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Phylogenetic Position of the Copepod-Infesting Parasite *Syndinium turbo* (Dinoflagellata, Syndinea)

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Sequences were determined for the nuclear-encoded small subunit (SSU) rRNA and 5.8S rRNA genes as well as the internal transcribed spacers ITS1 and ITS2 of the parasitic dinoflagellate genus *Syndinium* from two different marine copepod hosts. *Syndinium* developed a multicellular plasmodium inside its host and at maturity free-swimming zoospores were released. *Syndinium* plasmodia in the copepod *Paracalanus parvus* produced zoospores of three different morphological types. However, full SSU rDNA sequences for the three morphotypes were 100% identical and also their ITS1—ITS2 sequences were identical except for four base pairs. It was concluded that the three morphotypes belong to a single species that was identified as *Syndinium turbo*, the type species of the dinoflagellate subdivision Syndinea. The SSU rDNA sequence of another *Syndinium* species infecting *Corycaeus* sp. was similar to *Syndinium turbo* except for three base pairs and the ITS1—ITS2 sequences of the two species differed at 34—35 positions. Phylogenetic analyses placed *Syndinium* as a sister taxon to the blue crab parasite *Hematodinium* sp. and both parasites were affiliated with the so-called marine alveolate Group II. This corroborates the hypothesis that marine alveolate Group II is Syndinea.

Key words: dinoflagellates; ITS; morphology; parasites; phylogeny; SSU rDNA; Syndinea; Syndinium; zoospore.

Introduction

Syndinium is a genus of dinoflagellate (or dinoflagellate-like) parasites infecting marine, planktonic copepods and radiolarians. The first hosts found to be infected with *Syndinium* were the copepods *Paracalanus parvus* Claus infected with *Syndinium turbo* Chatton in the NW Mediterranean Sea and *Clausocalanus arcuicornis* Dana and *Corycaeus elongatus* Dana infected with *Syndinium* spp. (Chatton 1910). Afterwards, the host range and geographical distribution of *Syndinium* spp. have been documented to be wide (Hollande and Enjumet 1955; Ianora et al. 1987; Jepps 1937;

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Kimmerer and McKinnon 1990). *Syndinium* spp. has a devastating effect on its host, which is literally devoured from inside by the parasite — a pathology characteristic of parasitoids in insects (e.g. Doutt 1959). At times, *Syndinium* spp. may contribute considerably to the mortality of its host population (Kimmerer and McKinnon 1990).

The life cycle of *S. turbo* is not known in detail. Briefly, the parasite develops a multicellular plasmodium inside its host until it finally occupies nearly the entire body cavity. At maturity the host is killed and numerous free-swimming zoospores escape. Originally, two types of spores were described: macrospores and microspores, both of *Gymnodinium*-like shape (Chatton 1910). Cell dimensions were reported as being 12 μm long and 5 μm wide, and 16—20 μm in diameter for the microspores and macrospores, respectively. In that report it was suggested that the macrospore transformed into an *Oxyrrhis*-like cell. In his monograph on parasitic dinoflagellates, Chatton (1920) described *S. turbo* in more detail. Herein he considered the *Oxyrrhis*-like cell as a third type of spore, "spore a rostre" — or rostrate spore. Each infected copepod always released only one type of *Syndinium* zoospore. At first Chatton (1920) was in doubt whether the rostrate spores were produced by *Syndinium* or by another parasite in *P. parvus*, *Atelodinium*. In an appendix, however, Chatton (1920, p. 467) stated that he had now confirmed "without possible doubt" that the rostrate spores originated from *Syndinium* plasmodia. In a few cases, a *Syndinium*-like parasite has been reported from *Paracalanus* spp. as *Atelodinium* sp. (Armeni-Agiovlassiti 1996; Ianora et al. 1987; Kimmerer and McKinnon 1990). However, the genus *Atelodinium* cannot be justified (Chatton and Soyer 1973) and based on pathology and morphology there is little doubt that the parasite reported from *Paracalanus* spp. in these cases was *Syndinium* (see also Ianora et al. 1990).

Based on the polymorphism of *Syndinium* spores, Chatton (1922) later concluded that the three different spore types were three separate species, and named the macrospores, microspores and rostrate spores, *S. turbo*, *S. minutum*, and *S. rostratum* respectively. They were all parasites of the same host, *P. parvus*. Additionally, a *Syndinium* species found in *Corycaeus venustus* was named *S. corycaei* (Chatton 1922). In a posthumous work (Chatton 1952), new generic names were mentioned for *S. rostratum* and *S. corycaei: Synhemidinium rostratum* and *Cochlosyndinium corycaei* respectively. Sournia et al. (1975), however, considered the descriptions of *Synhemidinium rostratum* and *S. minutum* invalid and interpreted *Cochlosyndinium* as a subgenus of *Syndinium*. Whether other *Syndinium* species infect other copepod species still needs to be resolved. In addition to copepods, several radiolarians and one amphipod are known as hosts for *Syndinium* spp. or *Syndinium*-like parasites (Hollande and Enjumet 1955; Manier et al. 1971).

In current taxonomy schemes, dinoflagellates (Dinoflagellata) are divided into two subdivisions: Dinokaryota and Syndinea (Fensome et al. 1993). Syndinea comprises one single order, Syndiniales, of exclusively parasitic species that, among other things, lack most characters defining the typical nucleus of the Dinokaryota. While the phylogeny of Dinokaryota has been investigated in a series of studies (e.g. Daugbjerg et al. 2000; Saldarriaga et al. 2004; Saunders et al. 1997), the phylogeny of Syndinea has received only little attention. Morphological studies on members of Syndinea are few and only representatives of two syndinian genera, *Amoebophrya* and *Hematodinium*, have been investigated by use of molecular techniques (Gruebl et al. 2002; Gunderson et al. 1999).

Recent investigations of environmental rDNA sequences from marine picoplankton have revealed two "new" groups of dinoflagellate-related alveolates referred to as marine alveolate Group I and Group II (López-García et al. 2001; Moon-van der Staay et al. 2001). Members of these seemingly new alveolate groups are unknown with respect to their morphology, the only exception being the syndinian parasite *Amoebophrya* that belongs to the marine alveolate Group II (Moon-van der Staay et al. 2001; Moreira and López-García 2002). Another syndinian, the blue crab parasite *Hematodinium* sp., also seems affiliated with Group II (Saldarriaga et al. 2004). Thus, the only two syndinian genera from which SSU rDNA sequences are known both belong to marine alveolate Group II and it is, therefore, tempting to suggest that this group and Syndinea are, in fact, the same group of organisms (Saldarriaga et al. 2004).

The two primary goals for this work were (1) to determine the taxonomic and phylogenetic position of *Syndinium* based on SSU rDNA sequences and (2) to clarify whether the three morphologically different types of zoospores originally described from *S. turbo* belong to the same species by comparing rDNA (SSU and 5.8S) and ITS1 and ITS2 sequences of these zoospores.

Results

Syndinium ex *Paracalanus parvus*

Paracalanus parvus in the late stages of *Syndinium* infection was easily recognized because the parasite caused the copepod body to become swollen and opaque. When parasite plasmodia matured, the hosts died and numerous free-swimming, colorless zoospores escaped through cracks in the exoskeleton of the dead host (Fig. 1 A). Often the *Syndinium* zoospores were visible moving inside a living and actively swimming host. Dozens of live, *Syndinium*-infected *P. parvus* were observed from summer until late autumn and these were all adult females or juvenile females at late developmental stages.

Zoospore Morphology

Three morphologically distinct zoospores were released from *Syndinium*-infected *P. parvus* (Figs 2 A—D, 3), but all zoospores originating from one individual copepod were always of the same morphological type. The different types of zoospores could be distinguished by use of a stereomicroscope at 50 × magnification due to differences in size, gross morphology, and motility.

Live macrospores were 8—12 μm long and 5—8 μm wide with an asymmetrically gymnodiniod shape and two flagella (Figs 2 A—B, 3 A—D). They possessed a wide left-hand transverse groove, a cingulum, originating from the insertion of the anterior flagellum, proceeding around the dorsal side of the cell and ending at the ventral side close to the cell's antapex (Fig. 3 A—D). The posterior flagellum was shorter than the cell, whereas the anterior flagellum was slightly longer than the cell (Fig. 3 A—C). The insertion of the posterior flagellum marked a sulcus-like posterior depression, extending from below the proximal part of the cingulum to the posterior end of the cell where it fused with the distal part of the cingulum (Fig. 3 A—B). A well-defined, curved apical groove extended from the insertion of the anterior flagellum to the cell apex (Fig. 3 D). Cell motion was moderate and live cells could also be seen lying on the bottom of the culture vessel. Macrospores typically survived for 1—2 days in filtered seawater at 20—25 °C.

Live microspores measured 8—10 μm in length and 2—4 μm in width (Fig. 2 C). In the light microscope, a characteristic refractile body was visible in the posterior end of cells (Figs 2 C, 3 E). This structure typically disappeared or shrunk during SEM preparation (Fig. 3 F—H). Cells had a twisted shape with a very wide, cingulum-like, circumferential groove that proceeded from the insertion of the flagella around the cell in a left-hand spiral, ending near the cell's antapex (Fig. 3 E, H). Microspores had two flagella. The posterior one was approximately of similar length as the cell, the anterior one was 3—4 times longer (Figs 2 C, 3 E—F). Cells were highly motile and

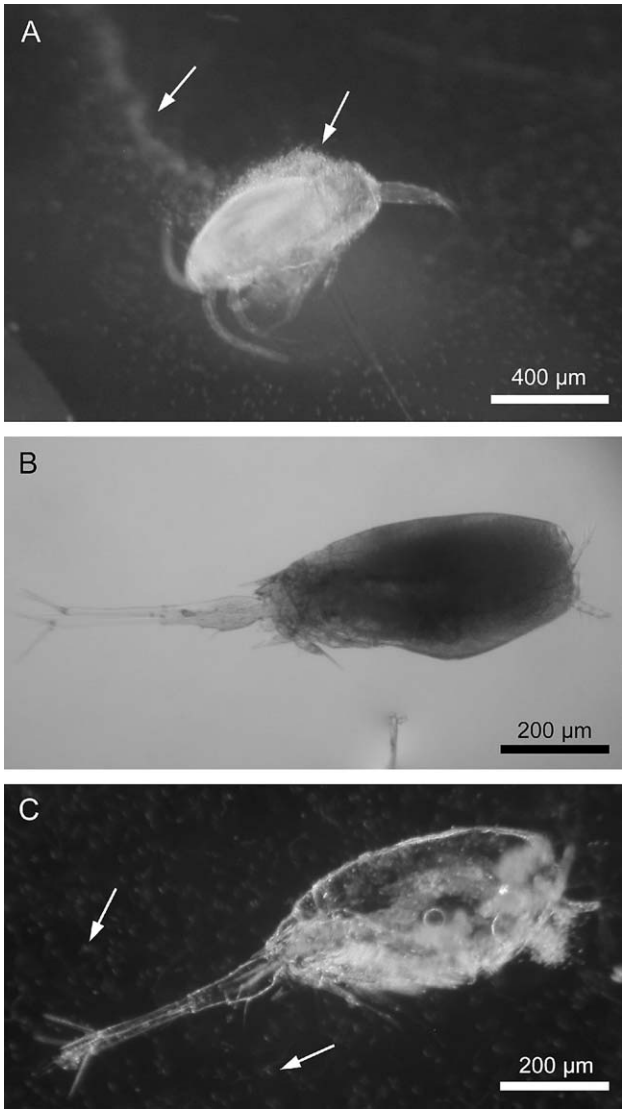

Figure 1. **A.** Live *Syndinium* zoospores (arrows) leaving a recently dead host, *Paracalanus parvus*. **B.** *Corycaeus* sp. infected with *Syndinium*. *Syndinium* infection can be recognized by the unusually dark body content of the host. **C.** Same copepod as in B, but 24 h later. *Syndinium* zoospores (arrows) have now abandoned the host.

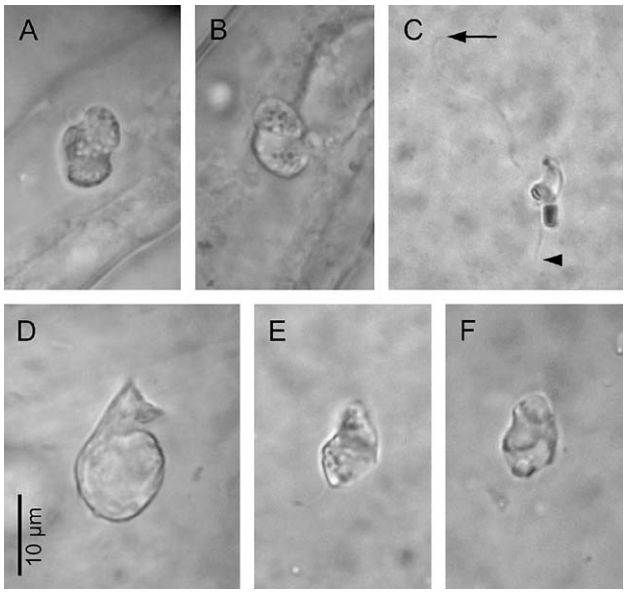

Figure 2. **A,B.** *Syndinium* ex *Paracalanus parvus* macrospores; **A.** Lateral view; **B.** Dorsal view. **C.** *Syndinium* ex *Paracalanus parvus* microspores. Tip of anterior flagellum is marked with an arrow, tip of posterior flagellum is marked with an arrowhead. Note the refractile body at the cell's posterior end. **D.** *Syndinium* ex *P. parvus* rostrate spores, lateral view. **E,F.** *Syndinium* ex *Corycaeus* sp. zoospores. Scale bar applies to A—F.

were rarely seen lying motionless on the bottom of the culture vessel. The microspores generally stayed viable for only 5—8 h in filtered seawater.

The rostrate spore was the largest spore type with cell length and width of approximately 16—20 µm and of 10—12 µm, respectively, for live cells (Fig. 2 D). These were nearly drop-shaped, i.e. spherical with the anterior part of the cell truncated into a beak-like projection. One-third of a cell length below the apex, a transverse depression or groove was located that seemed not to completely encircle the cell (Fig. 3 I). There was a weak marking of a longitudinal groove extending ventrally from the transverse groove towards the posterior end of the cell without reaching the antapex. The rostrate spores possessed two flagella that were both approximately similar to the cell in length (Fig. 3 I—J). The anterior flagellum was inserted in the transverse groove, the posterior flagellum in the longitudinal groove. Rostrate cells contained numerous trichocysts that could be seen extruded in fixed material (Fig. 3 K). Live cells had limited mobility and after having left their host, most rostrate spores congregated in a sessile cell cloud in proximity of the dead host. The peculiar cell shape was not evident in all cells. Some cells were almost spherical, but of similar diameter, and even cells with a more well-defined cell morphology tended to loose their shape and become spherical while still kept in the original culture container. Live rostrate spores could be observed several days after they had left their host. Cells of all three morphotypes generally shrunk by approximately 50% when prepared for SEM (Figs 2,3).

Syndinium ex *Corycaeus* sp.

Only two *Syndinium*-infected *Corycaeus* sp. were observed and these were both females. Infected hosts were of similar shape as healthy copepods, but could be distinguished due to their unusually dark body content (Fig. 1 B). Zoospores from both hosts were collected for DNA extraction and were, therefore, not studied in much morphological detail (Fig. 2 E,F). Few characters were recognizable on these zoospores when seen in the light microscope. They were of approximately similar size as macrospores of *Syndinium* ex *P. parvus*, but otherwise it was not possible to detect any close morphological similarity between these zoospores and any of the three types originating from infected *P. parvus*.

Sequences

Two partial sequences were obtained from each *Syndinium* zoospore morphotype (macrospore, microspore, and rostrate spore) originating from *P. parvus*, i.e. from a total of six hosts. All six sequences were 100% identical along the >700 bp partial sequences obtained. Three PCR products (one from each morphotype) were further sequenced to obtain full-length SSU rDNA sequences and these were 100% identical. From these three samples, the ITS1, ITS2, and 5.8S rDNA were also sequenced, and these were identical at all positions except four, i.e. 2 to 3 substitutions were found when comparing any two types of zoospores pair wise (in total three substitutions in ITS1 and one in ITS2).

Complete SSU rDNA sequences of two *Syndinium* ex *Corycaeus* sp. were achieved from two individual hosts and sequences ITS1—ITS2 were obtained from one of these. The two SSU rDNA sequences of *Syndinium* ex *Corycaeus* sp. were 100% identical and were similar to that of *Syndinium* ex *P. parvus* except for three substitutions. Comparing ITS1 and ITS2 sequences of

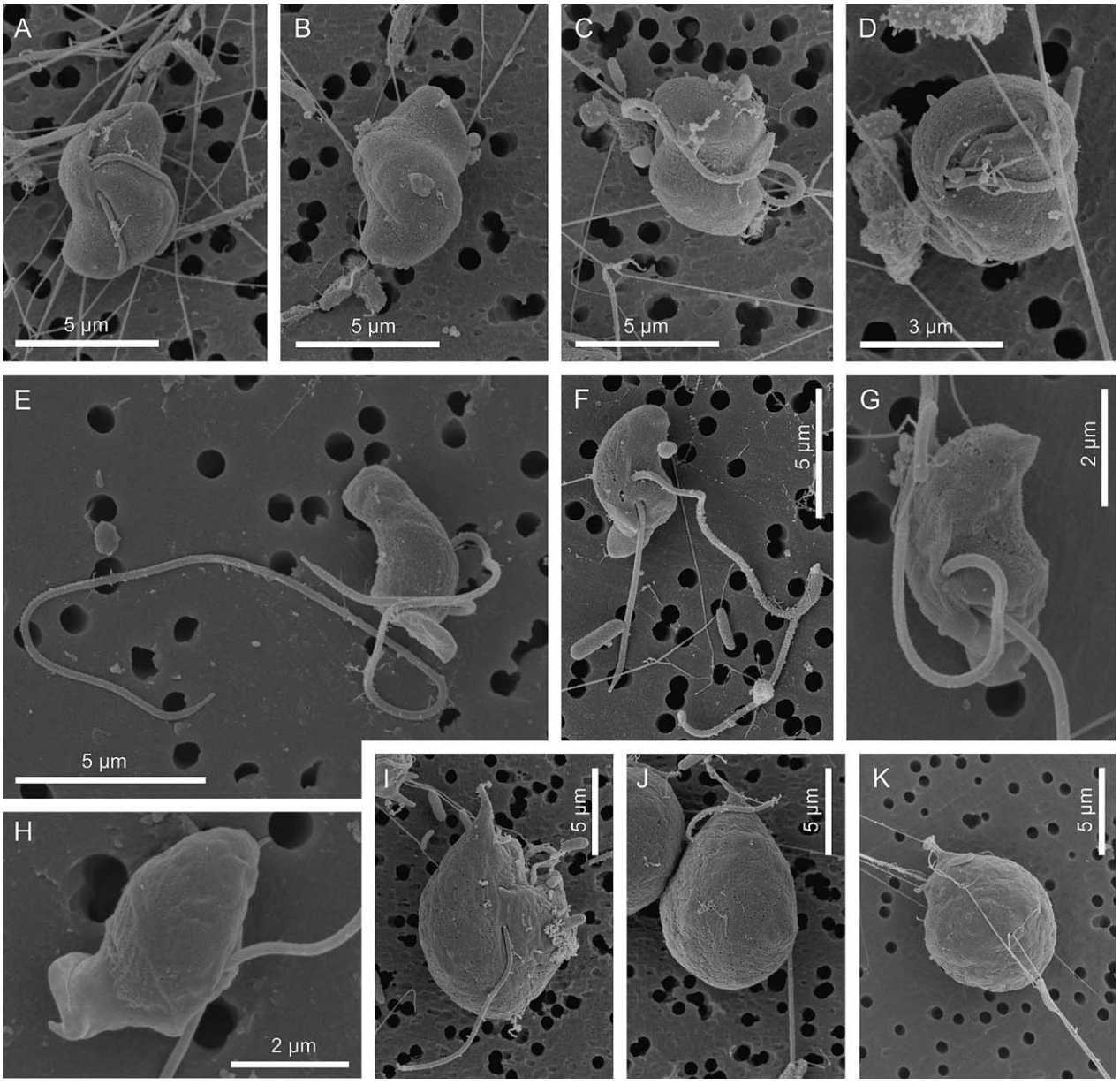

Figure 3. SEM pictures. **A—D.** *Syndinium* ex *Paracalanus parvus* macrospores. **A.** Ventral view; **B.** Lateral/antapical view; **C.** Dorsal view; **D.** Apical view. **E—H.** *Syndinium* ex *P. parvus* microspores. **E.** Dorsal view of the whole cell with intact flagella and refractile body; **F,G.** Ventral view of two different cells; **H.** Lateral/apical view. **I—K.** *Syndinium* ex *P. parvus* rostrate spores. **I.** Ventral view; **J.** Dorsal view; **K.** Lateral view.

Syndinium ex *P. parvus* and *Syndinium* ex *Corycaeus* sp. showed that they were considerably less similar than the respective SSU rDNA: there were 34—35 divergent positions out of a total of 470 bp in the ITS1 and ITS2 regions (17—18 substitutions out of 210 in ITS1 and 17 out of 260 in ITS2). 5.8S rDNA sequences from *Syndinium* ex *P. parvus* and *Syndinium* ex *Corycaeus* sp. were identical, all 150 bp long. BLAST searches run on any of the two different SSU rDNA sequences revealed SSU rDNA sequences from *Hematodinium* sp., marine alveolate Group II, and dinoflagellates as closest matches. A BLAST search run on the 5.8S rDNA sequence showed similarity with apicomplexan, perkinsid, and ciliate 5.8S rRNA genes, even though only roughly half of the

5.8S rRNA gene matched 5.8S rRNA genes of these organisms. On the other hand, no close similarity with any dinoflagellate 5.8S rRNA genes was found. Results of BLAST searches performed on any of the ITS1 and ITS2 sequences did not yield any significant match.

Phylogenetic Analyses

Syndinium ex *P. parvus* and *Syndinium* ex *Corycaeus* formed a clade in the maximum-likelihood tree (Fig. 4) and this was supported (100% values) by Bayesian posterior probabilities and neighbor-

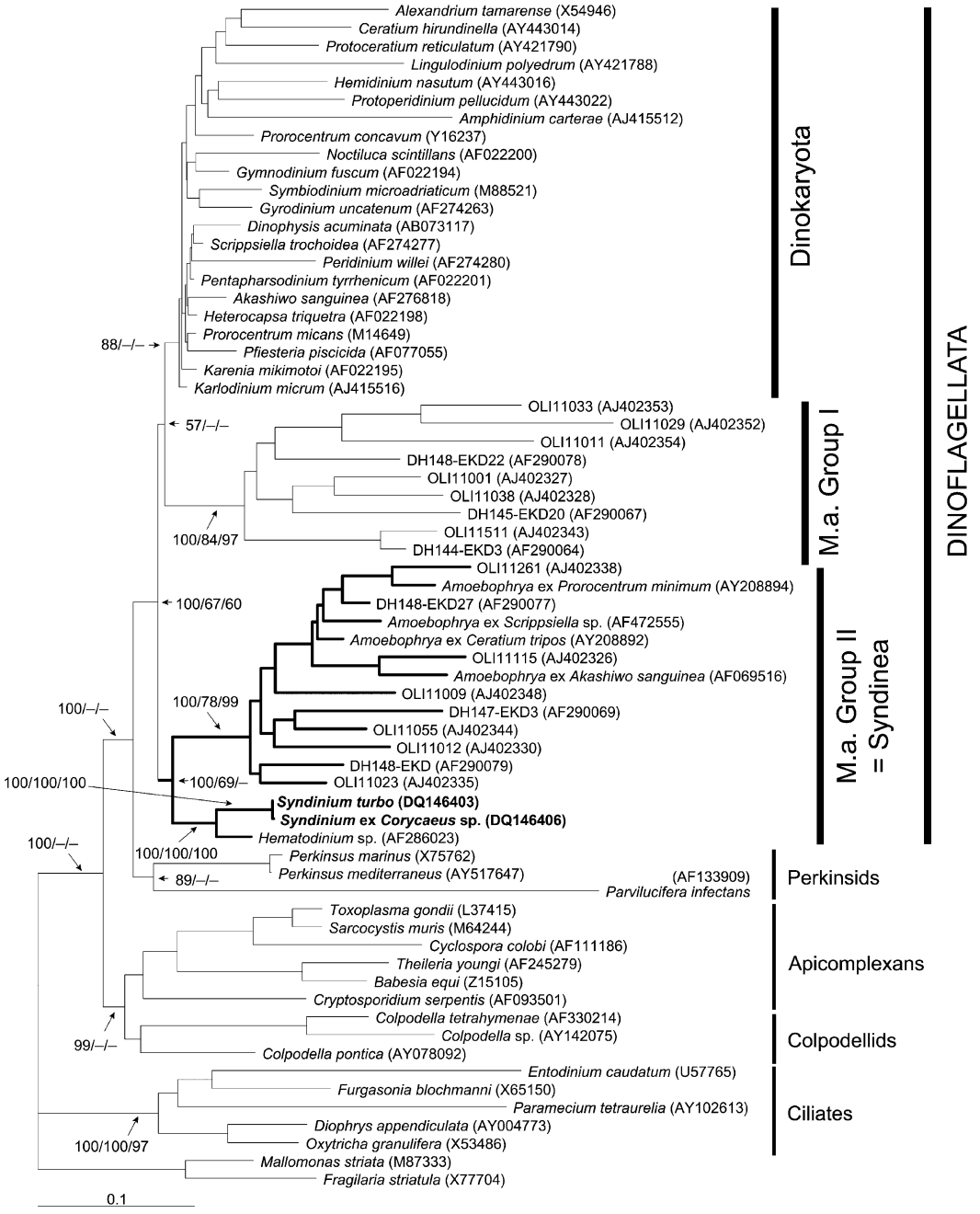

Figure 4. Maximum-likelihood phylogenetic tree based on SSU rDNA sequences of representative alveolates and two stramenopiles as outgroup. Numbers shown at most relevant nodes are, respectively, Bayesian posterior probabilities (mean of four separate analyses) and neighbor-joining and maximum-parsimony bootstrap values. GenBank accession numbers are given in parentheses.

joining and maximum-parsimony bootstrap values. *Hematodinium* sp. formed a sister clade to *Syndinium* spp. with the same 100% support. The *Syndinium*/*Hematodinium* group branched as a sister group to the marine alveolate Group II with highest support (100%) in Bayesian posterior probabilities, moderate support by neighbor-joining and negligible support in the maximum-parsimony analysis (Fig. 4). Both the marine alveolate Groups I and II were well supported (78—100%). The marine alveolate Group I branched as a sister group to Dinokaryota, but with negligible support (Fig. 4). The marine alveolate Groups I and II together with Dinokaryota formed a group with moderate to good support in all analyses (60—100%).

Discussion

All three morphotypes of *Syndinium* zoospores from *P. parvus* had evident morphological resemblances with the illustrations by Chatton (1920, 1952) depicting three types of *Syndinium* zoospores that all originated from plasmodia in *P. parvus*. Based on the identity in SSU rDNA sequences and the close similarity of ITS1 and ITS2 sequences, we conclude that all three types of zoospores represent one species, *S. turbo*. As a result, the final conclusion by Chatton (1922) that three species of *Syndinium* infect *P. parvus* should be rejected, implying that *S. turbo* produces three morphologically distinct types of zoospores as originally proposed by Chatton (1910).

The *Syndinium* species infecting *Corycaeus* sp. had similar SSU rDNA as *S. turbo* except for three substitutions. In our opinion, such minor difference in the SSU rDNA is not sufficient to prove that the two parasites should be considered different species. However, *Syndinium* ex *Corycaeus* sp. also differed markedly from *S. turbo* in the ITS1 and ITS2 regions and had a different host species and, therefore, we conclude that it is a different species from *S. turbo*. Chatton (1922) described *Cochlosyndinium corycaei* as a parasite of *C. giesbrechti* Dahl (as *C. venustus*). The host of *Syndinium* ex *Corycaeus* in the present study could not be identified to species level due to insufficient material, but it was undoubtedly not *C. giesbrechti*. The parasite may, nevertheless, have been *Cochl. corycaei*, but this needs confirmation.

The role of the three different morphological types of zoospores in *S. turbo* is puzzling. The life cycle of *S. turbo* is largely unknown, but it is likely that it includes the production of sexual anisogametes, since such morphologically differentiated gametes are known for both free-living (Pfiester 1984) and parasitic (Cachon and Cachon 1987) dinoflagellates. Dinoflagellate life cycles can be complex, in particular among the parasitic species (Coats 1999; Drebes 1984). A complete sexual life cycle is known for very few parasitic species and, in general, for only approximately 1% of all morphologically described dinoflagellate species (Elbrächter 2003). It may be possible that the life cycle of *S. turbo* contains stages that have yet not been fully recognized, e.g. dormant stages or zoospores of dissimilar ploidy. Not only do the size and shape vary among *S. turbo* zoospore morphotypes, but also the motility of these varies. It is plausible that the function of the rostrate spores is not simply to spread the parasite through infection of a new host, since the poor motility of this spore type does not create optimal odds for reaching a new host; however, based on present limited knowledge, it would be premature to speculate further on the nature of these morphotypes. Attempts to infect healthy copepods with *Syndinium* sp. by keeping the potential new hosts in suspensions of *Syndinium* sp. zoospores have been unsuccessful (Kimmerer and McKinnon 1990). Chatton (1910) tried to mix similar as well as dissimilar *S. turbo* zoospores in order to see possible signs of sexuality, i.e. cell division or fusion of possible gametes, but this was also unsuccessful. Both these experiments were reproduced in the present study, but likewise without observing any re-infection or cell fusion.

Our phylogenetic analyses confirmed the affiliation between *Syndinium* and the syndinian parasites *Amoebophrya* and *Hematodinium* as previously proposed in systematic schemes based on morphological data (Fensome et al. 1993). This supports the hypothesis that marine alveolate Group II is equivalent to Syndinea (Saldarriaga 2004). The *Syndinium*/*Hematodinium* clade does branch out at the base of the marine alveolate Group II clade (Fig. 4), but nevertheless, the data presently available support that these two clades together form Syndinea. Future studies on DNA sequences from other morphologically known syndinians may resolve this relationship in more detail. Having identified marine alveolate Group II as Syndinea, it will now be interesting to gather information on the morphology of members of marine alveolate Group I to determine whether it is a new group of dinoflagellate or dinoflagellate-affiliated alveolates or, alternatively, an assemblage of organisms of which some members may

already be described morphologically without having been analyzed genetically.

It has been questioned whether marine alveolate Groups I and II should be considered as dinoflagellates, implying that they may be sister groups to the dinoflagellates (Moreira and López-García 2002). Determining the exact composition of the group of organisms to be named dinoflagellates may be an artificial discussion, but clearly, members of marine alveolate Group II/Syndinea do differ both genetically and morphologically from the "core dinoflagellates", the Dinokaryota sensu Fensome et al. (1993). There is, however, good support for a close evolutionary relationship between Dinokaryota and both marine alveolate Groups I and II (Fig. 4; López-García et al. 2001), and at least two morphologically known members of marine alveolate Group II/Syndinea, *Syndinium* (Chatton 1920; Hollande and Enjumet 1955; this study) and *Amoebophrya* (Cachon 1964), produce zoospores with one or more typical dinoflagellate features such as cingulum- and sulcus-like grooves, an apical groove, and a transverse and a longitudinal flagellum. These facts suggest a close relationship between the "core dinoflagellates" (Dinokaryota) and Syndinea and, thereby, support the current taxonomic scheme. However, *S. turbo* microspores also possess characters that are not typical for dinoflagellates: they have flagella of unequal lengths and a refractile body at the cell's posterior end. Both these characters are present in *Hematodinium* sp. microspores (Appleton and Vickerman 1998) as well as in spores of the perkinsid parasite *Parvilucifera infectans* (Norén et al. 1999). The latter observations, thus, give support to the theory that Syndinea and presumably also marine alveolate Group I are sister groups to the dinoflagellates. Future studies must address the phylogeny of major groups within Dinoflagellata and more morphological and molecular knowledge is needed, in particular about the parasitic species, in order to achieve a better overall understanding of the phylogeny of dinoflagellates and dinoflagellate-affiliated organisms.

Methods

Materials: Copepods were collected in the NW Mediterranean Sea off Port Olímpic, Barcelona, Spain, using a 100 μm mesh size plankton net pulled vertically from the bottom (38 m) to the surface. Samples were kept in thermo boxes during transport to the laboratory were they were manipulated at ambient seawater temperature (24—26 °C). Live *Syndinium*-infected animals could be identified by their body shape and/or color (Ianora et al. 1987) and were isolated and transferred individually to 1 ml of 0.2 μm-filtered seawater in 3 ml polycarbonate multiwell containers (Falcon). The infected animals were then observed for 2—12 h until parasites matured and developed into free-swimming zoospores. These were then collected with a capillary pipette while carefully avoiding collecting any fragments of the now dead copepod. Prior to collection, zoospores were observed in more detail using a compound microscope to confirm which type of spore was sampled. Infected *P. parvus* were collected on August 13, 2003 and *Corycaeus* sp. on October 10, 2004.

Scanning electron microscopy (SEM): *Syndinium* zoospores from individual hosts were fixed for at least 2 h in glutaraldehyde (final concentration: 2%) in unbuffered seawater. Fixed cells were retained on 0.8 μm pore size polycarbonate filters (Whatman), washed with distilled water, and dehydrated through a graded ethanol series. Parallel samples were post-fixed for 30 min in OsO_4 (final concentration: 5%) in unbuffered seawater prior to washing and dehydration. Filters were finally critical point dried (BAL-TEC CPD 030 Critical Point Dryer), sputtered with gold (Polaron SC500 sputter coater), and viewed in a Hitachi S-3500N scanning electron microscope operating at 5 kV.

DNA extraction and amplification: *Syndinium* zoospores were collected from 8 host organisms: 6 *P. parvus* (2 hosts releasing each zoospore morphotype) and 2 *Corycaeus* sp. In the order of 200—500 zoospores generated by each single host were retained on 0.22 μm pore size Durapore filters (Millipore) that were placed in 0.75 ml lysis buffer (40 mM EDTA, 50 mM Tris—HCl, 0.75 M sucrose) and stored at −70 °C until DNA extraction. Nucleic acid extraction started with the addition of lysozyme (1 mg ml^{-1} final concentration) to the filters that were then incubated at 37 °C for 45 min. sodium dodecyl sulfate (SDS, 1% final concentration) and proteinase K (0.2 mg ml^{-1} final concentration) were then added and the filters were incubated at 55 °C for 60 min. Nucleic acids were extracted twice with phenol—chloroform—isoamyl alcohol (25:24:1) and the residual phenol was removed once with chloroform—isoamyl alcohol (24:1). Nucleic acid extracts were finally washed and concentrated in Centricon-100 concentrators (Millipore), recovered in 50 μl of sterile MilliQ water, and stored at −70 °C until analysis.

PCR amplification: SSU rRNA genes were amplified by polymerase chain reaction (PCR) with the eukaryotic primers EukA and EukB (Medlin et al. 1988). The 50 μl PCR mixture contained 5 μl DNA extract as template, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 1.25 units *Taq* DNA polymerase (Promega) and the PCR buffer supplied with the enzyme. The PCR cycle, run in an automated thermocycler (MiniCycler, MJ Research), was as follows: an initial denaturing step at 94 °C for 3 min, 30 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 3 min, and a final extension at 72 °C for 10 min.

The PCR mixture for amplifying ITS1, ITS2, and 5.8S rDNA was as above except for the primers which were 1209f (Giovannoni et al. 1988) and ITS4 (White et al. 1990). The cycle for this reaction was: initial denaturing at 94 °C for 1 min, 30 cycles of denaturing at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1.5 min, followed by a final extension at 72 °C for 7 min (Zechman et al. 1994).

Sequencing: Amplified PCR products were purified with the Qiagen PCR purification kit and sequenced with a Big Dye Terminator Cycle Sequencing kit (v.3) (PE Biosystems) and an ABI PRISM model 3100 (V3.3) automated sequencer (Applied Biosystems). The original PCR primers plus three additional eukaryotic-specific primers were used to sequence the complete SSU rRNA gene, and Euk1209f, ITS2 (White et al. 1990), and ITS4 were used to sequence ITS1, 5.8S rDNA, and ITS2. Sequence reads were aligned and assembled using ChromasPro software. The 5′ end of the LSU rRNA gene was identified and removed by comparing with a LSU rDNA sequence from *Prorocentrum minimum* (Pavillard) Schiller (Lenaers et al. 1989). Ends of 5.8S rDNA sequences were identified through comparison with a selection of perkinsid, apicomplexan, and ciliate 5.8S rDNA sequences from GenBank. *Syndinium* sequences were submitted to GenBank under the accession numbers DQ146403 to DQ146406.

Phylogenetic analysis: The two different complete SSU rDNA sequences obtained here were aligned using ClustalW 1.82 (Thompson et al. 1994) with a selection of alveolate sequences from databases: 22 dinoflagellates belonging to Dinokaryota plus 9 marine alveolate Group I, 13 marine alveolate Group II, and *Hematodinium* sp.; 3 perkinsids; 6 apicomplexans; 3 colpodellids; and 5 ciliates. In addition, two stramenopile sequences were included as outgroup. Highly variable regions of the alignment were located and removed using Gblocks (Castresana 2000) with parameters optimized for rDNA alignments (minimum length of a block: 5; allow gaps in half positions), leaving 1550 informative positions. Maximum-likelihood analysis was carried out with PAUP 4.0b10 (Swofford 2002), with the general time-reversible model assuming a discrete gamma distribution with six rate categories and a proportion of invariable sites. Parameters were estimated from an initial neighbor-joining tree. Bayesian analysis was carried out with MrBayes v3.0B (Huelsenbeck and Ronquist 2001) using the same model as above but with four rate categories in the gamma distribution. Bayesian posterior probabilities were computed by running four chains for 1,000,000 generations using the program default priors on model parameters. Trees were sampled every 100 generations. A total of 1000 or 1500 trees were discarded as "burn-in" in different runs upon examination of the log likelihood curve of the sampled trees, so that only trees in the stationary phase of the chain were considered. Neighbor-joining bootstrap values from 1000 replicates were calculated with PAUP following the same model as for the maximum-likelihood analysis. Maximum-parsimony bootstrap values from 1000 replicates were computed with PAUP using a heuristic search method with a tree-bisection-reconnection branch swapping option with random taxon addition.

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