The morphology of *Chrysochromulina rotalis* sp. nov. (Prymnesiophyceae, Haptophyta), isolated from the Skagerrak

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Chrysochromulina rotalis sp. nov. was isolated from surface water in the Skagerrak (58°11'N, 09°06'E) using the serial dilution culture method. The cells are saddle shaped with the appendages inserted subapically on the ventral side of the cell. The coiling haptonema measures 2-4 times the length of the flagella when extended. The cells contain two golden brown chloroplasts. The periplast is covered by two types of scale with dimorphic scale faces. The scales constituting the outer layer bear a short spine supported by four struts, the inner layer scales lack protrusions.

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INTRODUCTION

The haptophyte genus *Chrysochromulina* Lackey has a worldwide distribution and more than 50 species have been described, 38 of them have been reported from Scandinavian waters (e.g. Throndsen 1969; Leadbeater 1972a, 1972b; Espeland & Throndsen 1986; Kuylenstierna & Karlson 1997; Eikrem & al. 1998). In addition a number of undescribed forms or species has been observed in the area (e.g. Eikrem & al. 1998; Jensen 1998). Representatives of the genus are widespread and the number of species worldwide may exceed 100 (Thomsen & al. 1994). The first species of *Chrysochromulina*, *C. parva* Lackey was described from freshwater (Lackey 1939) and further species were not added until the 1950s when Parke and Manton began to describe marine species of *Chrysochromulina* (e.g. Parke & al. 1955, 1956, 1958, 1959). They introduced the use of electron microscopy in haptophyte research and ultrastructural details of the scales covering the cell body became the main taxonomic criterion for species identification in *Chrysochromulina*. However, they stressed the importance of light microscopic observations and in fact some *Chrysochromulina* species may be identified in the light microscope (e.g. *C. parkeae* Green & Leadbeater and *C. spinifera* (Fournier) Pienaar & Norris). Since then a number of additional authors have contributed to the now long list of formally described *Chrysochromulina* species (e.g. Green & Leadbeater 1972; Hällfors & Niemi 1974; Estep & al. 1984; Hällfors & Thomsen 1985; Moestrup & Thomsen 1986; Kawachi & Inouye 1993).

The cells of the *Chrysochromulina* species have two golden brown chloroplasts, two flagella, a haptonema and a scale covered periplast. The cells may vary in size from c. 4 µm (e.g. *C. apheles* Moestrup & Thomsen and *C. minor* Parke & Manton) to c. 25-30 µm (e.g. *C. birgerii* Hällfors & Niemi and *C. parkeae*). Their shape is quite variable and they may be spherical, oblong or saddle shaped. In some species the haptonema may be very long (e.g. *C. campanulifera* Manton & Leadbeater and *C. cymbium* Leadbeater & Manton) and in most species it has the ability to coil. In a few species (e.g. *C. spinifera* and *C. parkeae*) it is short (c. 2-5 µm) and rigid. The appendages may be inserted apically or subapically and in the saddle shaped species they are inserted ventrally.

The saddle shaped species of the genus *Chrysochromulina* all possess long coiling haptonemata. The group has been reviewed recently (Eikrem & Moestrup 1998) and at present 13 described species are known (Eikrem & al. 1998), *C. rotalis* sp. nov. included. The present species is one of a number of previously undescribed species encountered in Skagerrak in 1990, as part of a project under the Norwegian Research Programme on Harmful Algae.

MATERIAL AND METHODS

Chrysochromulina rotalis was isolated from a serial dilution culture (Throndsen 1978) inoculated with surface water collected in the Skagerrak (58°11'N, 09°06'E; off Hirtshals, Denmark) on 30 June 1990.

The culture was grown in IMR 1/2 medium (Eppley & al. 1967) enriched with 10 nM selenite at about 15 °C under white fluorescent light with a quantum flux of about 100 µmol photons $m^{-2}s^{-1}$ and 16:8 h L:D cycle.

The cells were studied live under a Nikon Microphot FX fitted with phase contrast and differential interference contrast optics and electronic flash. Stained whole-mounts were prepared according to Moestrup (1984) and some of the preparations were shadowed with gold-palladium in an Edwards Speedivac 12 E6 coating unit, angle c. 30°. Thin sections were prepared according to the following protocol; 10 ml of culture was fixed with 4 drops of 4 % osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 8) for 3 h and subsequently centrifuged to form a pellet, then rinsed 3 × 15 min in sodium cacodylate buffer and 2 × 10 min in distilled water. The samples were left overnight in 2 % aqueous uranyl acetate. Thereafter the cells were rinsed in distilled water and dehydrated in an ethanol series starting at 30 % and gradually rising to 96 %. The dehydration was concluded with 4 × 10 min in 100 % ethanol and 2 × 10 min in propylene oxide. The pellets were left over night in a 1:1 mixture of propylene oxide and Epon (Burke & Geiselman 1971) embedding resin. Finally the cells were given 3x1h in undiluted Epon before they were polymerized at 50 °C for 12 h. The sectioning was carried out on a Sorvall Ultra Microtome MT 5000.

Sections and whole-mounts were viewed in a Jeol 1200ex and 100cx at the EM laboratories for Biosciences, Department of Biology, University of Oslo.

RESULTS

Diagnosis: Cells saddle shaped measuring 4-6 µm with two chloroplasts. Two flagella, usually equal 8-18 µm. Haptonema 22-80 µm long, coiling. Periplast covered with dimorphic scales, in two layers; outer layer scales slightly elongate, 450-570 × 350-470 nm, with spine supported by four decurrent struts, spine approximately equalling the scale radius; inner layer scales slightly elongate, 400-550 × 320-460 nm, without protrusions. Proximal scale faces with concentric fibrils and distal faces with radiating ribs.

Latin diagnosis: Cellulae ephihippoideae, 4-6 µm in longae et latae chloroplastis binis. Flagellae duae plerumque aequales, 8-18 µm longa. Haptonema 22-80 µm longum, contractum gyros formans. Periplastus squamis dimorphis in stratis duobus dispositis tectus, squamae strati exterioris aliquantum elongatae 450-570 × 350-470 nm, spinam centralem 4 tigillis suffultam ferentes, longitudo spinae quasi radium squamae aequans. Squamae strati interioris parum elongatae, 400-550 × 320-460 nm, inermes. Superficies proximalis squamarum ordinationem concentricam fibrillarum, superficies distalis ordinationem radiatam praebentes.

Holotype: Fig. 8; (EM graph from a section of Embedding no. 15, block P16, at Dept. Biology, University of Oslo, Norway).

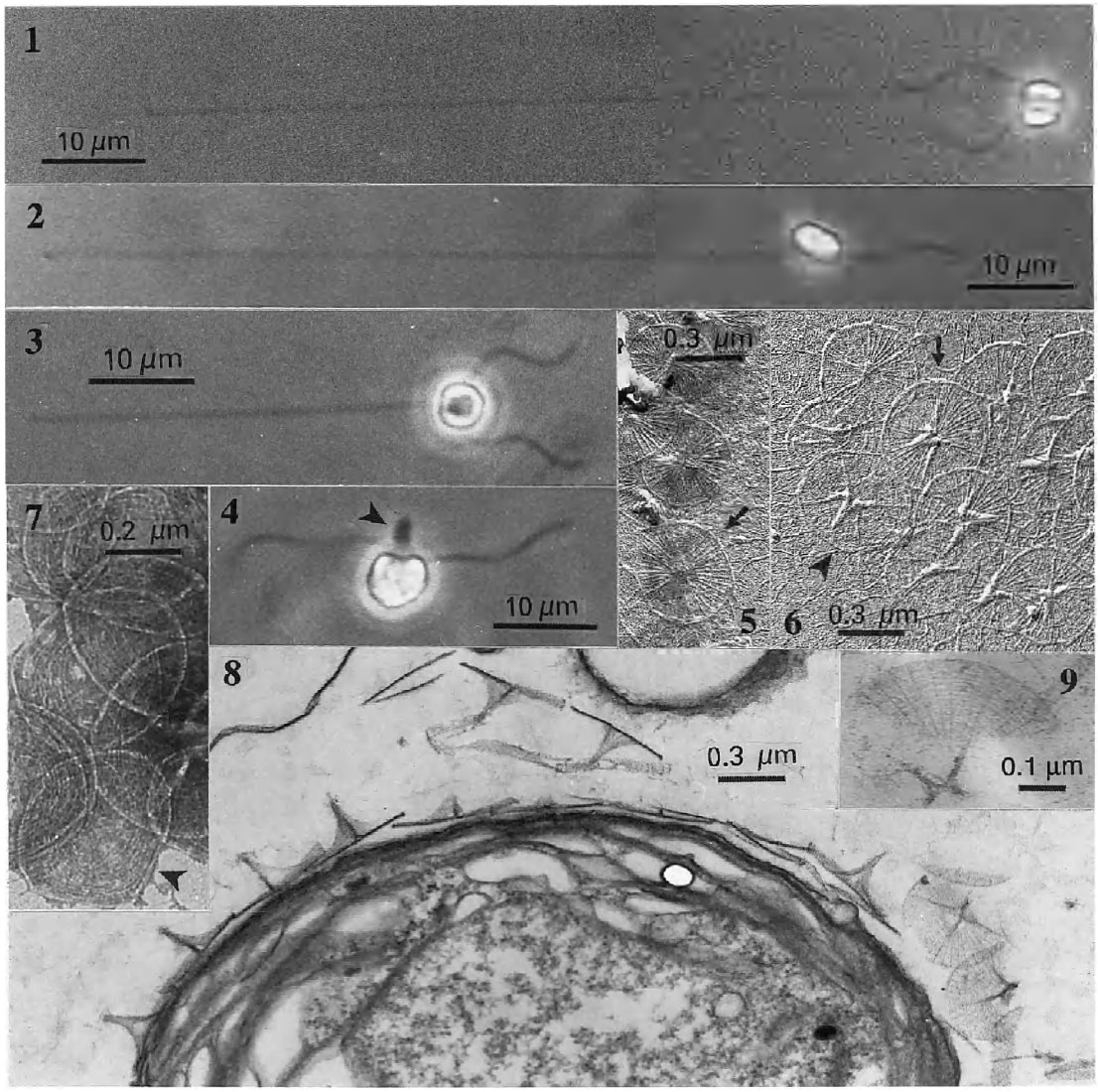
Type locality: Skagerrak; 58°11'N, 09°06'E; off Hirtshals, Denmark

Etymology: rotalis = having wheels (referring to the scales)

Observations

The cells of *C. rotalis* are typically saddle shaped with two golden brown parietal chloroplasts. Each chloroplast has a pyrenoid (not illustrated) which can barely be seen in the light microscope. The outline of the cell is apple shaped in ventral or dorsal views (Figs 1, 3 & 4), the length and width being almost equal, measuring 4-6 µm. In lateral view (Fig. 2) the outline is bean shaped, the dorsiventral axis being approximately half of the cell length. As can be seen in Fig. 2, the cell axis is kept at an angle of approximately 30° to the plane of the haptonema and flagella. The two flagella are usually equal to subequal in length, measuring 8-18 µm. The haptonema is long, normally 22-80 µm when extended (Figs 1-3), and easily visible also when coiled (Fig. 4 arrowhead). During normal swimming the flagella beat homodynamically along the sides of the cell, pushing the cell with the haptonema pointing forward (Figs 2 & 3), or they are reversed while the haptonema is dragged behind the cell (Fig. 1). This is also the case when the haptonema attaches temporarily to the cover slip surface.

The periplast is covered with two types of scales; plate scales and spine scales (Figs 5-8). The distal faces of both types have concentric fibrils which may be revealed in negative stained (Fig. 7) or shadow cast preparations (Fig. 6). The proximal faces have radiating ribs most easily seen in shadow cast preparations (Figs 5 & 6), but also evident in sections; in Fig. 9 showing a glancing section of an embedded scale both the concentric pattern of the proximal side and the radial pattern of the distal side can be seen. The scales of the outer layer bear short spines approximately equal to or shorter than the radius of the scales (Figs 6 & 8). The spines are supported by four decurrent struts.



Figs 1-9. *Chrysochromulina rotalis* sp. nov. Figs 1-4. Light micrographs (phase contrast) of living cells; Fig. 1. Cell with attached haptonema. Fig. 2. Lateral view of swimming cell. Fig. 3. Dorsal view of swimming cell. Fig. 4. Resting cell with coiled haptonema (arrowhead). Figs 5-7. Electron micrographs of whole-mounts; Figs. 5-6. Shadow-cast preparations; Fig. 5. Proximal face of plate scales with radiating ribs (arrow). Fig. 6. Proximal (arrow) and distal (arrow head) faces of spine scales. Fig. 7. Negative stained preparation showing concentric fibrils of distal face of plate scale (arrowhead). Figs 8-9. Electron micrographs of thin-sections; Fig. 8. Part of scale covered periplast. Plate and spine scales in cross section. Fig. 9. Detail of scale showing radiating ribs overlaying concentric fibrils.

DISCUSSION

Disregarding the spine, the scales of *Chrysochromulina rotalis* resemble those of the saddle shaped *C. simplex* (Estep & al.) Birkhead & Pienaar, as we know the latter from Scandinavian waters, i.e., with scales of one size only (figs 11-13 in Birkhead & Pienaar 1995; fig. 35 in Eikrem & al. 1998). The type described by Estep (Estep & al. 1984) as well as the emended diagnosis by Pienaar and Birkhead include larger scales with two central perforations in addition to the small scales. Cells with this kind of scale (Estep & al. 1984 fig. 15) have only been observed once in Norwegian waters (fig. 49a in Eikrem & al.1998). This organism may represent another

morphotype, perhaps another species. The question of species delimitation is further complicated in the Prymnesiophyceae by the different morphology of various stages in the life cycle of a species. This has been demonstrated for coccolithophorids e.g., species of *Hymenomonas* (Rayns 1962), *Coccolithus* (Parke & Adams 1960) and *Emiliania* (Braarud 1963), and has also been shown for *Chrysochromulina polylepis* Manton & Parke (Edvardsen & Paasche 1992).

The saddle shaped species share a number of ultrastructural features (Eikrem & Moestrup 1998) and also according to genetical information they cluster in one group (e.g. Simon & al. 1997; Medlin & al. 1997, including *C. rotalis* as P 16). The ornamentation of the scales covering the periplast is the character used in species identification, but the variation in scale ornamentation between the saddle shaped species is considerable (e.g., Eikrem & Moestrup 1998) and may not be used as a unifying character of the group. Similarity in scale ornamentation may indicate a close relationship between species, and in the case of *C. cymbium* and *C. strobilus* Parke & Manton which only differs in minor details of scale structure (Leadbeater & Manton 1969 a,b), this has been confirmed by genetic analysis (Medlin pers. commn). On the other hand, large differences in scale morphology do not necessarily indicate a remote relationship between species as exemplified by the marked differences in scale morphology between the two alternate stages of *C. polylepis*. This emphasizes the importance of using several criteria when characterizing a species, and future investigations should preferably be based on unialgal/clonal cultures which will provide material for fine structural and genetic analysis, as well as for experimental physiology and autecology.

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REFERENCES

Birkhead M, Pienaar RN. 1995. The taxonomy and ultrastructure of *Chrysochromulina simplex* (Prymnesiophyceae). *Phycologia* 34:145-156.

Braarud T. 1963. Reproduction in the marine coccolithophorid *Coccolithus huxleyi* in culture. *Pubblicazioni della Stazione zoologica di Napoli* 33:110-116.

Burke CN, Geiselman CW. 1971. Exact anhydride epoxy percentages for electron microscopy embedding (Epon). *Journal of ultrastructure research*. 36:119-126.

Edvardsen B, Paasche E. 1992. Two motile stages of *Chrysochromulina polylepis* (Prymnesiophyceae): Morphology, growth, and toxicity. *Journal of Phycology* 28:104-114.

Eikrem W, Jensen MØ, Moestrup Ø, Throndsen J. 1998. An illustrated key to the unmineralized prymnesiophycean flagellates of Scandinavian marine waters with special reference to the genus *Chrysochromulina*. In: Jensen MØ. 1998. *The genus* Chrysochromulina *(Prymnesiophyceae) in Scandinavian coastal waters - diversity, abundance and ecology* [PhD thesis]. University of Copenhagen. Part 5. p 1-36.

Eikrem W, Moestrup Ø. 1998. Structural analysis of the flagellar apparatus and the scaly periplast in *Chrysochromulina scutellum* sp. nov. (Prymnesiophyceae, Haptophyta) from the Skagerrak and the Baltic. *Phycologia* 37:132-153.

Eppley RW, Holmes RW, Strickland JDH. 1967. Sinking rates of marine phytoplankton measured with a fluorometer. *Journal of experimental marine biology and ecology.* 1:191-208.

Espeland G, Throndsen J. 1986. Flagellates from Kilsfjorden, southern Norway, with description of two new species of Choanoflagellida. *Sarsia* 71:209-266.

Estep KW, David PG, Hargraves P, Sieburth JMcN. 1984. Chloroplast containing microflagellates in natural populations of North Atlantic nanoplankton, their identification and distribution; including a description of five new species of *Chrysochromulina* (Prymnesiophyceae). *Protistologica* 20:613-634.

Green JC, Leadbeater BSC. 1972. *Chrysochromulina parkeae* sp. nov. (Haptophyceae), a new species recorded from S.W. England and Norway. *Journal of the Marine Biological Association of the United Kingdom* 52:469-474.

Hällfors G, Niemi Å. 1974. A *Chrysochromulina* (Haptophyceae) bloom under the ice in the Tvärminne archipelago, Southern coast of Finland. *Memoranda Societatis pro Fauna et Flora Fennica* 50:89-104.

Hällfors S, Thomsen HA. 1985. *Chrysochromulina brachycylindra* sp. nov. (Prymnesiophyceae) from Finnish coastal waters. *Nordic Journal of Botany* 5:499-504.

Jensen MØ. 1998. Seasonal dynamics of *Chrysochromulina* species (Prymnesiophyceae, Haptophyta) in Danish coastal waters: diversity, abundance and ecology. In: Jensen MØ. 1998. *The genus* Chrysochromulina *(Prymnesiophyceae) in Scandinavian coastal waters - diversity, abundance and ecology* [PhD thesis]. University of Copenhagen. Part 4. p 1-38.

Kawachi M, Inouye I. 1993. *Chrysochromulina quadrikonta* sp. nov., a quadriflagellate member of the *Chrysochromulina* (Prymnesiophyceae = Haptophyceae). *Japanese Journal of Phycology* 41:221-230.

Kuylenstierna M, Karlson B. 1997. Checklist of phytoplankton in the Skagerrak-Kattegat. *http://www.marbot.gu.se/SSS/SSShome.htm*

Lackey JB. 1939. Notes on plankton flagellates from the Scioto River. *Lloydia* 2: 128-143.

Leadbeater BSC. 1972a. Fine structural observations on six new species of *Chrysochromulina* (Haptophyceae) from Norway, with preliminary observations on scale production in *C. microcylindra* sp. nov. *Sarsia* 49:65-80.

Leadbeater BSC. 1972b. Identification, by means of electron microscopy of flagellate nanoplankton from the coast of Norway. *Sarsia* 49:107-124.

Leadbeater BSC, Manton I. 1969a. New observations on the fine structure of *Chrysochromulina strobilus* Parke & Manton with special reference to some unusual features of the haptonema and scales. *Archiv für Mikrobiologie* 66:105-120.

Leadbeater BSC, Manton I. 1969b. *Chrysochromulina camella* sp. nov. and *C. cymbium* sp. nov., two new relatives of *C. strobilus* Parke & Manton. *Archiv für Mikrobiologie* 68:116-132.

Medlin LK, Kooistra WHCF, Potter D, Saunders GW, Andersen RA. 1997. Phylogenetic relationships of the golden algae (haptophytes, heterokont chromophytes) and their plastids. *Plant systematics and evolution (Suppl.)* 11:187-219.

Moestrup Ø. 1984. Further studies on *Nephroselmis* and its allies (Prasinophyceae). II. *Mamiella* gen. nov. Mamiellaceae fam. nov. Mamiellales ord. nov. *Nordic Journal of Botany* 4:109-121.

Moestrup Ø, Thomsen HA. 1986. Ultrastructure and reconstruction of the flagellar apparatus in *Chrysochromulina apheles* sp. nov. (Prymnesiophyceae = Haptophyceae). *Canadian Journal of Botany* 64:593-610.

Parke M, Adams I. 1960. The motile (*Crystallolithus hyalinus* Gaarder & Markali) and non-motile phases in the life history of *Coccolithus pelagicus* (Wallich) Schiller. *Journal of the Marine Biological Association of the United Kingdom* 39:263-274.

Parke M, Manton I, Clarke B. 1955. Studies on marine flagellates II. Three new species of *Chrysochromulina*. *Journal of the Marine Biological Association of the United Kingdom* 34:579-609.

Parke M, Manton I, Clarke B. 1956. Studies on marine flagellates III. Three further species of *Chrysochromulina*. *Journal of the Marine Biological Association of the United Kingdom* 35:387-414.

Parke M, Manton I, Clarke B. 1958. Studies on marine flagellates IV. Morphology and microanatomy of a new species of *Chrysochromulina*. *Journal of the Marine Biological Association of the United Kingdom* 37:209-228.

Parke M, Manton I, Clarke B. 1959. Studies on marine flagellates. V. Morphology and microanatomy of *Chrysochromulina strobilus* sp. nov. *Journal of the Marine Biological Association of United Kingdom* 38:169-188.

Rayns DG. 1962. Alternation of generations in a coccolithophorid, *Cricosphaera carterae* (Braarud & Fagerl.) Braarud. *Journal of the Marine Biological Association of the United Kingdom* 42:481-484.

Simon N, Brenner J, Edvardsen B, Medlin LK. 1997. The identification of *Chrysochromulina* and *Prymnesium* species (Haptophyta, Prymnesiophyceae) using fluorescent or chemiluminescent oligonucleotide probes: a means for improving studies on toxic algae. *European journal of Phycology* 32:393-401.

Thomsen HA, Buck KR, Chavez FP. 1994. Haptophytes as components of marine phytoplankton. In: Green JC, Leadbeater BSC, editors. *The Haptophyte Algae. Systematics Association* Special Volume No. 51. Oxford: Clarendon Press. p 187-208.

Throndsen J. 1969. Flagellates of Norwegian coastal waters. *Nytt Magasin for Botanikk* 16:161-214.

Throndsen J. 1978. The dilution culture method. In: Sournia A, editor. *Phytoplankton manual. Monographs on oceanographic methodology* 6 Paris: UNESCO. p 218-224.

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