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Mesopedinella arctica (Pedinellales) II. Phylogeny of *Mesopedinella*, including a cladistic analysis of Dictyochophyceae

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Mesopedinella arctica, Daugbjerg a marine phytoflagellate, was recently described ultrastructurally and observed to possess characters common to the Pedinellales Zimmermann, Moestrup et Hällfors. To infer the phylogenetic position of this new genus within the Pedinellales, including all well-circumscribed genera, and to study relationships among the three orders in the Dictyochophyceae Silva, a cladistic analysis was performed on a data matrix of 33 ultrastructural characters. The Pedinellales proved to be monophyletic and *Mesopedinella* to be closely related to *Pedinella hexacostata* Wyssotzki. *Pseudopedinella tricostata* (Rouchijajnen) Thomsen, with three chloroplasts, was the most basal pedinellid suggesting that six chloroplasts is the advanced state. The heterotrophic pedinellids form a monophyletic group, suggesting that there was a single loss of chloroplasts within this cluster. Within the Dictyochophyceae, the silicoflagellate *Dictyocha speculum* Ehrenberg was the most divergent taxon. The cladistic analysis constitutes an independent hypothesis of phylogenetic relationships of the Dictyochophyceae to which reconstructions based on molecular data can be compared.

INTRODUCTION

Mesopedinella arctica, a marine phototrophic nanoflagellate from Arctic Canada, was recently characterized by light and electron microscopy. The general ultrastructure of the cell resembles that of the Pedinellales, but the cells also possess morphological features incompatible with other known genera (Daugbjerg 1996). Based on the presence of at least two synapomorphic characters—microtubule-supported tentacles which arise from the nuclear envelope and a ring-like structure outside the axoneme (not observed in all pedinellids)—the Pedinellales, Rhizochromulinales O'Kelly et Wujek and Dictyochales Haeckel were recently proposed to form a natural phylogenetic cluster, comprising the class Dictyochophyceae (Moestrup 1995).

To examine the phylogenetic relationships of *Mesopedinella* within the Pedinellales and to infer relationships of orders within the Dictyochophyceae, a cladistic analysis was performed based on a data matrix comprising 33 ultrastructural characters. The analysis included well-circumscribed genera of Pedinellales, the amoeboid *Rhizochromulina marina* and the silicoflagellate *Dictyocha speculum.* Bourrelly (1957) included the two sessile freshwater genera *Cyrtophora* Pascher and *Palatinella* Lauterborn in the Pedinellales and family Pedinellaceae. Due to lack of information these were not included in the present analysis.

Recently, technological advancements in molecular biology, notably sequencing of PCR products of conserved genes, have resulted in a significant increase in the number of studies addressing protist evolution and phylogeny. Most of these studies are based on sequences of the nuclear-encoded SSU rDNA gene. Often the phylogenetic hypotheses are based solely on nucleotide sequences as there have been comparatively few cladistic studies based on morphological characters. Hence, the second purpose of this study was to establish a basis for comparisons between independent phylogenetic hypotheses based on morphological and molecular characters, respectively. At present such comparisons are biased as molecular data are not yet available for all described genera of dictyochophytes.

MATERIALS AND METHODS

Cladistic analysis

Eight genera (10 species) of pedinellids, *Rhizochromulina marina* and *Dictyocha speculum,* were scored for 33 ultrastructural characters. Lists of characters with their scores are given in Tables 1 and 2, respectively. Characters were predominantly based on those published by Smith & Patterson (1986). Additional ultrastructural information was taken from Hibberd & Chrétiennot-Dinet (1979); Pedersen *et al.* (1986); Koutoulis *et al.* (1988); Thomsen (1988); Moestrup & Thomsen (1990); O'Kelly & Wujek (1995) and Daugbjerg (1996).

Characters were treated as unordered and analyzed using the heuristic search option in PAUP version 3.1.1 (Swofford 1993) with 100 random additions of taxa in tree bisection–reconnection. Following this search strategy, characters were reweighted using the rescaled consistency index until the same number of equally parsimonious trees was found twice in a row. To examine the evolution of ultrastructural characters, these were mapped on a phylogenetic tree using MacClade version 3.05 (Maddison & Maddison 1992).

Outgroup

A recent phylogenetic analysis based on sequences of SSU rDNA suggested that the lack of chloroplasts in the heterotro-

Table 1. Characters used in cladistic analysis

1. Number of protruding flagella (one = 0, two = 1).
2. Immature flagellum with paraxonemal rod (1), or without the rod (0).
3. Basal extension of flagellum with tuft of tripartite hairs.
4. Basal swelling on mature flagellum in connection with photoreceptor apparatus.
5. Basal bodies almost parallel (0) or orthogonal (1).
6. Basal bodies attach directly or close to nucleus.
7. Flagellar scales present.
8. Transitional ring(s) located above (0) or below (1) transitional plate or absent (2).
9. Crescents occur below basal bodies.
10. Amorphous masses in vicinity of basal body.
11. Extrusomes present.
12. Dictyosome in anterior half of the cell (0) or in posterior half (1).
13. Oval flat body scales.
14. One (0), two (1), three (2), six (3) or many (4) chloroplasts.
15. Outer chloroplast membrane continuous with outer nuclear membrane.
16. Pyrenoid present.
17. Pyrenoid bulging.
18. Pyrenoid with broad tubular intrusions.
19. Posterior end of cell with vacuolar system.
20. Vacuolar system simple (0) or complex (1).
21. Trailing stalk present or sometimes present.
22. Trailing stalk contractile.
23. Trailing stalk supported by triad microtubules.
24. Trailing stalk with sphincter.
25. Cytoskeletal microtubules in triads (0), in bundles (1) or different (2).
26. Microtubules emanating from nuclear surface.
27. Cytoplasmic microfibrillar system present.
28. Cell with tentacles (pseudopodia).
29. Triad caps present.
30. Phototrophic (0), mixotrophic (1) or heterotrophic (2) mode of nutrition.
31. Tentacles normally extend anteriorly.
32. Central pair of microtubules ends above transitional plate.
33. A fibre-like structure present between the central pair of microtubules and the transitional plate.

phic pedinellids *Pteridomonas* Penard and *Ciliophrys* Cienkowski was due to secondary loss (Cavalier-Smith *et al.* 1995) as the sister group to the Pedinellales was phototrophic. Following this finding, *Ochromonas danica,* a chloroplast-bearing chrysophyte, was selected as the outgroup.

RESULTS AND DISCUSSION

Characters and cladistic analysis

Ultrastructure of the flagella and flagellar apparatus has been considered significant for elucidating protist relationships (e.g. Preisig 1989; O'Kelly 1992; Inouye 1993). Twelve of 33 characters compiled for a cladistic analysis of the Dictyochophyceae were flagellar features, whereas other characters pertained to the general ultrastructure of the cell including chloroplasts and the presence of a posterior vacuole system/trailing stalk (a specific character of pedinellids). The heuristic search, followed by reweighting of characters according to a rescaled consistency index, found six equally parsimonious trees for which a strict consensus tree is shown in Fig. 1.

The six most parsimonious trees differed in the branching of clades containing *Pseudopedinella elastica*/*Pseudopedinella pyriformis* and *Parapedinella reticulata*/*Ciliophrys infusionum.* Therefore, the consensus tree shows unresolved relationships of these pedinellids. This phylogenetic analysis suggests that the Pedinellales form a monophyletic group within the Dictyochophyceae. *Rhizochromulina marina* is a sister taxon to the Pedinellales and *Dictyocha speculum* is the most divergent taxon within the Dictyochophyceae. *Mesopedinella arctica* is closely related to *Pedinella hexacostata* and together they form a sister group to *Apedinella radians.* According to this analysis, the genus *Pseudopedinella* Carter is paraphyletic with *Pseudopedinella tricostata* (bearing 3 chloroplasts) branching off as the basal pedinellid. The heterotrophic pedinellids cluster together, arguing for a single secondary loss of chloroplasts within the Pedinellales. *Actinomonas* Kent and *Pteridomonas* are closely related. The unresolved position of *Parapedinella reticulata* probably is due to the

Table 2. Character matrix used in the cladistic analysis

Pedinellid taxa included in the analysis	Character no.[1] (1 5 10 15 20 25 30)
Ochromonas danica Pringsheim	10011000000000110??0?0???200000?00
Pseudopedinella elastica Skuja	0100010201010311111111101?100011
Pseudopedinella pyriformis Carter	01000102?1010311111111??01?000???
Pseudopedinella tricostata (Rouchijajnen) Thomsen	01000102010102110111 1?11011000?1?
Apedinella radians (Lohmann) Campbell	0100010111111311111101?11011110011
Pedinella hexacostata Wyssotzki	010001 0?11110310??10110001?101111
Mesopedinella arctica Daugbjerg	010001021101131 0??10100001101 0?11
Actinomonas mirabilis Kent	0100011211110?????1111110111 02111
Pteridomonas danica Patterson et Fenchel	0100011111110?????111111011102111
Ciliophrys infusionum Cienkowski	0100011?01110???????1???01?1020??
Parapedinella reticulata Pedersen et Thomsen	01????1???1?1???????0??????1?20??
Dictyocha speculum[2] Ehrenberg	001001020100040100 0?0???110100010
Rhizochromulina marina[3] Hibberd et Chrétiennot-Dinet	0000010 1?1010001000?0???110100011

1 = yes/present; 0 = no/absent; ? = unknown or not applicable.

[1] As in Table 1.

[2] Armoured stage.

[3] Zoospore.

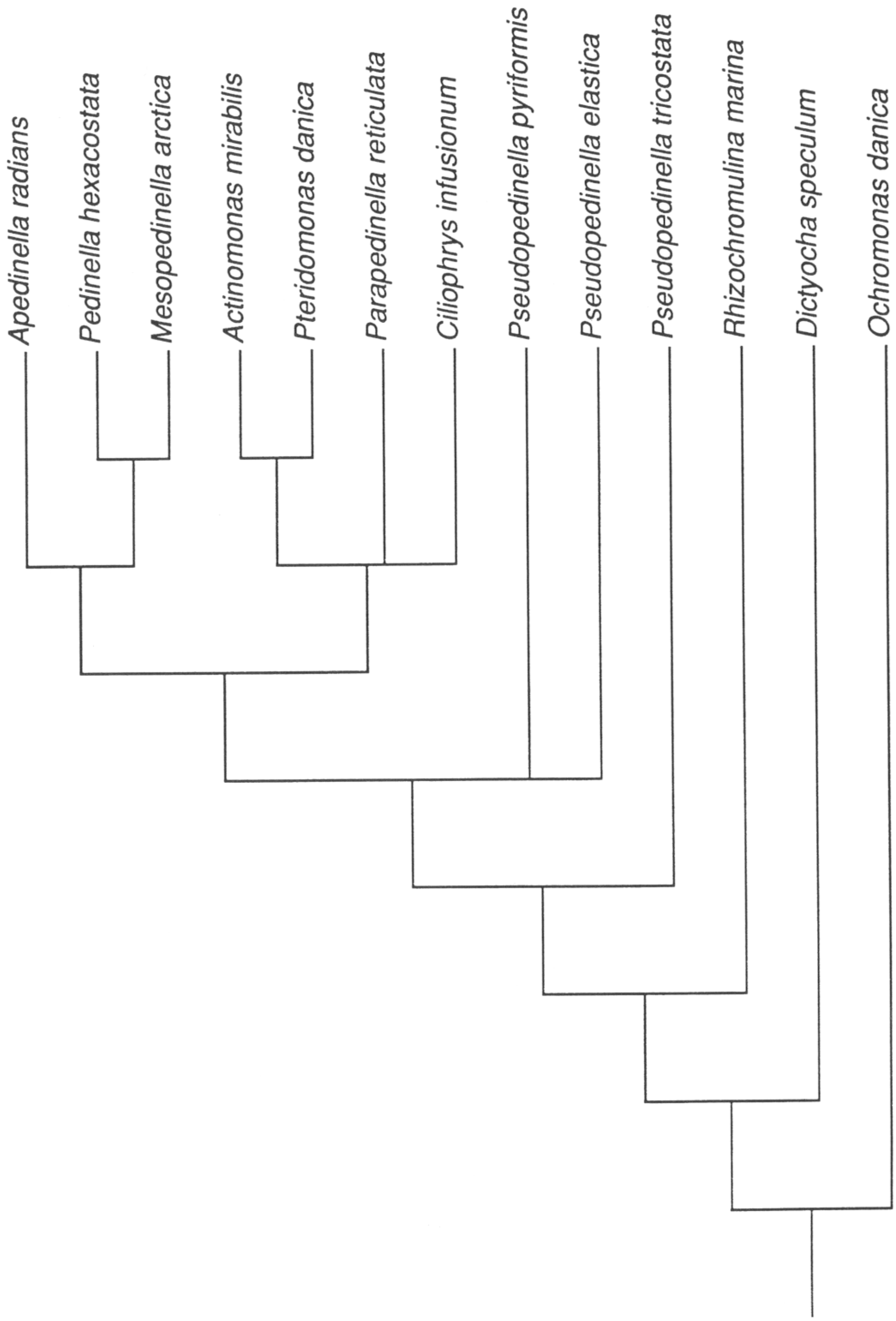

Fig. 1. Cladistic analysis of the Dictyochophyceae based on 33 ultrastructural characters. Strict consensus tree of the six most parsimonious trees (53 steps long). The phototrophic chrysophyte *Ochromonas danica* was designated as the outgroup.

limited available information, because this species has only been described from material studied in the light microscope and in EM whole mounts (Pedersen *et al.* 1986).

Phylogeny of *Mesopedinella*

The cladistic analysis suggests that *Mesopedinella arctica* and *Pedinella hexacostata* represent the most advanced genera within the phototrophic pedinellids. The close relationship between the two taxa was expected as they share two unique ultrastructural features: chloroplasts without pyrenoids and a simple posterior vacuolar system not encircled by the Golgi apparatus (Swale 1969; Daugbjerg 1996). *Apedinella radians,* the sister taxon to *Mesopedinella* and *Pedinella,* possesses two types of body scales. One type, the flat oval body scales, is morphologically similar to scales of *Mesopedinella.* The ancestor to *Mesopedinella* may therefore have lost the spine scales typical of *Apedinella* but retained the oval scales while the ancestor to *Pedinella* lost both types of body scales.

Comparison with other phylogenetic analyses

Relationships of the Dictyochophyceae identified by the cladistic analysis shown in Fig. 1 can be compared only to few other studies of which Smith & Patterson (1986) is the most detailed. Smith & Patterson compiled a data matrix consisting of 198 predominantly morphological characters to analyze the phylogeny of heliozoans and other protozoa, including six genera (7 species) of pedinellids. Although their focal point was not centred around the pedinellids, the heterotrophic genera were shown to form a monophyletic cluster in five of the six different phylogenetic methods applied, including 25 taxa and all characters (Smith & Patterson 1986). Hence, the consensus of their analyses strongly argues for a single secondary loss of chloroplasts. In contrast, the phototrophic pedinellids formed a paraphyletic group in four of six reconstructions based on all 198 characters. The paraphyly was caused by the position of *Pedinella hexacostata* which appeared as sister taxon to the heterotrophic pedinellids (Smith & Patterson 1986).

Patterson (1986) presented a tree of pedinellid phylogeny based on identification of synapomorphies characterizing each taxon (cf. his fig. 4.24). This approach suggests monophyly of heterotrophic pedinellids with *Pteridomonas* as the most divergent taxon. Among the phototrophic pedinellids included by Patterson, *Apedinella* branched off as the first whereas *Pedinella* was the sister taxon to the aplastidic genera. The overall branching pattern of phototrophic pedinellids is different from those reported by Smith & Patterson (1986) and Fig. 1 of the present paper. Particularly, the phylogenetic position of *Apedinella* and *Pseudopedinella* differs.

Few molecular studies have addressed the phylogeny of the Dictyochophyceae. A recent study based on SSU rDNA nuclear genes from six dictyochophytes suggested a polyphyletic origin of the Pedinellales with *Ciliophrys infusionum* more closely related to *Rhizochromulina* cf. *marina* than to the clade containing *Pteridomonas danica, Apedinella radians* and *Pseudopedinella elastica* (figs 1, 2 in Cavalier-Smith & Chao 1996). The *Ciliophrys/Rhizochromulina* clade was supported by bootstrap values above 98% in both neighbor-joining and parsimony analyses. This is surprising as it is contradicted by a number of distinct morphological differences pertaining to the host. The morphological differences between *Rhizochromulina* and *Ciliophrys* not commented on by Cavalier-Smith & Chao (1996) include the microtubule-supported tentacles, which in *Ciliophrys* are interconnected to form triads whereas in *Rhizochromulina* they are in bundles of up to seven (O'Kelly & Wujek 1995). A paraxonemal flagellar rod is absent in *Rhizochromulina* but present in a reduced form in *Ciliophrys* (Moestrup & Andersen 1991). *Ciliophrys* possesses tentacles with extrusomes, lacking in the vegetative stage of *Rhizochromulina. Rhizochromulina* has both a vegetative amoeboid stage and uniflagellated zoospores whereas *Ciliophrys* is known only to have uniflagellated stages. Furthermore, the tree presented by Cavalier-Smith & Chao (1996) implies that aplastidic pedinellids lost their chloroplasts at least twice in the course of their evolution. If analysis of the SSU rDNA gene reflects phylogeny, all of the ultrastructural characters listed above must have evolved at least twice. This introduces a number of unlikely (non-parsimonious) events. It will be of interest to examine how the branching pattern changes when SSU rDNA sequences are included from more species, particularly *Actinomonas, Pedinella* and *Mesopedinella.* The phylogenetic tree based on the ribosomal gene suggests that the silicoflagellate *Dictyocha speculum* is the most divergent taxon (Cavalier-Smith & Chao 1996), in agreement with Fig 1.

Evolution of ultrastructural characters

To study the evolution of ultrastructural characters as inferred by the cladistic analysis, one of the six most parsimonious trees was selected by chance for mapping of characters (Fig. 2). According to the cladistic analysis, it is most parsimonious to suggest that the ring-like structures below the transitional plate of the transition region have evolved independently three times, twice in the Pedinellales (*Apedinella* and *Pteridomonas*) and once in *Rhizochromulina* (character 8). If this character has a multiple origin then the presence of ring-like structures below the transitional plate should not be used as a diagnostic character when defining Pedinellales and Rhizochromulinales. Except for *Parapedinella reticulata,* pedinellids possess a posterior trailing stalk although this may be lacking in some cells (e.g. Daugbjerg 1996). The absence of a stalk in *Parapedinella* is most parsimoniously explained as a secondary loss (character 21). The lack of tentacles in *Pseudopedinella pyriformis, Pseudopedinella tricostata* and *Mesopedinella arctica* are also best explained as secondary losses (character 28), as *Dictyocha* and *Rhizochromulina* both produce these structures. This cladistic analysis suggests that the minute annular scales present on the protruding flagellum have evolved once in the ancestor giving rise to the monophyletic heterotrophic pedinellid lineage, and are therefore a synapomorphic character (character 7).

Phylogenetic hypotheses based on SSU rDNA gene sequences suggested that the sister group to the Dictyochophyceae has a phototrophic mode of nutrition (Cavalier-Smith *et al.* 1995; Cavalier-Smith & Chao 1996). The chloroplasts in the pedinellid ancestor possessed pyrenoids with broad tubular intrusions (character 18). The bulging of the pyrenoid occurred after the split leading to *Pseudopedinella tricostata* and all other pedinellids (character 17) and the lack of pyrenoids in *Pedinella* and *Mesopedinella* is best explained as a secondary loss in the ancestor giving rise to this lineage.

If the reconstruction of the Pedinellales shown in Fig. 1 is correct, then the presence of three chloroplasts is the ancestral state in the Pedinellales. Pedinellids with six chloroplasts may therefore have evolved from a trichloroplastic ancestor in which the chloroplasts divided without cell division. The number of chloroplasts in the Dictyochophyceae may have evolved from many, as in *Dictyocha* (Moestrup & Thomsen 1990), to a single in *Rhizochromulina* (Hibberd & Chrétien-not-Dinet 1979), and then to three and six in the phototrophic pedinellids.

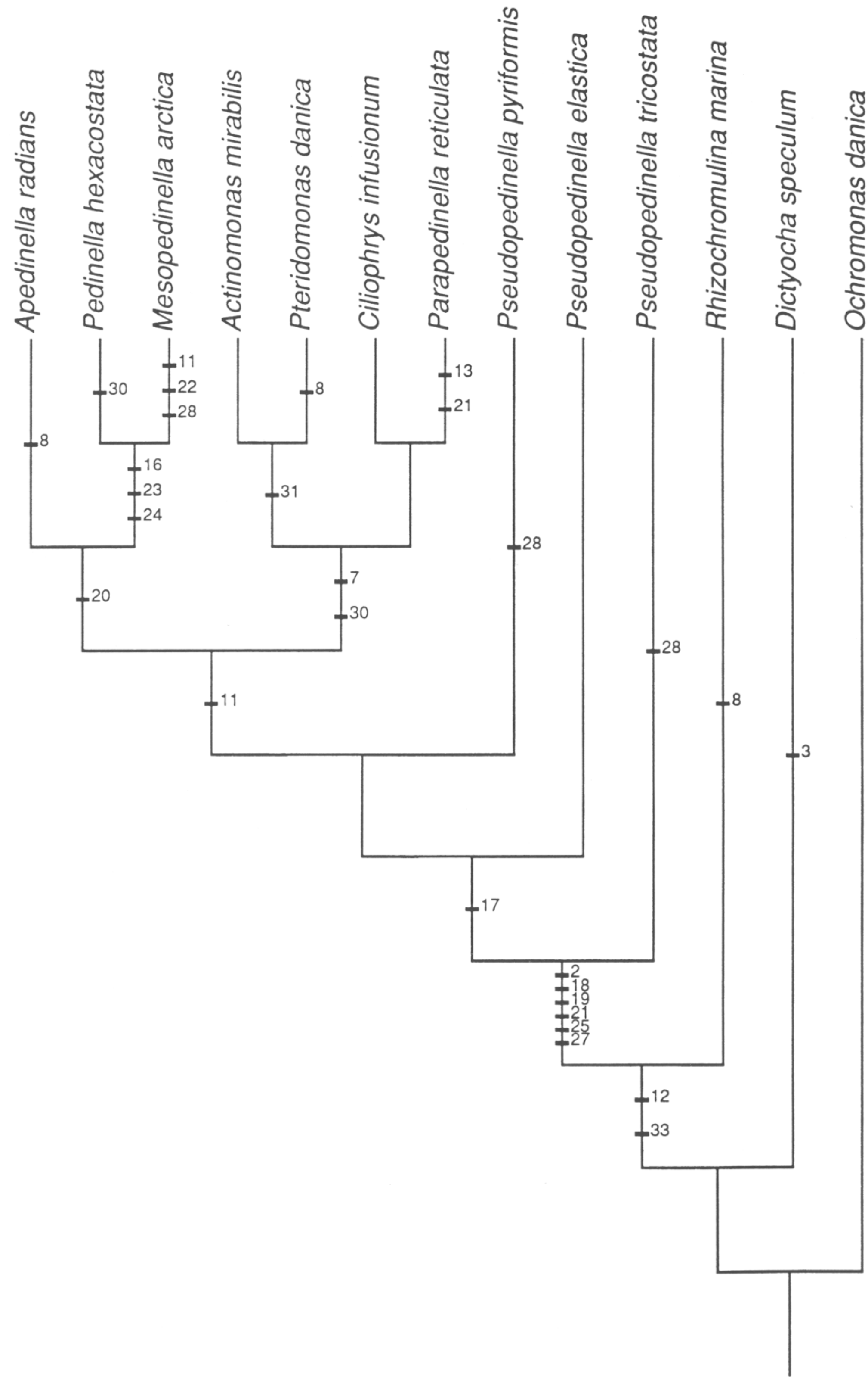

Fig. 2. Evolution of ultrastructural characters as inferred from the cladistic analysis. Characters are mapped on one of the six most parsimonious trees. See Table 1 for explanation of characters.

CONCLUSION

The phylogenetic reconstruction of the Dictyochophyceae based on a cladistic analysis of ultrastructural data supports the classification of Moestrup (1995) and O'Kelly & Wujek (1995). Moestrup (1995) speculated that the Rhizochromulinales represent an intermediate group between the Pedinellales and the Dictyochales, lacking a silicified skeleton as in pedinellids, yet possessing bundles of microtubular tentacles similar to those observed in silicoflagellates.

In addition to examining the phylogenetic position of the newly described phytoflagellate *Mesopedinella,* the analysis of the Dictyochophyceae based on ultrastructural information will hopefully serve as a platform for comparison with future phylogenies based on molecular data.

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