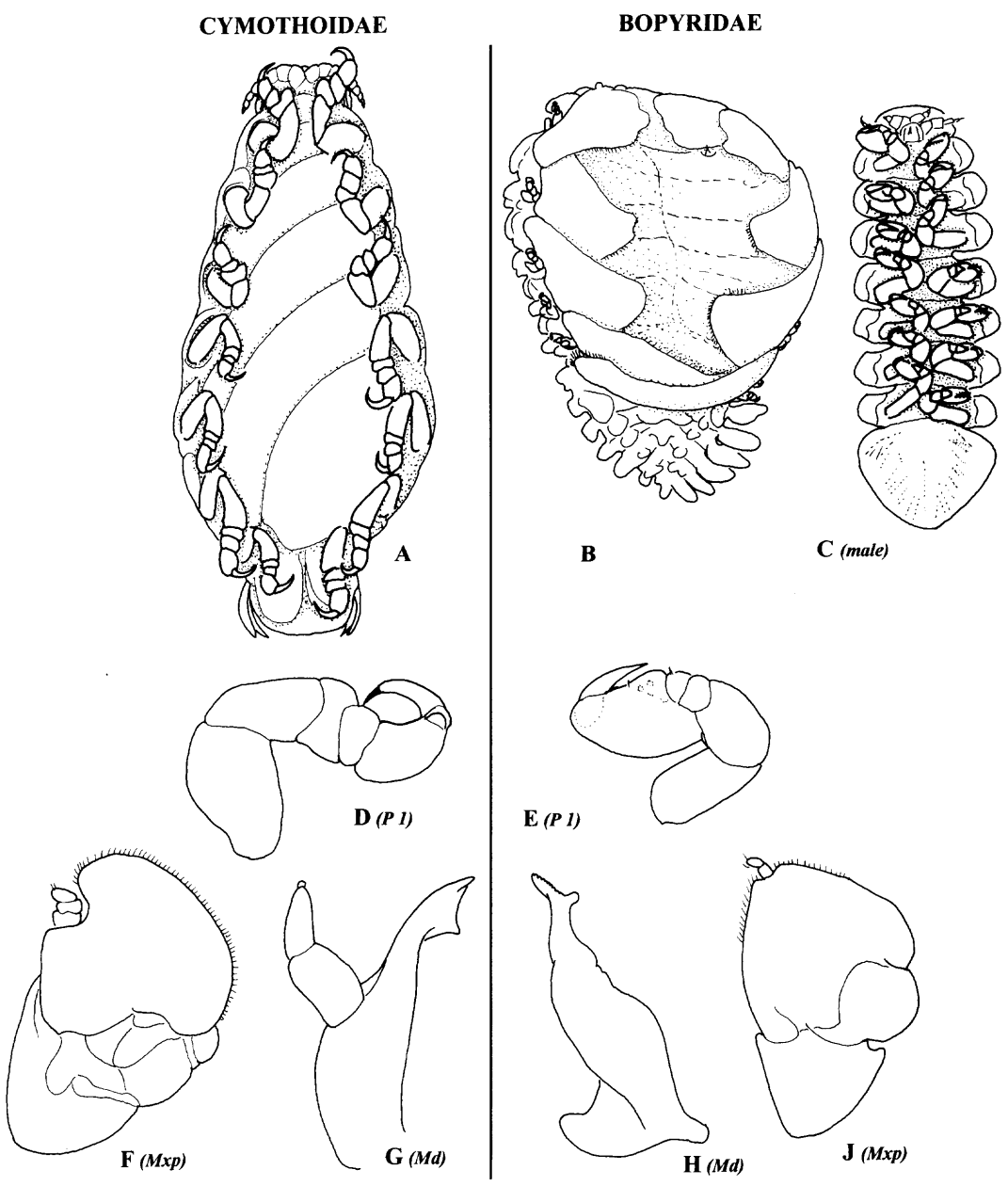

Fig. 11. Comparison of some morphological characters of cymothoids and bopyrids. A and B: ovigerous females in ventral view, C: dwarfish male in ventral view, enlarged in comparison with B (A: *Ceratothoa oestroides*, after Montalenti 1948: length of adult females: 18 - 30 mm; B, C: *Argeia atlantica*, after Markham 1977: length of adult male: 3.3 mm; length of adult female: 7.4 mm). D-J: selected appendages, namely pereopod 1 (*P 1*), mandible (*Md*) and maxilliped (*Mxp*). D: P1 of *Ceratothoa sp.* (after Nunomura 1981);); E: P1 of *Argeia atlantica* (after Markham 1977); F: Mxp of *Ceratothoa banksii* (after Bonnier 1900); G: Md of *Cymothoa excisa* (after Monod 1969); H: Md of *Bopyrus fougerouxi* (after Bonnier 1900); J: Mxp of *Pleurocryptella formosa* (after Bonnier 1900).

animals can rest for a long time after a good meal (e.g., Johnson 1976a, b; Wägele 1990).

Aegids are more similar to cymothoids in the structure of their appendages: they are adapted to cling to living fishes (Sars 1879; Brusca 1983; Wägele 1990). Their anterior pereopods (1-3) have an acute, hook-like dactylus. The mandibles of aegids have a much smaller pars incisiva in comparison with cirolanids, they are not able to cut meat but they destroy tissue of their host to suck blood. Interestingly, in contrast to stinging gnathiids or paranthurids the mandibles are not really acute, they are not suitable for a simple piercing movement. Also, neither cymothoids nor bopyrids have

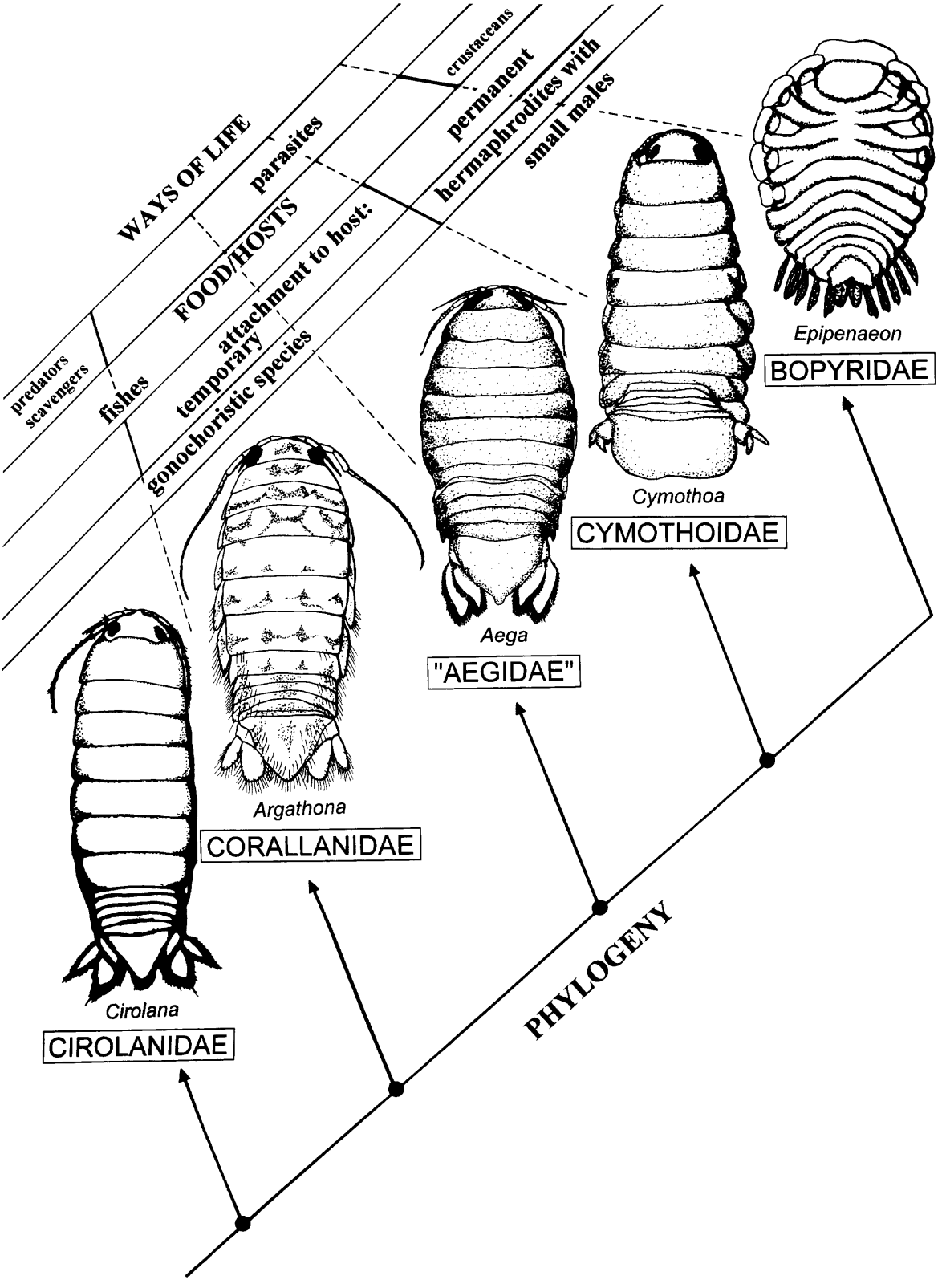

Fig. 12. Evolutionary scenario as obtained when ways of life are mapped on the phylogeny of part of the Cymothoida (lineage leading to Bopyridae). The taxon Tridentellidae, not shown here, also belongs to this lineage as close relative of the Corallanidae and Aegidae (the exact phylogenetic relationships of these families are not resolved).

needle-like mandibles but always conserve a small transverse pars incisiva (Fig. 11).

The less derived cymothoids look at first sight like aegids (Fig. 12), but they differ in having also hooked claws on pereopods 4 to 7, not only on pereopods 1–3 (Fig. 11A). While aegids fall off their host when they are satiated, cymothoids stay on their host, some growing to asymmetrical shapes in adaptation to life in gill chambers, for example. The animals do not suck continuously but bite several times through their host's skin, some mature females do not feed (Brusca 1978 a, b). Their life cycle is more specialized in comparison with the foregoing taxa: all cymothoids are facultative or protandrous hermaphrodites (e.g.; Bullar 1876; Legrand 1950; Szidat 1965; Brusca 1978 a,b), all postembryonic developmental stages live on fishes, with few exceptions (see below). While immature stages are able to swim, the adults reduce the swimming setae on their pleopods and stay on their host, in contrast to aegids. Males are smaller than females (Fig. 10). In *Nerocila californica* all juveniles mature into males when they settle on a fish, and they transform into a female when a second male arrives (Brusca 1978a). In some species males are neotenous and dwarfish in comparison to females (Szidat 1966).

The evolution that can be reconstructed from these observations clearly leads from cirolanid-like taxa to highly specialized fish parasites (see e.g. Menzies et al 1955; Brusca 1981; Wägele 1989). Some early steps of this evolution also have been discussed by Delaney (1989), who analyzed characters of the Tridentellidae and Corallanidae in comparison with cirolanids but did not consider the other taxa of the suborder Cymothoida. The results of our analyses indicate that the bopyrids, which all are parasites of crustaceans, evolved from ancestors that were parasitic on fishes, as proposed by zoologists of the nineteenth century and by Kussakin (1979), Bruce (1981) and Wägele (1989). All evidence presented in this study confirms this idea.

The change of hosts from fishes to crustaceans is not as surprising as it looks at first sight: corallanids have been found on palaemonid and atyid decapod crustaceans (Riek 1953, 1966) and sucking on mysids (Guzman et al 1988) and also the cymothoid *Telotha henselii* lives on palaemonids (Castro 1985). We must assume that Bopyridae and Cymothoidae had a last common ancestor that was a parasite on fishes and from which one line of descendants specialized more and more to suck hemolymph of crustaceans, giving rise to modern Bopyridae. Most bopyrids are probably hermaphrodites with small males (Fig. 10), an adaptation to sessility on hosts also present in cymothoids and therefore probably a character of the last common ancestor. While all cymothoid females pass through a male stage (Fig. 10), bopyrids show more variations. Immature specimens usually can develop into males or females, depending on the presence of another indivual when they arrive on a host. It has however often been overlooked that at least in the well-studied species *Ione thoracica* (Fig. 10) males can transform into females in the same way as cymothoids (Reverberi and Pitoti 1942), and young females also can change sex.

All pereopods of bopyrids are prehensile with acute claws, a character shared only with cymothoids. Maxillipeds of ovigerous females are very similar in both taxa (Fig. 11), with a broadened leaf-like maxilliped and a tiny palp of three or fewer articles. The mandible of bopyrids is not an acute stilett, as in other stinging isopods, but has distally a transverse incisor, as present in cymothoids (Fig. 11). Sessile adults lose their pleopodal swimming setae in both groups. One could argue that all these characters are convergent adaptations to parasitism, but probability of convergence is considered to be low by us, because in other isopod taxa alternative solutions are possible (see e.g. life-cycle of gnathiids: Wägele 1988). The similarity of the ssu rDNA can not be explained as adaptation to parasitism.

Taxonomic consequences

Early authors of the nineteenth century (Milne-Edwards 1840; Gerstaecker 1893; Kossmann 1880) placed bopyrids near cymothoids and cirolanids, implying that the parasites of crustaceans and fishes (bopyrids and cymothoids) evolved from carnivorous cirolanid-like ancestors. Fritz Müller (1871) rejected this idea and postulated a closer relationship with the terrestrial isopods (Oniscidea), mainly because of the presence of short antennulae, reduction of mandibular palps, a short telson and styliform uropods. He united oniscids and bopyrids in the group "Ligioiden". Kossmann (1880) rightly pointed out that these characters are not very specific and they are present in other crustaceans, too. Instead, he named a tribe "Cirolanidea" composed of carnivorous or parasitic isopods (Serolidae, Cirolanidae, Cymothoidae, Bopyridae, Cryptoniscidae). Many authors simply kept the parasites of crustaceans in a separate suborder (e.g., Giard and Bonnier 1888).

Most authors of the twentieth century got used to the separation of the bopyrids from all other isopods in the suborder Epicaridea (e.g., Richardson 1905; Nierstrasz 1931; Shiino 1939; Bourdon 1963; Gruner 1966; Kussakin 1967; Schultz 1969; Bowman and Abele 1982; Kensley and Schotte 1989; Roman and Dalens 1999), usually without comments on the phylogeny of these animals. Opinions on the phylogenetic position within the Isopoda dif-

fer greatly: Monod (1922) thought that bopyrids and the Valvifera are sistergroups. After studying the embryology of isopods Strömberg (1972) concluded that bopyrids belong to a monophylum together with the Flabellifera (which include among others the Cymothoidae) and the Gnathiidea. Schmalfuss (1989) in a study on the phylogeny of terrestrial isopods places the Epicaridea in a sistergroup-relationship with a group composed of Gnathiidea + Anthuridea + Flabellifera + Valvifera. The notion of earlier authors that bopyrids are derived from a cymothoid-like ancestor is found in topologies published by Kussakin (1979), Bruce (1981), and Wägele (1989).

The traditional classification of the isopod parasites of crustaceans as a separate suborder "Epicaridea Latreille, 1831" is not based on phylogeny reconstructions but is a pure typological separation of a group that is easily identified due to the derived morphology of these species. The results of Wägele (1989) and our new data show that these parasites are the sistergroup of extant Cymothoidae, which traditionally are classified as family within a suborder. We propose to classify the former "Epicaridea" simply as family Bopyridae, as suggested by Wägele (1989), and to keep the former family Bopyridae Rafinesque, 1815 as a subfamily. This consequence is necessary to get a classification that is compatible with the assumed order of divergence events.

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