OF UROPODS AND ISOPOD CRUSTACEAN TREES : A COMPARISON OF "GROUNDPATTERN" AND CLADISTIC METHODS

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CLADISTICS
COMPUTERISED METHODS
PHYLOGENETICS
CRUSTACEA
ISOPODA

ABSTRACT. – In a recent paper, Wägele (1994) attacked widely used computer-assisted cladistic methods for estimating phylogenetic trees, specifically those used in isopod phylogeny. This paper evaluates his alternative method, based on the allegedly "Hennigian" determination of groundpatterns, and compares it with empirical cladistic methods. Wägele's groundpattern method for determining phylogenies is logically circular, because it finds monophyletic groups that were assumed in the assembly of the groundpatterns. The method is also unscientific because it does not test the hypotheses that it proposes. Trees obtained using this method are likely to be unparsimonious because characters are not evaluated globally. As examples of how Wägele's method fails, and how cladistic methods are more rigorous, three cases from isopod phylogeny are discussed in some detail : the distribution of character states in the uropods, the sister groups of the Protognathiidae, and the relationships of taxa in the Microcerberidae.

CLADISTIQUE
MÉTHODES INFORMATIQUES
PHYLOGÉNIE
CRUSTACEA
ISOPODA

RÉSUMÉ. – Dans une publication datant de 1994, Wägele critique les méthodes cladistiques informatiques par la reconstruction des arbres phylogénétiques, et en particulier celles qui ont été utilisées pour la phylogénie des Isopodes. Cet article évalue une méthode alternative basée sur l'élaboration de "groundpatterns" méthode prétendue "hennigienne", puis la compare avec les méthodes cladistiques empiriques. La méthode du groundpattern de Wägele pour reconstruire les phylogénies est circulaire, car elle retrouve les groupes monophylétiques qui étaient déjà présumés dans l'ensemble des groundpatterns. Ce procédé est également peu convaincant car il ne teste pas l'hypothèse qu'il propose. Les arbres ainsi obtenus ne sont pas parcimonieux car les caractères ne sont pas évaluées globalement. Trois exemples de la phylogénie des Isopodes sont discutés en détail afin de montrer les faiblesses de la méthode de Wägele et comment les méthodes cladistiques sont plus rigoureuses : la distribution des états des caractères des uropodes, les groupes frères des Protognathiidae et les relations entre taxons chez les Microcerberidae.

INTRODUCTION

Phylogenetic research has seen stormy times in the last century, with various methods of inference holding the scientific community's attention, and then being replaced by more explicit and rigorous techniques. Computer assisted cladistic methods are now commonly used for inferring the branching structure of evolution, and in the absence of time machines, we may not have much better in the near future. Some European workers nevertheless prefer an allegedly "Hennigian" style of "argumentation," and belabour "well interred criticisms" of cladistic methods (Cannatella, 1991 :

377; see also Janvier, 1991). This rift between empirical cladists and neohennigians seems unlikely to go away for largely sociological reasons (Nelson, 1993), but this paper clarifies the methods of one neohennigian practitioner with respect to maximum parsimony cladistic methods.

Wägele (1989a) presented an explicit branching diagram of the phylogeny of major taxa in the Isopoda based on a detailed discussion of character states, but without global optimisation of the characters. Brusca and Wilson (1991) compared Wägele's tree with cladograms found using a well defined data matrix. Wägele's tree was found to be unparsimonious and therefore a less probable hypothesis of phylogeny. The most parsimonious

trees found by Brusca & Wilson (1991) also suggested different paths of character evolution and a different classification from those of Wägele (1989a). Recently, Wägele (1994; Wägele et al., 1995) has dismissed the Brusca and Wilson (1991) cladograms for the isopod crustaceans as "simplistic" and "based on methodological error." Wägele's premise is that the cladogram (in his opinion) was wrong, so the method must be wrong. Because Wägele, in a series of papers (Wägele, 1994, 1995; Wägele et al., 1995; Wägele and Stanjek, 1995), attacks analytical paradigms in modern phylogenetics, his initial 1994 paper is discussed in some detail here. In so doing, I clarify the differences between empirical cladistic methods that use global parsimony analysis and Wägele's (1989a; 1994) method of phylogenetic construction, which depends on what he calls "groundpatterns" (not to be confused with Wagner's (1980) groundplan divergence method). Wägele (1994) is in the same vein as an earlier critique of computer assisted cladistic methods (Lorenzen and Sieg, 1991), which was shown to be seriously flawed and invalid (Pleijel et al., 1992; Meier and Whiting, 1992; Haszprunar, 1992). The inadequacies of Wägele's "groundpattern" method are explained below, with a few examples from isopod phylogeny. Wägele (1994) is not answered point by point, because several issues he raises will be dealt with in later papers. In the following, "Wägele" means the paper of Wägele (1994), and page citations are from that work.

GROUNDPATTERNS

The notion of groundpatterns is central to Wägele's (1989a; 1994; Wägele et al., 1995; Wägele and Stanjek, 1995) method of constructing allegedly "Hennigian" phylogenies. A groundpattern of a taxon is an assemblage of presumed ad hoc, a priori ancestral character states. The groundpattern represents either a terminal taxon or an hypothetical ancestor within a phylogenetic tree. The construction of a groundpattern apparently relies on phylogenetic information external to the analysis at hand. That is to say, early in the analysis (e.g. Wägele's Fig. 1), polarities are determined for each character based on a priori knowledge of hypothetical relationships. The groundpattern is then constructed from these assumed plesiomorphic features of group of taxa. Wägele (p. 82) states that a groundpattern is "... reconstructed by analysis of the phylogeny of subordinated taxa after the reconstruction of the groundpatterns of these subordinated taxa." Groundpatterns, therefore, are "'basal node characters' or 'ancestral states'" that are "reconstructed in a previous analysis" (p. 85).

Wägele's groundpattern analysis differs from the determination of ancestral states often used in empirical cladistic analyses. Ancestral character states are generally constructed for entire ingroups or for terminal taxa using outgroup analysis (Maddison et al., 1984) or using ontogenetic information (Nelson, 1978). Lundberg rooting (Lundberg, 1972; Swofford, 1990) is sometimes used in cases where suitable outgroups are unavailable to root an undirected tree (e.g. for the Onychophora: Reid, 1996). In Lundberg rooting, undirected trees obtained in a parsimony analysis are rooted using a hypothetical ancestor, defined only by those character states for which an a priori determination of polarity is possible. Many or most characters for a hypothetical ancestor in Lundberg rooting need not be defined. Wägele, on the other hand, believes groundpatterns should be constructed sequentially for all characters during each step of a phylogenetic analysis to build a tree.

The greatest weakness of groundpatterns is how one determines their membership. One must rely on arguments of monophyly to assemble the taxa of a groundpattern. A groundpattern thus assumes that which is being sought in a phylogenetic analysis; i.e., it provides data for a hypothesis of relationships, but is dependent on that hypothesis for its description. This method is also error prone because Wägele (1989a; 1994) seems to rely on previously published ideas for determining ground pattern membership. For example, his groundpattern grouping for a sister group of the Asellota includes the Calabozoidae, because van Lieshout (1983) in her original nonphylogenetic paper highlighted what she thought were asellotan features. The Protognathiidae is grouped with the Gnathiidae because Wägele assumes that the two groups are closely related using results from an earlier paper (Wägele and Brandt, 1988). Wägele (1989a, 1990; 1994) believes that the Microcerberidae are nested within the Asellota based on an earlier analysis (Wägele, 1983a), and therefore does not test the possibility that his or other classifications of this group may be incorrect (Brusca & Wilson, 1991). These issues are treated in the sections below.

Wägele (pp. 102-103) is concerned that computerised cladistics fails to recognise the "encaptic order" of the taxa, by which he means taxa nested within other taxa are used in a single analysis as separate entities. This "encaptic order" is an essential part of the groundpatterns, because some groundpatterns have other groundpatterns nested within them. Brusca & Wilson (1991) tested hypotheses of relationship by using both subordinate taxa and more inclusive taxa as separate entities in the same analysis, such as the Microcerberidae and the Asellota. In Wägele's (1983a, 1989a) classification, the Microcerberidae are a derived member of the Asellota. Wägele finds Brusca &

Wilson's (1991) use of the two taxa disturbing because it violates his groundpattern groupings. Nevertheless, if one decides that one taxon is nested within another and excludes the first taxon from the analysis (as Wägele does), one cannot test the validity of that nesting.

PHYLOGENETIC "ARGUMENTATION" USING GROUNDPATTERNS

The "Hennigian" analysis is "carried out 'descending' step by step, starting with small taxonomic units,..." (p. 84). Wägele (1994) and Sieg (oral communication, 1990 Crustacean Conference, Brisbane Australia) claim that all possible trees need not be considered because the characters determine the phylogeny. "It is a mistake to belief [sic] that relationships of a large number of taxa can be explored only with computer programs. The number of possible taxa [sic] combinations decreases rapidly with each correctly identified synapomorphy" (p. 104). The operative words in the last quote are "correctly identified synapomorphy." For Wägele, a synapomorphy becomes "correct" because he uses a preconceived notion of phylogeny. In this view, one simply builds the tree by adding larger and larger blocks of taxa, associated by the groundpattern characters (e.g., his Fig. 4). Therefore, that the number of hypothetically possible trees increases polynomially with the number of taxa (Felsenstein, 1978) is not an issue, because each synapomorphy limits the number of possible trees to a very small set. This method is similar to that used in the first, non-definitive step of a computerised analysis : finding a starting tree (= hypothesis) upon which to swap branches (e.g., the "closest" method in PAUP).

Wägele's groundpattern method runs afoul of homoplasy and parsimony – if you have independent characters that provide conflicting evidence of descent, how do you resolve a phylogeny? Global homoplasy is not a problem in Wägele's "descending reconstruction" method because the characters are not optimised across the entire tree but only among the groundpattern groupings previously determined. Therefore, a potentially homologous character state appearing in other presumptive clades/groundpatterns has no significance. Global parsimony is ignored; in fact parsimony itself is largely ignored. Such "argumentation" allows Wägele (1989a; 1994), for example, to assert that the uropod is repeatedly modified from the groundpattern state of the tail-fan form (see below for further discussion). Wägele claims he is using parsimony in his method (e.g. the nearly unintelligible section on "evolutionary parsimony", p. 101), but his groundpattern

grouping method denies global parsimony, i.e., across the entire tree, and makes no attempt to minimise homoplasy.

Wägele appears to confuse "synapomorphy" with "autapomorphy" (e.g., pp. 91, 93, 98; also in Wägele, 1995 : 45-46), but his misuse of the latter term may clarify the underlying method of groundpattern analysis. Ordinarily one uses the word "autapomorphy" for a unique derived character that is found only in one terminal taxon. An autapomorphy is therefore uninformative about cladistic relationships, although it may define a single terminal taxon. Wägele uses "autapomorphy" to refer to a derived character shared by several terminal taxa being analysed, where most systematists would use the term "synapomorphy", a shared derived character. Wägele is misusing the term, but in the context of his groundpattern analysis, however, "autapomorphy" refers to a hypothetical taxon with an apomorphic feature. This then is the essence of groundpattern analysis : synapomorphies are converted into autapomorphies by coalescence of terminal taxa into a single groundpattern; the terminal taxa are thus removed from consideration. During the groundpattern tree reconstruction method, the analytical universe (set of analysed taxa) is reduced at each node deeper into the tree. Wägele's use of terms indicates this is being done during the analysis. The method fails at this point because falsifiability and parsimony are denied. By sequentially reducing the effective tip taxon number during a groundpattern analysis, Wägele's method increasingly removes parts of the tree from testing with the parsimony criterion. Consequently, a phylogeny estimated by this method is not scientific in the Popperian sense. Parsimony is simply ignored – no attempt at global minimisation of character state transitions is made. In contrast, empirical cladistic methods do not change the analytical universe during analysis; a cladogram derived by these methods, therefore, is a simultaneously parsimonious hypothesis of relationships for all taxa included.

Wägele's attack (p. 85) on the concept of the OTU (operational taxonomic unit) comes from the same source as his inability to tell a synapomorphy from an autapomorphy. Wägele asserts that OTU "smokescreens the indispensable reconstruction of groundpatterns." He dislikes this concept because an OTU presumes that all taxa will be used in the construction of a tree, while he thinks that only his groundpatterns should be used to construct the tree. By sequentially reducing the set of taxa or groundpatterns during tree building, he simplifies his analysis but, as pointed out above, fails to achieve a global solution.

The appearance of different, more parsimonious topologies in Brusca & Wilson (1991) is therefore not surprising. Wägele's groundpattern method is

logically circular. Although he accuses Brusca & Wilson (1991) of this error, no such error was committed (see below). Circular logic is simply where the data used to choose a hypothesis depend on the same hypothesis for their existence. In a phylogenetic analysis using groundpatterns, this occurs when a hypothesis of relationship is used to determine the data that are then used to choose the same hypothesis. Wägele (1989a; 1994; Wägele et al., 1995; Wägele and Stanjek, 1995; Wägele, 1995), therefore, sequentially assembles presumed monophyletic groups, determines their groundpattern states and finishes with a global hypothesis that contains the previously determined groups. No scientific test occurs in Wägele's groundpattern method: he simply piles taxa together in an *ad hoc* fashion.

CLADISTICS AND PARSIMONY ANALYSIS

How cladistic computer programs work

In the "subjectivity of computer cladistics" (p. 84), Wägele criticises 'computer cladistics,' but lists things inherent in any phylogenetic method. Wägele also claims that cladistic computer programs "calculate the tree." In this, he appears to misunderstand how a maximum parsimony analysis simultaneously and globally uses the information in all characters. In empirical cladistic methods, trees are chosen on the basis of their ability to parsimoniously explain the distribution of character states among all taxa with the fewest possible *ad hoc* hypotheses of character change. Using parsimony analysis, a universe of all possible solutions is systematically narrowed down to the fewest equally most probable solutions. Therefore, trees are not calculated, but are tested with the data using the parsimony criterion. Modern cladistic methods are most assuredly not "phenotypic" (p. 97) [*sic* – "phenetic" may be the meant]. Wägele thus confounds phylogenies and phylogenetically informative data, and may not be aware of the epistemological implications of his own groundpattern analysis.

Using the criterion of maximum parsimony, a cladogram (representing the branching order of a phylogeny) is chosen by observing how characters change on its branches. Although fully elucidated in several sources (e.g. Wiley, 1981; Swofford, 1990; Swofford & Olsen, 1990; Forey et al., 1992), the following simplified explanation is provided for comparison with Wägele's method. First, the character changes are plotted on a tree so that the number of changes or transitions are minimised. Next, the changes are summed for all characters on all branches, providing the total

number of transitions for the tree, the "tree length." The first tree in an analysis is retained for comparison with other trees. How the first tree is obtained is unimportant, except for optimising the time needed to find the shortest trees. Then another tree is obtained by some method (variations of branch re-arrangements), and the number of character changes summed as before. The new tree length is compared with the previous sum of character transitions, and the shortest tree of the two is retained. This process is continued until all shortest trees are found. During this tree comparison process, the characters are not used to calculate the tree, as Wägele (also quoted from Neff, 1986) implies, but the characters are used to choose a tree topology. The tree topology is constructed independently of the characters. But because the distribution of character states reflects the evolutionary process, the tree that is most congruent with the characters will be the best estimate of the phylogeny.

In parsimony methods, the simplest hypothesis is chosen as the most probable. The Popperian point of view asserts that the most probable complex hypothesis is that which is rejected the fewest times, thus suggesting a criterion for minimisation. For computer assisted cladistic applications, the appropriate criterion to be minimised is tree length, the sum of all hypothetical character changes (i.e, evolutionary transitions). For the method to work, the trees must be chosen independently of the character data, or the method becomes circular or at least starting point dependent (i.e., different starting points yield different results). For this reason, I prefer random starting tree topologies (available in the computer programs PAUP – Swofford, 1990 and PHYLIP – Felsenstein, 1993), because no assumptions are made about the distribution of character states during the initial tree construction. A tree must succeed over other possible trees based only on its parsimonious explanation of the data. Computer programs figure in this process because many tree topologies must be tested, and topologies with even small numbers of taxa may have millions of possible trees (Felsenstein, 1978). Wägele (p. 81) believes that computer programs are used as "black boxes" but anyone who uses a particular program should understand what the program is doing, or the interpretation of the results becomes equivocal. Most cladists do not have a "blind belief" in computer programs and, in fact, are constantly alert for programming algorithms that may violate the underlying cladistic logic (e.g. Luckow & Pimentel, 1985; Platnick, 1989; Coddington and Scharff, 1995). Much effort has been devoted to evaluating the accuracy of computerised phylogenetic methods (Hillis, 1995; Huelsenbeck, 1995; Li and Zharkikh, 1995; Miyamoto and Fitch, 1995). Although cladistic computer programs do not provide Wägele's "hand calculated"

results, one should not suspect the programs are wrong (Pleijel *et al,* 1992; Meier and Whiting, 1992). Wägele's groundpattern method may be the problem. His method cannot be heuristically useful because it determines tree structure from a priori hypotheses of relationship, and does not make independent comparisons of alternative trees. Computers may indeed be "black boxes" for Wägele because he appears to confuse maximum parsimony analysis with a distance analysis : Wägele (1995 : 45, his Fig. 3) discusses a distance tree in a parsimony context.

Necessity of polarising the characters

Despite Wägele's strident claims to the contrary, characters do not need to be polarised prior to a maximum parsimony cladistic analysis (Clark and Curran, 1986), except in Lundberg rooting or in some other parsimony methods that require some (but not all) characters to be polarised (e.g. Dollo or Camin/Sokal methods : Farris, 1977; Camin & Sokal, 1965). The character states must be homologous, but *a priori* choice of the direction and the pattern of changes is not necessary to find a parsimonious tree. In many cases, this is a strength of computer assisted cladistic analysis, because the *a priori* assignment of polarity to character transitions requires *ad hoc* arguments. While some character states may be objectively classified as apomorphic, in many cases one cannot be certain that an assessment of polarity and/or direction is correct, as happens for many characters of the Isopoda. Outgroup rooted maximum parsimony analyses are needed to achieve an unbiased assessment of the polarity of the characters simultaneously with that of the tree topology. The congruence of all characters on a parsimonious topology is an objective criterion for assigning apomorphies.

Use of outgroup taxa

In "Character valuation – an *a priori* source of mistakes", Wägele (p. 85-86) writes that using outgroups to provide "character polarity" is a "logical mistake : only the groundpattern of the ingroup contains the plesiomorphies that could be used for this procedure..." Wägele is apparently unaware of the implications of his statement. The groundpattern is a hypothetical construct that is obtained prior to the estimation of a phylogeny. The groundpattern does not contain anything because it does not exist – it is only a hypothesis similar to an *ad hoc* hypothetical ancestor, but with the difference that the latter does not require the monophyly of the ingroup to be certain. We cannot determine a groundpattern from inspecting

an animal and we cannot be certain that the groundpattern has anything to do with the ancestral states of a taxon. A phylogenetic estimate based on a predetermined hypothesis of descent must be circular in construction, and is one weakness of Wägele's method.

Wägele also claims (p. 84) that the use of outgroup taxa in an analysis is a subjective procedure, despite widespread opinion to the contrary (Maddison *et al.,* 1984; Kitching, 1992; Nixon and Carpenter, 1994). His belief is based on the assumption that one must be certain of the monophyly of the ingroup and the sister groups of the ingroup, using an a priori analysis. This assumption is incorrect. The use of outgroups in an analysis can test the monophyly of the ingroup, as was done in Wilson (1994) for the isopod family Janiridae. A more robust, objective analysis of relationships results from the inclusion of several outgroups (Maddison *et al.,* 1984), and decreases the chance that an inappropriate taxon will distort the rooting. Multiple outgroups provide the best ancestral optimisation at the outgroup node. Wägele also asserts that (p. 90) "Prior to the cladistic analysis, character analysis requires outgroup comparisons and – for terminal taxa – the reconstruction of ground patterns." Only in Wägele's idiosyncratic method (see also Wägele, 1995) is this necessary.

Character states of Terminal taxa

On p. 91 Wägele states : "To use computer programs, prior to the assemblage of a data matrix, groundpatterns must be reconstructed for all terminal taxa, whenever these are not species. To avoid mistakes, only groundpattern characters can be used for the data matrix." Groundpatterns are not necessary for phylogenetic analysis, and should be avoided because of the subjective element they introduce. The character states in a matrix are determined from the diagnoses of the terminal taxa. These are observed features, not idealised character states where the evolutionary direction has been interpreted, perhaps wrongly. If characters vary in the terminal taxa, as often happens, one has several options depending on the goals of an analysis.

1. Add terminal taxa to the tree to account for all variants (e.g. Struwe *et al.,* 1994). This is probably best alternative because it tests the monophyly of the terminal taxon. This method becomes computationally difficult when the number of variants is high in terminal taxa. Care must be taken to avoid combinations that do not occur in nature.

2. Use the multistate taxon option (as implemented in PAUP). This option allows the terminal taxa to have several character states during an

analysis, and can be used when polymorphism is observed in a species level taxon. This option may be used for higher level taxa, although one should demonstrate that the terminal taxon is monophyletic. This method is generalised by scoring the character as "unknown," allowing any state to be considered for a multistate taxon.

3. Use only the state found in the type species of the terminal taxon. This will guarantee precise results for a particular classification. This method does not test for nonmonophyly of a terminal taxon. Aberrant type species may also decrease the generality of such analyses.

4. Use the plesiomorphic state within the taxon, as was done in Brusca & Wilson (1991). This last option is closest to Wägele's "groundpattern" character analysis, but it is only used for terminal taxa and only for the characters where several states were observed in a terminal taxon. Wägele extends this method throughout the tree during tree construction, a procedure even less compatible with objective phylogenetic estimation. Maximum parsimony analysis makes no such attempts.

Tests of Cladograms

In his "Circular tree comparison" section (p. 103), Wägele appears to misunderstand phylogenetic arguments and even the nature of a circular argument. His Figure 11 shows a comparison of "dendrograms" from two different data sets, and asserts that this is circular argumentation, another "mistake" he finds in Brusca & Wilson (1991). Despite these assertions, independent data sets are valuable for comparison of phylogenies. The best test of a cladogram (a more accurate term than "dendrogram") is to use a new and different data set (Miyamoto and Fitch, 1995). If a tree represents a robust phylogenetic hypothesis, it will be corroborated by the new data. This is not circular argumentation, as Wägele complains, but is an independent test of the cladogram because the data are independent of the cladistic hypothesis. The groundpattern method, on the other hand, is inherently circular because the data are not independent of the tree : the investigator develops a groundpattern of characters based on a priori notions of the phylogeny ; the groundpattern is then used to build the phylogeny.

PHYLOGENETIC HYPOTHESES IN THE ISOPODA

The strength of empirical cladistic methods is that trees are analysed without preconceived notions about descent, using the characters to test and either reject or accept particular phylogenetic hypotheses. The character state data contain the phylogenetic information that one wishes to recover. Under a criterion of maximum parsimony, the data used by Brusca & Wilson (1991) rejected the hypothesis of Wägele (1989a), and provided support for a different topology (Fig. 1). Although all of Wägele's points are not addressed here, a few important examples are selected.

Uropods

Wägele highlights the optimisation of uropod character states as a major difference between his phylogeny (Wägele, 1989a) and that of Brusca & Wilson (1991). Two generalised states of this character can be identified. "Tail fan" uropods (figs. 2A-B) have broad, flat rami and short protopods (basal article), while styliform uropods (Fig. 2C-D) have elongate protopods and rami. In Wägele's opinion, the tail-fan uropod of isopods (Fig. 2A) is similar to those of the Eucarida (Fig. 2B), so this state must be the isopod "groundpattern." The styliform uropods seen in all basally derived isopods (Fig. 2D) and in all possible outgroups are only multiple convergences, reductions of the basic tail fan. Therefore, he concludes that the Brusca & Wilson (1991) phylogeny must be in error. Wägele's (1989a,b ; 1990 ; 1992a,b) theories regarding phylogeny, ecological adaptation and biogeography depend heavily on this idiosyncratic concept of uropod evolution. Nevertheless, the optimisation of the uropod states on either Wägele (1989a) or Brusca & Wilson (1991) trees (Fig. 1) results in an ancestral form that is unlike the Wägele isopod groundpattern uropod (Fig. 3).

Brusca & Wilson (1991) used multiple peracaridan outgroups to root the isopod cladogram, providing an objective status for the character states of the isopod outgroup node (Fig. 3). Most peracarid outgroups (Amphipoda, Cumacea, Mictacea, Tanaidacea) have styliform uropods. The taxa that emerge closest to the isopod part of the tree (Mictacea, Tanaidacea) have styliform (or non-tailfan) shaped uropods. The isopod taxa that branch off earliest in both versions of the phylogeny (Fig. 3) also have styliform uropods (e.g. Crenoicus, Fig. 2D). Parsimony demands that the simplest explanation, a styliform uropod, stands at the basal node (or "groundpattern") of the isopods.

Wägele, on the other hand, homologises the uropod of the Cirolanidae and other "flabelliferans" with basally derived malacostracans. Previous authors (Schultz, 1969 ; Hessler, 1969 : R372-373 ; Kensley and Schotte, 1989) have indicated that the Cirolanidae (Fig. 2A) and other flabelliferan families have the archetypical form of the Isopoda, primarily based on the fan-like
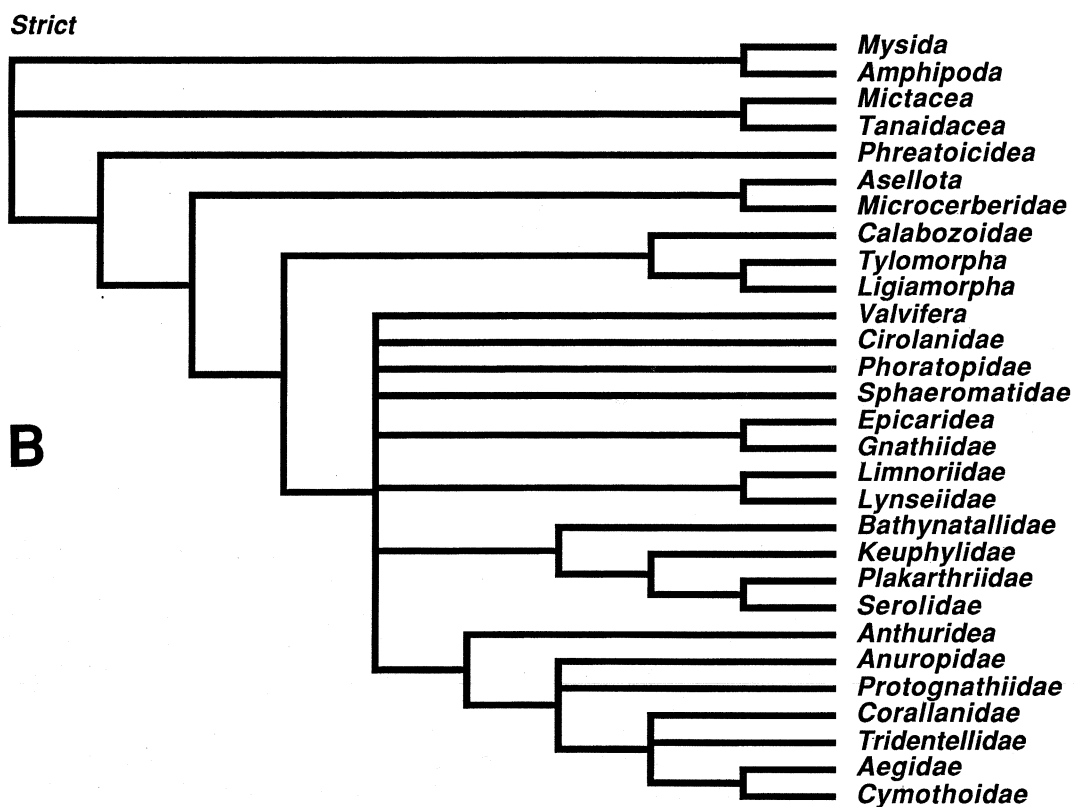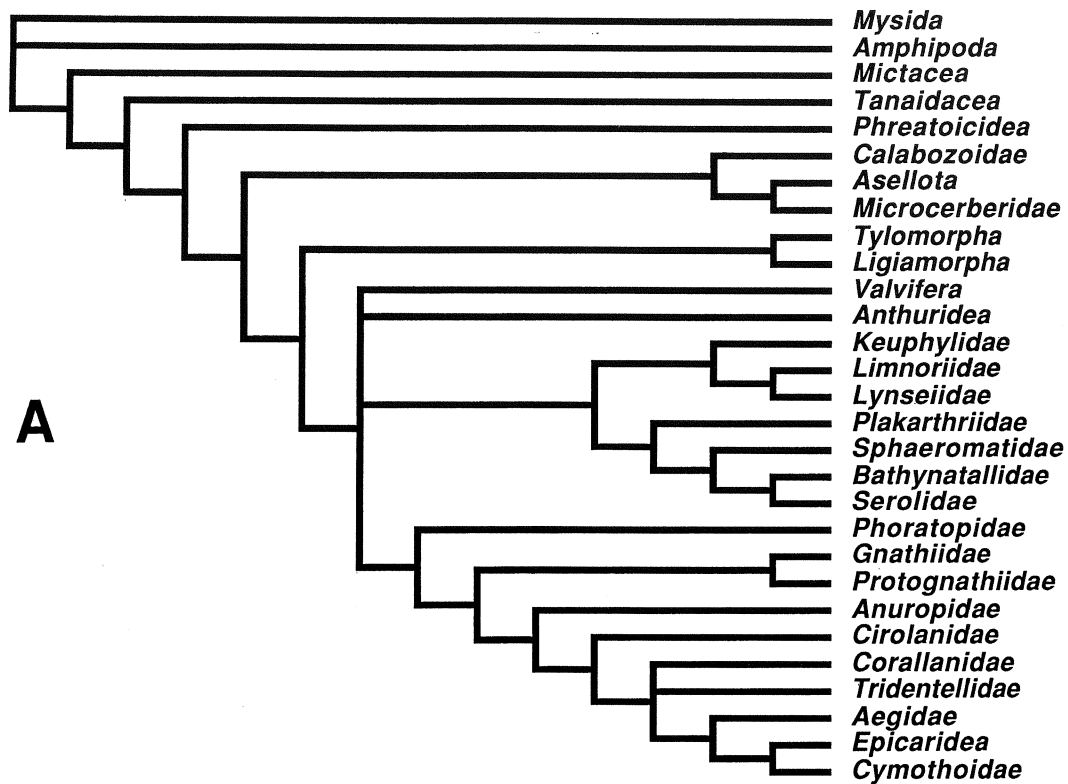
Fig. 1. – Two competing tree topologies for the Isopoda, with 4 peracaridan outgroups (Mysida, Amphipoda, Mictacea, Tanaidacea). A, topology of Wägele (1989a), as analysed by Brusca & Wilson (1991), tree length = 153. B, strict consensus tree from Brusca & Wilson (1991) of 16 equally parsimonious trees, tree lengths = 129. Trees drawn using PAUP (Swofford, 1990).
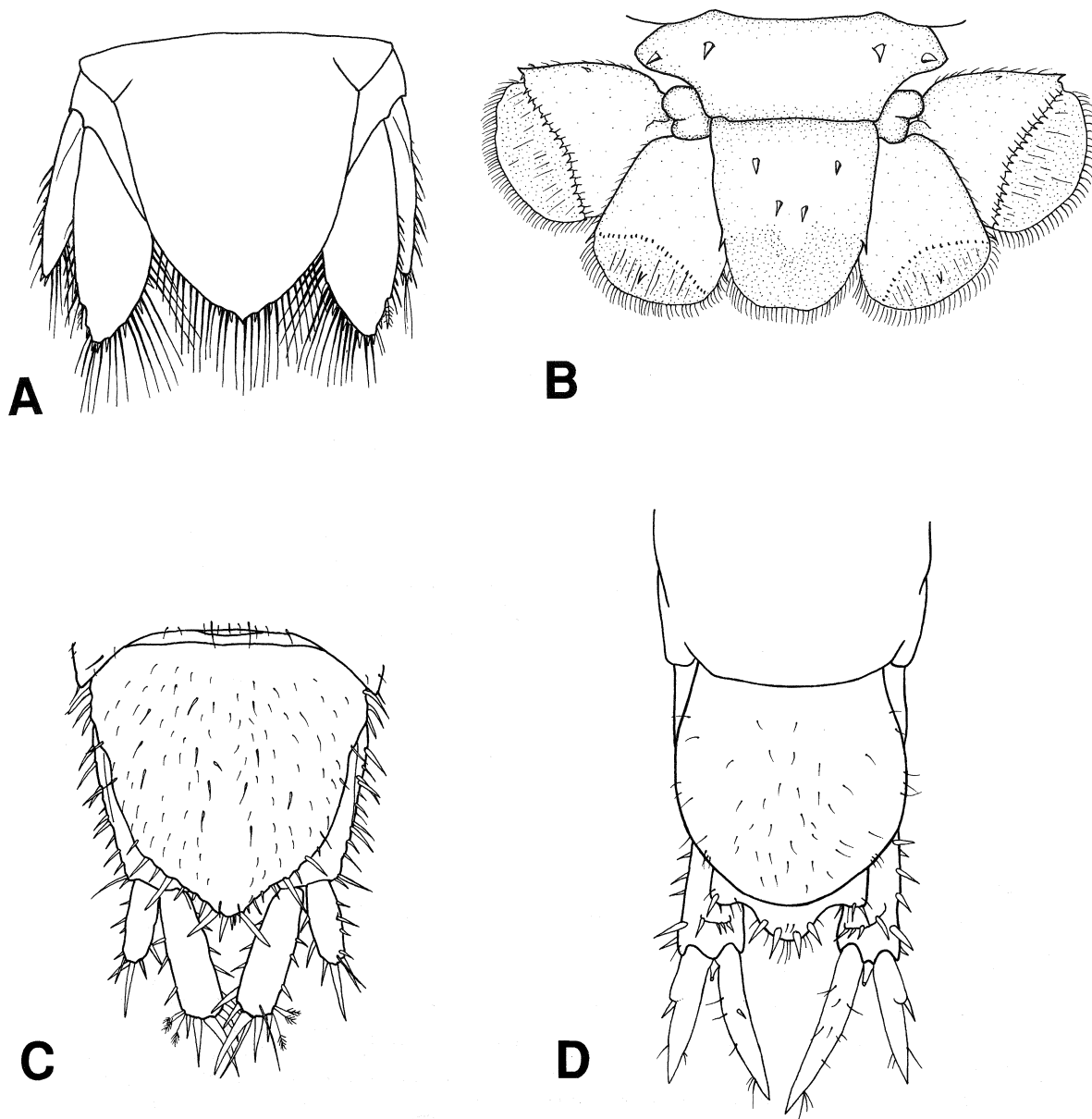
Fig. 2. – Uropods (terminal limbs) and pleotelsons (terminal body segments) compared. A, *Natatolana* (Isopoda, Cirolanidae) redrawn from Bruce (1986). B, *Euastacus* (Decapoda, Parastacidae) redrawn from Hale (1927); telson (terminal segment) and urosomite not fused. C, *Tainisopus* (Isopoda), redrawn from Wilson & Ponder (1992). D, *Crenoicus* (Isopoda, Phreatoicidae) redrawn from Wilson & Ho (1996). A & B have "broad & flattened uropods," and C & D have "styliform uropods."

uropods thought to be similar to the caridoid malacostracans. Both Wägele (1989a) and Brusca & Wilson (1991), however, show that the Cirolanidae have many apomorphic states compared to earlier derived isopods, so this family and its relatives cannot be "archetypical" in the sense that, as a clade, it appears early in isopod evolution. Brusca & Wilson (1991) tested this early derivation hypothesis and found it to be highly unparsimonious. This finding is in accord with the fossil record (Hessler, 1969; Schram, 1974):

the Phreatoicidea first appear in the Upper Carboniferous (Palaeozoic), while "flabelliferan" types do not appear until the Jurassic (Mesozoic). All taxa that branch off early in isopod evolution (Phreatoicidea, Asellota, Microcerberidea, Oniscidea) have styliform uropods (Fig. 3). Such a distribution of character states causes the uropod character to optimise to the styliform state at the ancestral node for all isopods. The uropodal state observed in the cirolanid clade, therefore, must be a reversion to a fan-like state (Fig. 3).
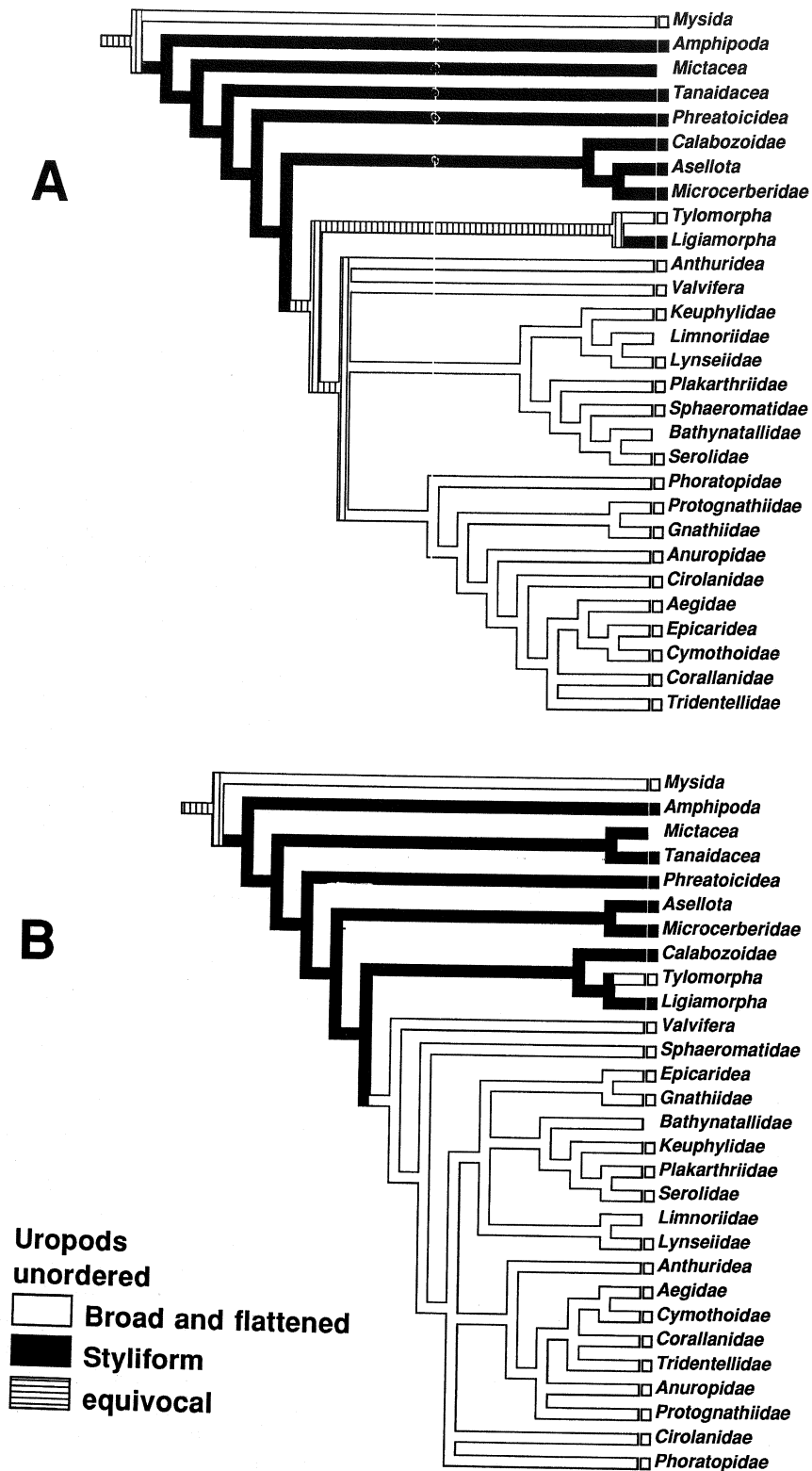
Fig. 3. – Distribution of uropod character states on two competing tree topologies for the Isopoda, with 4 peracaridan outgroups (Mysida, Amphipoda, Mictacea, Tanaidacea). Taxon names (state of terminal taxa) lacking small adjacent block indicates undefined or inapplicable states. A, topology of Wägele (1989a), as analysed by Brusca & Wilson (1991). B, One of 16 equally parsimonious trees of Brusca & Wilson (1991). Trees drawn using MacClade (Maddison & Maddison, 1992).

Wägele (1989a, 1994) believes that an uropodal tail fan occurs in the fossil tanaidacean *Cryptocaris hootchi* Schram, 1974. An inspection of Schram (1974), however, leads to different conclusions.

1. The published evidence for any reconstruction of the uropods appears weak. The photographed fossils show the telsonic region vaguely preserved the uropodal podomeres, although Schram indicates he can make them out.

2. Schram reconstructs the uropods as elongate with distal spines, quite unlike what one might regard as a tail fan. Schram's reconstruction differs in many details with other malacostracan tail fans such as those illustrated by Wägele (his Fig. 5). Although Schram (1974 : 102) indicates that the uropodal podomeres are "blade-like", his reconstruction shows them to be largely cylindrical. Moreover, caridean malacostracan fan-like uropods are not equipped with the elongate spines clearly shown in the *Cryptocaris* photographs and reconstruction.

Therefore, I conclude that the uropods of *Cryptocaris hootchi* do not form a "tail-fan", i.e., they are not homologous to those found in the eucarid Malacostraca (e.g., Euphausiacea or Decapoda).

How one interprets this reversal of the uropod form, alluded to in Wägele's text, is an important issue. Wägele interprets a reversal as a return to an ancestral state, with all its associated morphologies – an atavism. Another interpretation is that, although Brusca & Wilson (1991) have initially interpreted a character state as plesiomorphic, the appearance of a reversal in the optimisation of the character on a cladogram suggests that the feature is an entirely new state. In the discussion of the character distribution on the phylogenetic estimates for the isopods, Brusca & Wilson (1991 : ignored by Wägele) suggest that this latter interpretation is the case : the tail fan of cirolanids is not homologous with the tail fan of the decapods. Brusca & Wilson (1991) coded it as a plesiomorphic feature in the analysis to test this hypothesis, and so our coding is not "oversimplistic" as Wägele suggests. Because the tail fan-like uropod shows reversals in the isopod tree, they regarded this as a rejection of the initial hypothesis of homology. This is a strength of simultaneous analysis of all characters and taxa : hypotheses of homology are tested in their distribution on the shortest cladograms.

After quoting Brusca & Wilson (1991) on the standard method of evaluating characters as unordered, Wägele then asserts that "the analysis is numerical, and not phylogenetic" (p. 86). In this statement, Wägele demonstrates that he does not understand maximum parsimony and its function in phylogenetic analysis, nor does he accept the notion of homoplasy. He criticises our coding of uropods (0 = tail fan, 1 = styliform) in a simplistic fashion as a "double mistake" (p. 86). "Mistake no. 1" is that the polarity is not determined prior to the analysis, which I regard as a strength (no *ad hoc* hypotheses), rather than a mistake. "Mistake no. 2" is that the plesiomorphic state (uropods form a tailfan) is not identified correctly. Wägele's reference is not clear here because the 0 state is the *de facto* plesiomorphic state, although because Brusca & Wilson (1991) were not using Camin-Sokal parsimony (or irreversible parsimony) methods on any of the characters, changes in either direction are allowed. The cladograms of Brusca & Wilson (1991) therefore, indicate homoplasy in this character, which allows the tail fan seen in the Cirolanidae and other taxa to be interpreted as a "new" state, *a posteriori*.

The tail fan should be considered in the context of the Malacostraca. Some malacostracans have what is known as the "caridoid escape reaction," with numerous morphological adaptations for this behaviour, such as spiral abdominal musculature and the tail fan (Hessler, 1964; 1983). Although this behaviour and associated morphology occurs in the Decapoda, Syncarida and Euphausiacea, it is not well known in the Peracarida, and does not occur in the Hoplocarida (Kunze, 1981; in contradiction to Wägele, 1994 and Hessler, 1964). No known isopod has the caridoid escape reaction, and not surprisingly the musculature shows this as well (Hessler, 1964). If this behavioural/morphological complex is absent from all potential outgroups for the isopods, why does Wägele insist that the tail fan occurs in isopod groundpattern?

Wägele (1989a; 1994 : 93) indicates that muscles of the uropod and pleotelson in the Phreatoicidea are "shrimp-like", even though this taxon has a styliform uropod. Wägele's own evidence shows that the form of the uropod is independent of the positions of the musculature. If one accepts the nonindependence of these features, then they, too, are evidence that the ancestral form of the uropod was styliform, not a tail fan. Wägele believes the position of the anus provides additional support in the "long-tailed" vs "short-tailed" issue, although this is also an independent character. His data and those of Brusca & Wilson (1991) clearly show a variety of positions for the anus, unrelated to the form of the uropod. Wägele seems to argue that functionally related characters are phylogenetically locked – this proves to be not the case; the functionally related characters may be obtained sequentially, and largely independently. Attainment of a particular functional arrangement is a step-like process, with characters evolving separately. *Tainisopus* Wilson and Ponder, 1992 is a good example (Fig. 2C). This unusual isopod genus contains elongate, relatively unmodified isopods that have a broad pleotelson. Their uropods have the basal form, flattened sty-

liform, not the broad tail fan seen in the Cirolanidae. *Tainisopus* is a good swimmer (Wilson and Ponder, 1992), and yet does not have a tail fan.

Parsimony requires that the ancestral state for the Isopoda be a styliform uropod, i.e., not a tail fan. Wägele (1989a; 1994; see also Brusca & Wilson, 1991) indicates that the uropods are modified for many purposes and have many different morphologies within the isopods, so Wägele's evolution of the uropods becomes a weakly corroborated theory. Although one may decide that a feature must be plesiomorphic based on the distribution of states in some larger group of taxa, one may find that the concerted effect of many characters forces the feature to be a reversal. To deny the reversal is to deny parsimony.

How one interprets this rejection of homology is a matter for careful investigation of the characters involved. Wägele has done this but fails to parsimoniously interpret the data before him. He clings to his pet theory of the primacy of the tail fan homology, regardless of how much it is changed throughout the evolution of the peracarids. This tenacity may be based in his theoretical edifice based on an indefensible "just-so" story about ecological adaptations of the crustaceans to the evolution of fishes (Wägele, 1989b; 1992b). This case shows clearly that finding phylogenies using "groundpatterns" allows an investigator's preconceived ideas about evolution to colour the analysis.

Protognathiidae

An effective test of the trees of Wägele (1989a) and Brusca & Wilson (1991) comes from new information on the status of the genus *Protognathia* Wägele and Brandt, 1988. This taxon was originally proposed by Wägele and Brandt (1988) to be a "missing link" between the families Gnathiidae and Cirolanidae. The phylogeny of Wägele (1989a), not surprisingly, finds it to be a sister group of the Gnathiidae. Brusca & Wilson (1991) examined the descriptions of *Protognathia bathypelagica* (Schultz, 1977) and concluded that the Wägele and Brandt (1988) were in error about the maturity of the specimens. Other supposed synapomorphies of the protognathiid-gnathiid clade were shown by Brusca & Wilson (1991) to be not exclusively apomorphic. The strict consensus tree of Brusca & Wilson (1991) shows the Protognathiidae nested within a cirolanid clade as part of a three way polytomy that includes the Anuropidae and a corallanid-cymothoid clade (Fig. 1B, 3B). Gnathiidea is the sister group of the Epicaridea in a clade that is several branches removed from the Protognathiidae (Brusca & Wilson, 1991). This general topology is retained in recent cladograms resulting from analyses performed in-

cluding *Tainisopus* Wilson & Ponder 1992 (Wilson, in prep.).

Recently, new data on *Protognathia* corroborates Brusca & Wilson's (1991) conclusion that the original specimens were immature, a point ignored by Wägele. A new specimen clearly assignable to *Protognathia* has been reported from the Antarctic (Kussakin and Rybakov, 1995) : an adult male with the full complement of seven pereopods, not six as in the Gnathiidae. The primary synapomorphy between Protognathia and Gnathiidea proposed by Wägele and Brandt (1988) and Wägele (1989a), lack of the last pair of legs in an adult, proves to be homoplasy caused by a "mistake" in their groundpatterns. These new data corroborate Brusca & Wilson's (1991) tree, and rejects the classification and phylogeny proposed by Wägele (1989a).

Microcerberidae

In the "Failure to recognise the encaptic order" section, Wägele (p. 102) criticises Brusca & Wilson (1991) on their use of a terminal taxon (Microcerberidae) in the analysis that is nested within another (Asellota). Wägele ignored our justification for doing so. The status of the Microcerberidae is being treated separately (Wilson, in prep.), but Brusca & Wilson (1991) clearly state that the Microcerberidae do not have the defining synapomorphies of the Asellota, one of which is the geniculate copulatory appendage on male pleopod II. Therefore, the Microcerberidae were included separately – thus conflicting with Wägele's (1983a) unparsimonious (Wilson, 1987) phylogeny of the Asellota. Brusca & Wilson's (1991) results were not intended to "prove" the specific classification of the two taxa, as Wägele (p.103) asserts. Because the status of the Microcerberidae has been a much discussed issue within isopod phylogenetics, it was informative for Brusca & Wilson (1991) to include this taxon in their analysis, where it appeared as the sister group of the Asellota.

Wägele *et al.* (1995 : their Fig. 7) present a "Scheme showing conspicuous steps in the evolution of the Microcerberidae" (see Fig. 4A) based on their Table 1 : "Salient characters of species of the Microcerberidae." An inspection of this table shows that most of the characters are autapomorphies and therefore uninformative phylogenetically. Analysis of these data with Hennig86 (Farris, 1988) or PAUP ver. 3 (Swofford, 1990) results, not surprisingly, in 9 shorter trees (e.g Fig. 4B), an unresolved strict consensus tree (Fig. 4C), a majority of which are not congruent (Fig. 4D) with the preferred topology of Wägele *et al* (1995). Moreover, one can easily dispute the polarisations of the characters in their Table 1.
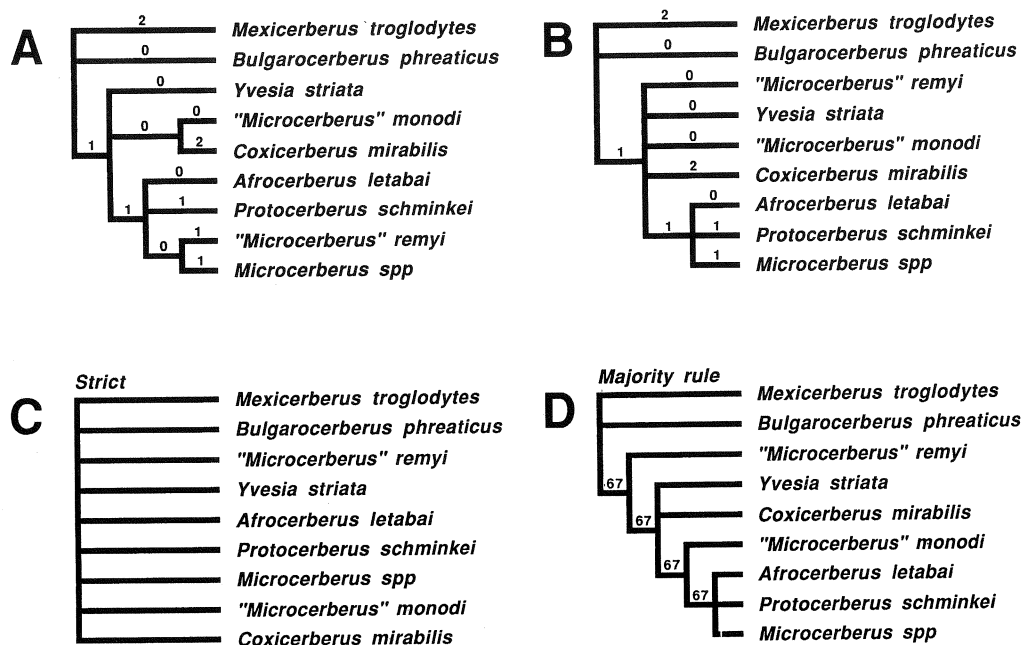
Fig. 4. Competing undirected cladograms for the Microcerberidae (Isopoda). A, topology from Wägele *et al.* (1995), tree length = 9 (or 3, uninformative characters excluded), with branch lengths (number of character state changes on a branch). B-D, topologies from 9 equally parsimonious trees found using the data of Wägele *et al.* (1995). B, one of 9 trees, length = 8 (or 2, uninformative characters excluded), with branch lengths. C, strict consensus tree. D, majority rule consensus tree, with branch percentages. Trees drawn using PAUP (Swofford, 1990).

For example, character 4 – the length of the fourth pleopod – depends on Wägele's assumption that microcerberids are asellotes : the plesiomorphic state would be short and covered by the previous pleopod, not long and protruding. By polarising the fourth pleopod character in a way that assumes asellotan ancestry, Wägele exposes the failure of his groundpattern method : it is unwilling to consider alternative hypotheses. Simply leaving the polarisation undecided, because either state could be plesiomorphic, is more objective.

Epistemological deficiencies in Wägele's groundpattern method lead to insupportable biogeographic schemes (Wägele's step no. 7, p. 83, in his "necessary steps in a phylogenetic analysis"). Wägele *et al.* (1995; also Wägele, 1983b, 1990) observe that the supposedly primitive species of Microcerberidae are found in fresh water, and that the derived species are marine. Wägele then jumps to the astonishing conclusion that microcerberids evolved in fresh water and then invaded the sea. His groundpattern method does not allow him to consider appropriate alternatives. Given that Wägele believes that microcerberids are asellotes, and that they are derived from asellotan taxa found only in fresh water (Wägele, 1983a), he concludes that the Microcerberidae plesiomorphically must be a fresh water group (Wägele, 1983b, Wägele *et al.*, 1995). A simpler alternative (abundantly demonstrated by the distribution of the Phreatoicidea : Birstein, 1962;

Schram, 1974; Banarescu, 1990; Williams, 1980) is that the Microcerberidae evolved in marine waters and colonised fresh water. This transition to fresh water occurred in taxa that retain some plesiomorphic features, such as the exopod on the uropod. The marine microcerberids continued to evolve, and the ancestors of the freshwater taxa became extinct in the ocean, or are not yet discovered there. Evidence for this scenario may be derived from the observation that the sister taxon of the Microcerberidae, the Atlantasellidae (see phylogeny in Wägele, 1983a; corroborated by unpublished data), is marine. If the outgroup is marine and the ingroup contains both marine and fresh water taxa, it is simplest to interpret their common ancestor as marine. According to Wägele's hypothesis, the Atlantasellidae, too, would have had to re-invade marine waters, despite this family occurring only in insular marine caves (Bermuda : Sket, 1979). The biogeographic data on this group are still too poor to be certain. Thus, Wägele's uncritical evaluation of the microcerberids as primitively freshwater cannot be supported by the data.

CONCLUSION

Wägele's groundpattern method is circular and nonscientific because it forsakes global corrobo-

ration of character distributions for *a priori* theories about phylogenetic descent. Phylogenetic trees constructed by Wägele's method are likely to be nonoptimal if they are evaluated cladistically using global parsimony. Wägele offers no strict algorithm to replace parsimony analysis, only a poorly characterised and subjective scheme of "argumentation." Groundpattern reconstruction methods cannot be recommended as a means to estimate phylogenies.

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