PHYLOGENETIC ANALYSIS OF COPEPOD ORDERS

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ABSTRACT

Ten orders of the subclass Copepoda were cladistically analyzed using 21 morphological characters. Platycopioida is the sister taxon of the Calanoida and this gymnoplean clade is the sister group of the podoplean clade, which consists of eight orders. Misophrioida is, in turn, the sister taxon of the clade that contains the rest of the podoplean orders. Interestingly, the four orders (Poecilostomatoida, Siphonostomatoida, Monstrilloida, and Cyclopoida) containing species symbiotic with aquatic animals are monophyletic. Harpacticoida, Mormonilloida, and Gelyelloida are monophyletic in a fully resolved cladogram. Comparisons were made between this newly reconstructed scheme of copepod phylogeny and the previously proposed schemes.

A cladistic analysis of the copepod orders was first conducted by Geoffrey A. Boxshall and presented in 1984 at the Second International Conference on Copepoda held in Ottawa, Canada. This original scheme of copepod phylogeny was reconstructed with 12 morphological characters. Later, Boxshall added 7 more characters to his analysis and the scheme constructed anew was adopted by Schram (1986, fig. 43-7). There is no topological difference between the two schemes. However, a close examination of the latter scheme (in Schram, 1986) revealed errors in the assignment of three character states. These are: assigning character 13 (loss of the endopod of the sixth thoracopod = leg 5) to Mormonilloida, character 14 (complete fusion of the first two abdominal segments) to Cyclopoida and Poecilostomatoida, and character 15 (no more than two segments on the antennal exopod) to Monstrilloida, Poecilostomatoida, and Cyclopoida.

Within the last four years, two new orders of Copepoda have been proposed: Platycopioida by Fosshagen and Iliffe (1985) and Gelyelloida by Huys (1988a). Each of these new orders consists of a single family which was assigned, prior to the proposal of its new status, to the Calanoida and Harpacticoida, respectively. This paper reports the results of my phylogenetic analysis of the ten copepod orders using 21 morphological characters.

CONSTRUCTION OF CLADOGRAM

Nine of the 21 selected characters listed in Table 1 are those used by Boxshall (in Schram, 1986). The 12 newly selected characters are coded: 6, 7, 10, 11, 13, 14, 15, 16, 17, 18, 19, and 21. As obvious from the coding of characters 9 through 11 and 16 through 18, I opted for unordering the transformation of character states in order to avoid a risk of predetermining the topology of the resultant cladogram (O'Grady and Deets, 1987; Dojiri and Deets, 1988). Therefore, part of the increase in the number of selected characters is due to this unordered manner of coding.

Based on Boxshall and Huys' (1989) work on the phylogenetic relationships of the maxillopodan taxa, the Mystacocarida is the sister group of the Copepoda. However, due to extreme specialization in many mystacocaridan appendages, it is very difficult, if not impossible, to use Mystacocarida as an outgroup to polarize the character states in the Copepoda. Although the Skaracarida, consisting of two extinct species recovered from the Upper Cambrian deposit in Västergötland, Sweden (Müller and Walossek, 1985), was recognized as the first outgroup of the Mystacocarida-Copepoda clade (Boxshall and Huys, 1989), the morphological gaps between the Skaracarida and the extant Copepoda are so great as to render the polarization of copepod characters impossible. Recently, a detailed account of another fossilized maxillopodan taxon of the Cambrian period, the Orstenocarida, was published by Müller and Walossek (1988). Nevertheless, according to Walossek (personal communication), Bredocaris admirabilis Müller, 1983, the sole representative of the Orstenocarida, is not as closely affiliated with the Mystacocarida-Copepoda complex as the Skaracarida.

With these difficulties in the selection of an outgroup, I opted to use Tiemann's (1984,

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Table 1. Characters used in the cladistic analysis (plesiomorphic/apomorphic). T = trunk somite, P = leg.

1 No flexure line between prosome-urosome/present between T6 and T7.
2 No flexure line between prosome-urosome/present between T5 and T6.
3 No carapace/present at posterior end of maxillipedal somite.
4 Heart present/lost.
5 Female abdomen 4-segmented/3-segmented.
6 Female abdomen 4-segmented/2-segmented.
7 Female genital openings ventral/dorsal or lateral.
8 Male antennules geniculate/nongeniculate.
9 Antennary exopod 7- or 8-segmented/1-segmented.
10 Antennary exopod present/lost.
11 Antenna and oral appendages present/lost.
12 Labrum free/fused with labium to form siphon (oral cone).
13 Mandibular palp well developed, biramous/reduced to lobe.
14 Maxillular protopod with endites/reduced, without endites.
15 Maxilla 5-segmented/2-segmented.
16 Maxillipedal endopod 5-segmented/3-segmented.
17 Maxillipedal endopod 5-segmented/2-segmented.
18 Maxillipedal endopod 5-segmented/lost (fused to basis?).
19 First exopodal segment in P2–P4 with one spine/two spines.
20 P5 biramous/uniramous (endopod at most a seta).
21 P5 present/lost.

fig. 10) "urcopepod" and combined its characters with those for the ancestral copepod envisioned by consensus opinions (Boxshall et al., 1984). Furthermore, following the selection process adopted by Ho (1986) for the characters with multiple derived states, the most primitive state of a character in an order was selected to represent the character of that order, even though the selected state is found in only very few members of the order. For instance, the mandibular palp

is typically lost in the Poecilostomatoida, but the order is given the apomorphic state for the character 13 (see Table 2) because a reduced palp is present in the genus Erebonaster (Humes, 1987).

The BRANCH AND BOUND algorithm from the phylogenetic computer package PAUP (version 2.4.1, written by David L. Swofford of the Illinois Natural History Survey) was used in my analysis of copepod phylogeny to generate the most parsimonious cladogram. Twelve equal-length shortest trees (27 steps) were obtained, with a consistency index of 0.778.

SELECTION OF CLADOGRAM

Four of the 12 cladograms (trees) are fully resolved, with an F-ratio of either 0.145 (Fig. 1) or 0.143 (Fig. 2). The remaining eight unresolved trees have lower F-ratios, ranging from 0.059 to 0.103. I discuss below why I opt for the tree reproduced in Fig. 1A.

The Gelyelloida, Harpacticoida, and Mormonilloida in the two trees shown in Fig. 2 are monophyletic, sharing an apomorphy (character 21) of losing leg 5 in the course of evolution. Leg 5 in the ancestral copepod is a biramous appendage with 3-segmented rami (Boxshall et al., 1984). The ancestral state of this character is found in some primitive calanoids (such as Epacteriscus and Bathycalanus) and platycopioids. In other copepods, this appendage is in various states of reduction: with less segments in each rami, loss of the endopod, reduction of the appendage to a few setae, or loss of the entire appendage.

The most primitive state of leg 5 in the harpacticoids is found in Longipedia. It has a much reduced protopod carrying a large, 1-segmented exopod and a small, 1-seg-

Table 2. Character data matrix. Code 9 refers to missing (unknown) data.

|	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Calanoida	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platycopioida	1	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Misophrioida	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Harpacticoida	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Mormonilloida	0	1	0	1	0	1	0	9	0	0	0	0	0	0	0	0	0	1	0	0	1
Gelyelloida	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Monstrilloida	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
Cyclopoida	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Poecilostomatoida	0	1	0	1	0	0	1	1	0	1	0	0	1	1	1	0	1	0	0	1	0
Siphonostomatoida	0	1	0	1	1	0	0	0	1	0	0	1	1	1	1	1	1	0	0	1	0
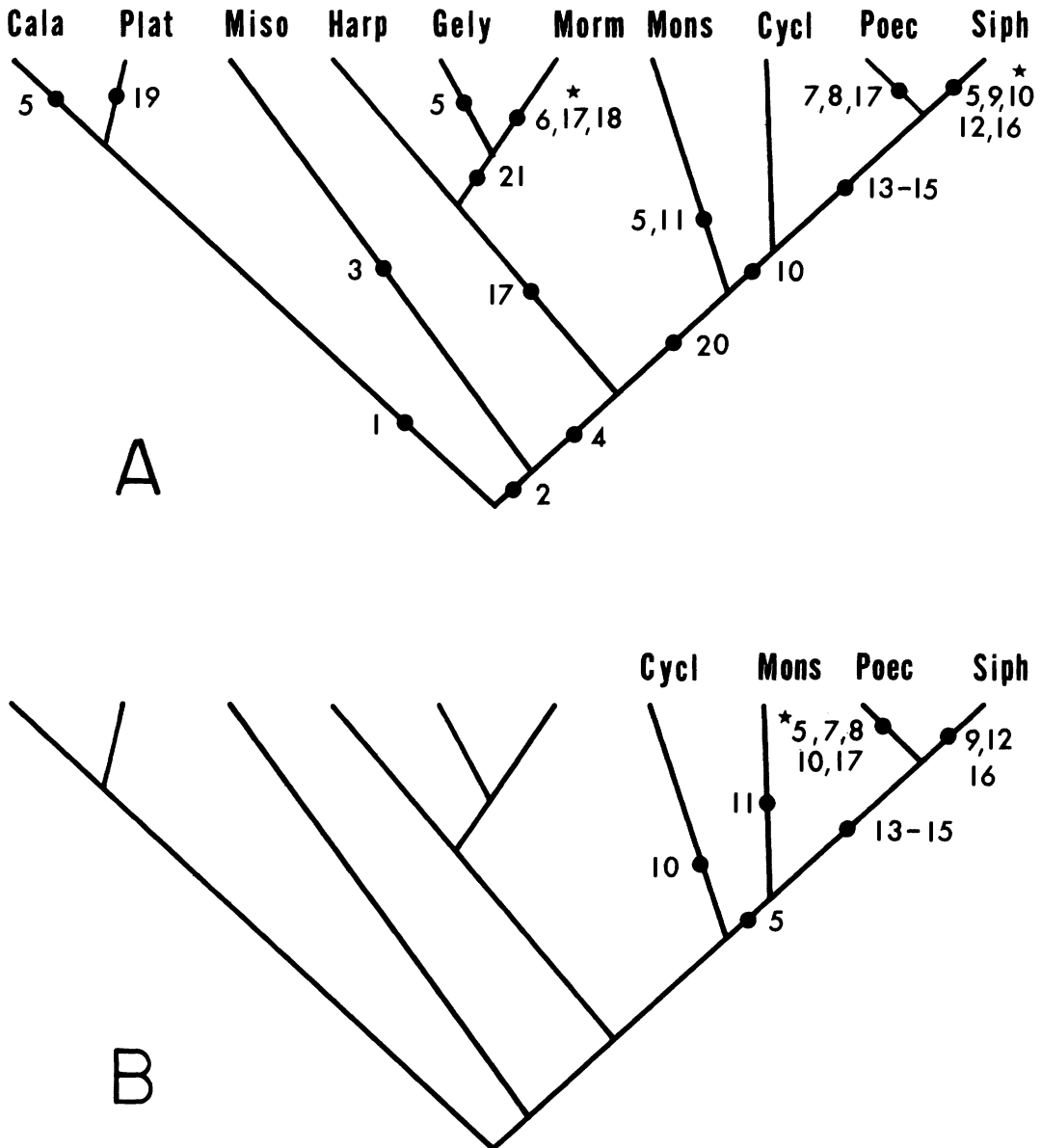
Fig. 1. Phylogenetic relationships of copepod orders. Two resolved trees with an F-ratio of 0.145. Topography and distribution of characters in B same as in A, unless otherwise indicated. Abbreviations of orders: Cala = Calanoida, Cycl = Cyclopoida, Gely = Gelyelloida, Harp = Harpacticoida, Miso = Misophrioida, Mons = Monstrilloida, Morm = Mormonilloida, Plat = Platycopioida, Poec = Poecilostomatoida, and Siph = Siphonostomatoida (* indicates reversal).

mented endopod. If the phylogenetic hypotheses represented in Fig. 2A, B are selected, one would have to accept an incomprehensible notion that leg 5 in the Harpacticoida was a redeveloped appendage, not an appendage in a reduced state. However, such difficulty is not encountered in accepting the phylogenetic hypotheses represented in Fig. 1A, B.

Gelyelloida, Harpacticoida, and Mormonilloida in both trees in Fig. 1 are monophyletic by sharing an apomorphy (character 17) of having a maxilliped with a 2-segmented endopod. The ancestral state of the endopod of the maxilliped is five segments, which is still exhibited in some species of calanoids, platycopioids, misophrioids, and cyclopoids. In these two fully
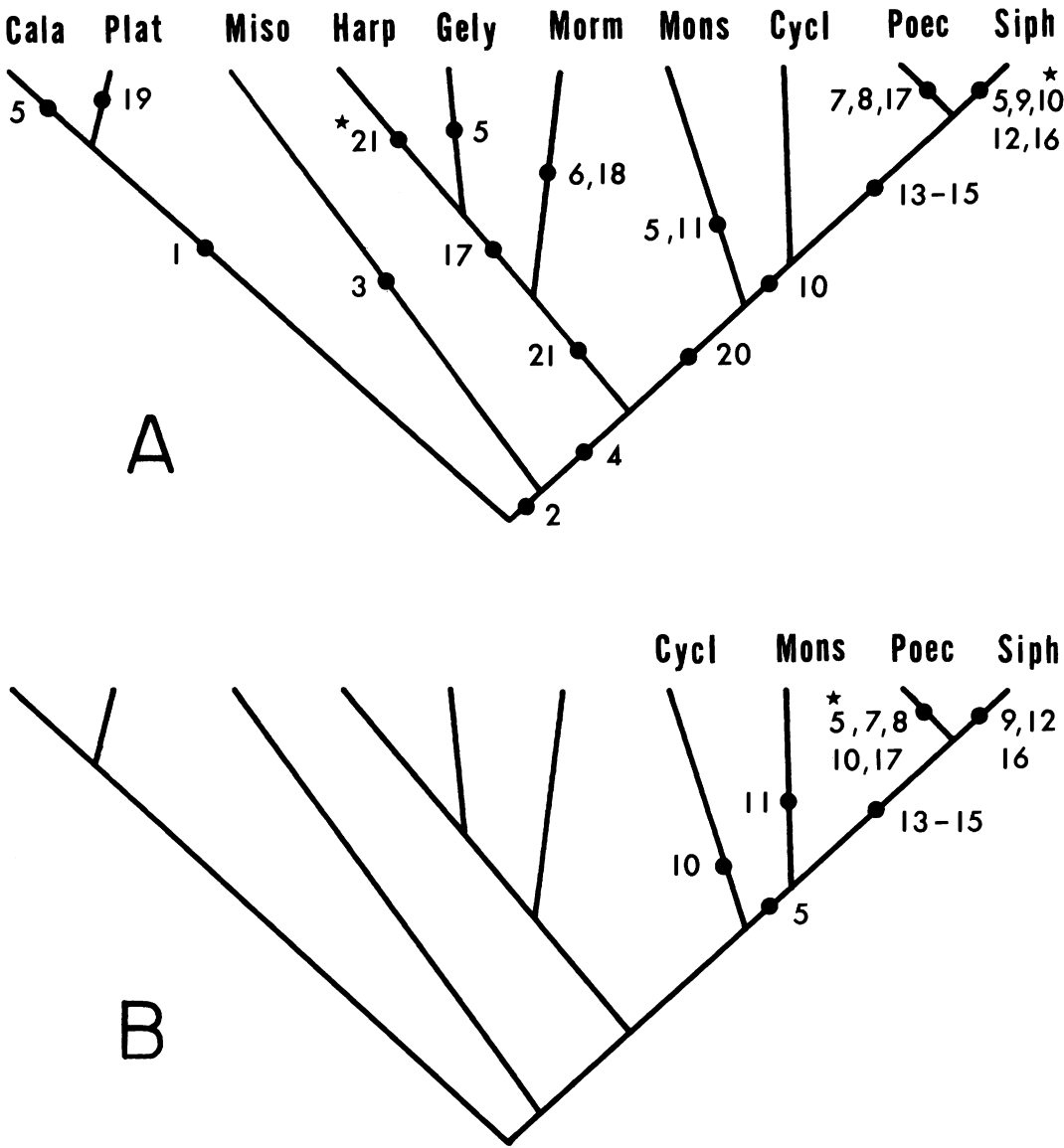
Fig. 2. Phylogenetic relationships of copepod orders. Two resolved trees with an F-ratio of 0.143. Topography and distribution of characters in B same as in A, unless otherwise indicated. For abbreviations of orders see legend in Fig. 1 (* indicates reversal).

resolved trees (Fig. 1A, B), the 2-segmented endopod of the maxilliped is lost (character 18) in the Mormonilloida, indicating a further reduction of the endopod of this appendage from the 2-segmented state. However, the reversal of character 17 on the Mormonilloida clade in these two trees is essentially a technical procedure due to the employment of an unordered manner of coding characters. It does not mean that the common ancestor of Mormonilloida car-

ried a pair of maxillipeds with multisegmented endopods. On the contrary, it infers that, in conjunction with character 18, Mormonilloida evolved from an ancestral form without an endopod on the maxilliped.

Character 10 (antennary exopod) is reversed on the siphonostomatoid clade shown in Fig. 1A. However, as in character 17 on the Mormonilloida clade, this reversal also results from the employment of the unordered manner of character coding; it does

not mean that siphonostomatoids carry a multisegmented antennary exopod. Most fish-parasitizing siphonostomatoids, except Lernaeopodidae, lack an exopod on their antennae. However, in those siphonostomatoid families symbiotic with marine invertebrates, possession of a rudimentary antennary exopod seems to be the rule. Unlike the one found in the misophrioids and the harpacticoids, the antennary exopod in Siphonostomatoida is an extremely reduced structure consisting of a single segment tipped with few setae (character 9). Combination of characters 9 and 10 on this clade would mean a true reversal of the antennary exopod in the course of siphonostomatoid evolution, changing from a state of missing (character 10) to a state of having one segment (character 9). Nevertheless, in this particular instance, it can be explained as the result of paedomorphosis.

Adults of Cyclopoida and Poecilostomatoida characteristically lack an antennary exopod. Interestingly, their first copepodids carry a rudimentary antennary exopod consisting of a single segment tipped with few setae. Both Dudley's (1966) work on Cyclopoida and Izawa's (1986) work on Poecilostomatoida clearly showed that the loss of the antennary exopod in the copepods of these two orders occurs in the second copepodid stage when it fails to continue the development of this part of the appendage. On the other hand, Kabata and Cousens (1973) and Kawatow et al. (1980), in their work on the life cycle of Lernaeopodidae, showed that the rudimentary antennary exopod appeared first in the free-swimming (first) copepodid stage and, unlike Cyclopoida and Poecilostomatoida, kept virtually unchanged throughout the remaining developmental (chalimus) stages, even after transformation into the adult. This is a genuine paedomorphosis with the larval (first copepodid) character persisting in the adult stage. Therefore, the combination of characters 9 and 10 on the siphonostomatoid clade in Fig. 1A does not represent a true reversal of character 10.

Since character 5 (female abdominal segments) on the poecilostomatoid clade in Fig. 1B is a true reversal, I select Fig. 1A as the best, fully resolved tree that contains no character reversal in the true sense.

Two of the eight unresolved trees repro-

duced in Fig. 3 have the lowest (best) value of F-ratio (0.059), which is the result of carrying no character reversal. According to the optimality criterion of Brooks et al. (1986), these two unresolved trees are to be selected. However, since the best, fully resolved cladogram shown in Fig. 1A does not contain a reversal of character in the true sense, I opt for the fully resolved tree as the preferred phylogenetic hypothesis.

PHYLOGENY

The concept of dividing the Copepoda into the Gymnoplea and Podoplea, a higher level of classification proposed by Giesbrecht (1892) and resurrected by Kabata (1979), is supported by the present analysis of the phylogeny. The former consists of two orders: Calanoida and Platycopioida. While the Calanoida is largely a group of planktonic copepods, the Platycopioida is composed of benthic copepods found primarily in marine caves (Fosshagen and Iliffe, 1988). This clade is distinguished by the possession of a major body flexure between the sixth and seventh trunk somites. The calanoid clade is identified by having a 3-segmented abdomen (character 5) and the platycopioid clade is recognized for bearing two spines on the first exopodal segment of legs 2, 3, and 4 (character 20).

The Misophrioida, a small group of copepods consisting of 16 marine species, is the most plesiomorphic member of the podoplean clade. For a long time this group was considered to be a deep-sea, near-bottom taxon (Boxshall, 1984), but in the last three years nine species have been reported from anchialine caves in the Atlantic and Indo-West Pacific (Boxshall and Iliffe, 1987; Huys, 1988b). It is characterized by the presence of a carapace (character 3). The remaining seven podoplean orders are united by the loss of the heart (character 4). The second offshoot of the podoplean clade, consisting of three orders, are descendants of an ancestor with only two segments in the endopod of its maxilliped (character 17). The Harpacticoida is the major taxon of this offshoot consisting of predominantly benthic copepods.

The recently proposed order, the Gelyelloida, in spite of its alleged affiliation with the Cyclopoida (Huys, 1988a), in this analysis proves to be the sister taxon of Mor-
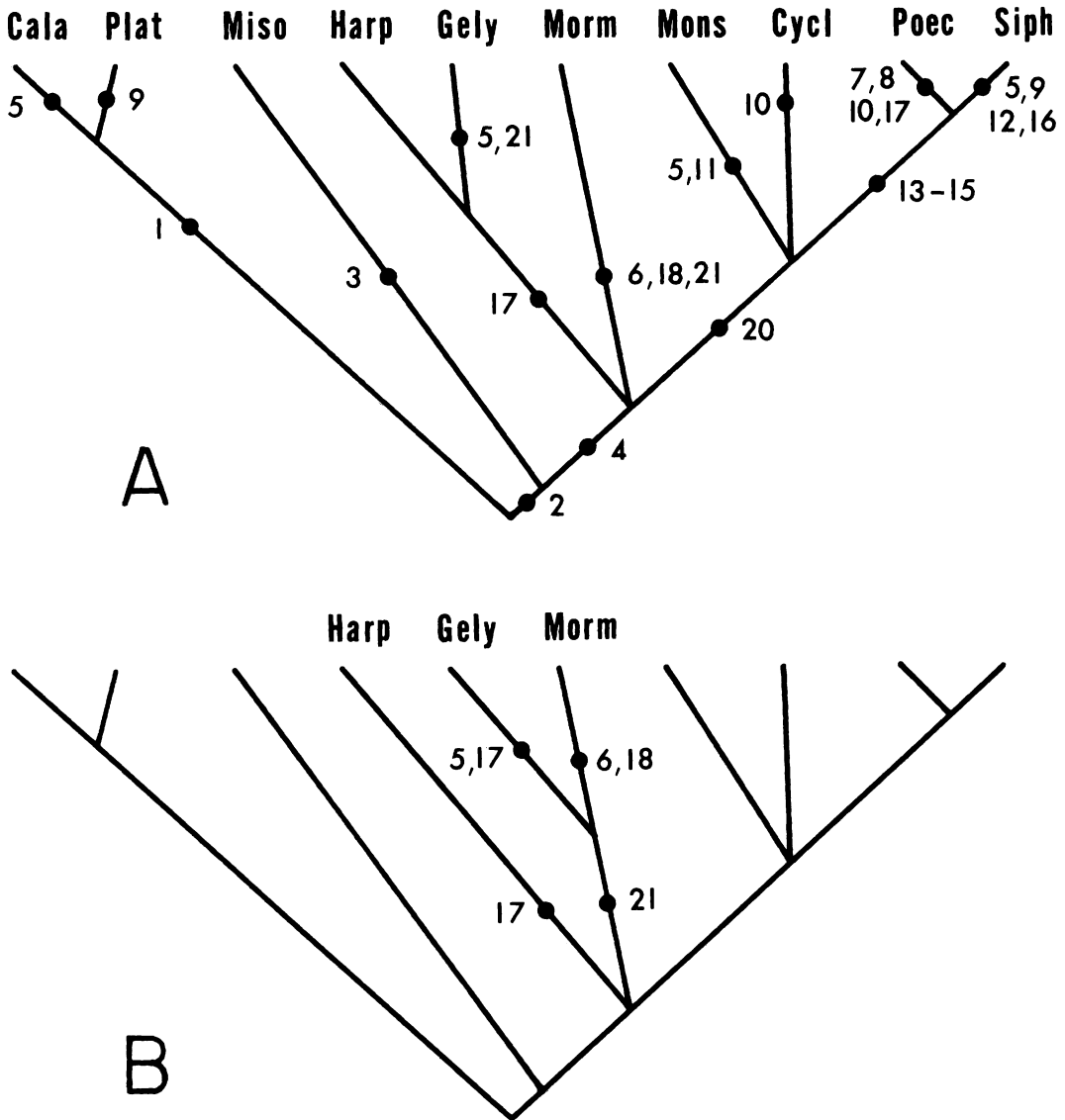
Fig. 3.   Phylogenetic relationships of copepod orders. Two unresolved trees with lowest value of F-ratio (0.059). Topography and distribution of characters in B same as in A, unless otherwise indicated. For abbreviations of orders see legend in Fig. 1.

monilloida. So far, the Gelyelloida and Mormonilloida are both known for only two congeneric species. Their nearest common ancestor is characterized by the loss of the fifth legs (character 21). *Mormonilla* is bathypelagic in the Atlantic Ocean (Boxshall, 1986), and *Gelyella* is a fresh-water inhabitant from subterranean water in European karstic systems (Huys, 1988b).

The remaining four orders of Copepoda, which contain species symbiotic with aquatic animals, are monophyletic. The

persistence of this relationship in the strict consensus tree (Fig. 4) reflects the true monophyly of these four orders. They descended from a common ancestor which had lost its endopodal segments on the fifth leg (character 20). The peculiar Monstrilloida, with parasitic larvae and planktonic adults, represents the first offshoot of this clade, which is characterized by lacking the second antenna and oral appendages in the adult (character 11). The next offshoot, the Cyclopoida, is represented by a group with
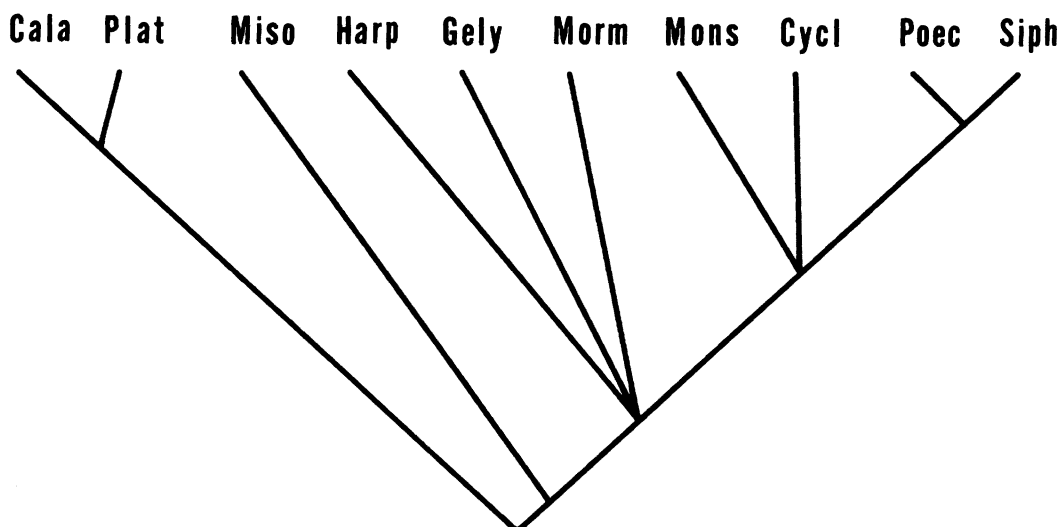
Fig. 4. Phylogenetic relationships of copepod orders. A strict consensus tree. For abbreviations of orders see legend in Fig. 1.

many free-living, fresh-water species. Ho (1986) envisioned that cyclopoids have invaded the fresh-water regime twice at two different stages of their evolution: once before the development of a terminal claw on the second antenna and again after the development of such a prehensile apparatus.

Siphonostomatoida and Poecilostomatoida are sister taxa that evolved from an ancestor with changes occurring in the oral appendages: the mandibular palp reduced to a lobe bearing setae (character 13), the maxillular protopod reduced to a lobe without endites (character 14), and the maxilla reduced to a 2-segmented appendage (character 15). These major changes in the oral appendages are viewed as preadaptations to the parasitic mode of life led by many members of these two orders.

COMPARISON OF PHYLOGENETIC SCHEMES

Between 1979 and 1984, three schemes of copepod phylogeny were proposed without the aid of cladistic analysis. The present scheme supports the "hypothetical phylogenetic tree of Copepoda" proposed by Kabata (1979, text fig. 23) at the stem where the Gymnoplea and Podoplea diverge. Kabata speculated that the podoplean line contained all orders of copepods except the Calanoida, but the sequence of their divergence from the podoplean stem differs considerably from the present scheme. Por's (1984,

fig. 19) "evolutionary scheme of the Copepoda" is very much like that of Kabata's, except most of the harpacticoid families with either phyllopodial or stenopodial maxillipeds were placed closer to the urcopepod prior to the divergence of the Gymnoplea and Podoplea.

The "tentative phylogenetic tree of copepod orders" proposed by Dussart (1984, fig. 1) is very different from the present scheme. It was reconstructed primarily on the basis of the naupliar musculature. Three major lineages were present in that scheme, one consisting of only the Harpacticoida, another containing the Poecilostomatoida and Calanoida, and the third embracing all the remaining orders. Dussart's scheme is not supported by Izawa's (1987) recent work on the ontogenetic features of poecilostomatoid nauplii. Izawa found that nauplii of the poecilostome "Cyclopoida bear resemblances to those of the Harpacticoida," but not to the Calanoida.

The major difference between Boxshall's (in Schram, 1986) phylogenetic scheme and the present one (Fig. 1A) is in the topology of the four orders that contain symbiotic species. The Siphonostomatoida is no longer a sister group of the clade containing the Poecilostomatoida, Monstrilloida, and Cyclopoida. Instead, it is the sister taxon of the Poecilostomatoida. This relationship is more in line with the common view that the

Siphonostomatoida and the Poecilostomatoida are more specialized (derived) than the Cyclopoida (see Kabata, 1979). Furthermore, this portion of the cladogram also reflects the system of copepod classification proposed by Lang (1948), which was considered one of the best post-Sarsian attempts (Kabata, 1979).

CONCLUSIONS

The phylogenetic hypothesis of copepod orders (Fig. 1A) presented here is derived from the cladistic analysis of the information available for adults, particularly adult females. It is not intended to be a definitive theory. It is presented here as a testable hypothesis that can be, and should be, corroborated by including more data. Information on the ontogeny, internal anatomy, and fine structures are all relevant for incorporation into this phylogenetic analysis.

Analysis of developmental patterns can yield useful information for reconstruction of copepod phylogeny. For instance, Ferrari (1988) discovered that the primary leg bud of leg 6 may first appear in podopleans at copepodid 3, copepodid 4, or copepodid 5, but never appears in gymnopleans. Regrettably, due to the lack of developmental information for Platycopioida, Gelyelloida, and Mormonilloida, this discriminant is not employable in the present study. Likewise, Izawa's (1987) excellent analysis on naupliar features is yet to be expanded to include all copepod orders.

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