Ultrastructure and phylogenetic position of *Chrysoculter rhomboideus* gen. et sp. nov. (Prymnesiophyceae), a new flagellate haptophyte from Japanese coastal waters

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We isolated a novel haptophyte alga from the coastal waters of the northern part of Japan. The cell is asymmetrical spindle-shaped and possesses two nearly equal flagella and a haptonema from the anterior tip. Two yellowish chloroplasts including immersed pyrenoids are situated asymmetrically. Two types of organic scales, small elliptical and large rhomboidal scales, cover the cell. The rhomboidal scale has two tubular projections at the longitudinal poles and closely attached to each other. The transition region of the flagellum includes only one transitional plate, which is probably homologous with the distal plate in other prymnesiophyceans. The flagellar apparatus of this alga has basic components found in other prymnesiophyceans. Developed root 1 (R1) is divided into two components that join again. The R1 extends toward the distal plate of the cell with associated endoplasmic reticulum (ER) and forms a cyttoplasmic tongue. A fibrous root, which has only been reported in some coccolithophorids, connects R1 and basal body 1. The proximal end of R1 is associated with the electron-dense plate and teeth-like structure. Four microtubules of the root 2 are arranged in an arc shape and have appendages. The free part of the haptonema includes five microtubules. In the traditional taxonomy, this alga is apparently a member of the genus *Chrysochromulina* because the cell possesses a long haptonema and no coccoliths. However, recent molecular phylogenetic studies have shown the polyphyly of the genus *Chrysochromulina*. Diagnostic characters of *Chrysochromulina* would be plesiomorphies of the Prymnesiophyceae. Phylogenetic analysis using 18S rDNA and *rbcL* sequences indicated that the alga reported here is not closely related to any other prymnesiophyceans, including *Chrysochromulina*, but suggested the basal position of the coccolithophorids. Based on these results, we propose *Chrysoculter rhomboideus* gen. et sp. nov. and *Chrysoculteraceae* fam. nov. for this unique haptophyte.

INTRODUCTION

The Haptophyta is an important algal group of photosynthetic primary producers especially in the open ocean. In addition to photosynthetic, some haptophytes exhibit phagotrophy and are notable consumers in the ocean (e.g. Parke et al. 1956; Kawachi et al. 1991; Jones et al. 1994). Haptophyte algae are also known to play an important role in carbon and sulphur cycles. Some species of the Haptophyta (Isochrysidales and Coccolithales; called the coccolithophorids hereafter) produce body scales composed of calcium carbonate and are known as coccoliths. Although the haptophytes play important ecological roles in nature, their diversity and evolution including the origin of the coccolithophorids are not well understood. Recent molecular phylogenetic studies suggest that there are many undescribed haptophyte algae in the ocean (Edvardsen et al. 2000; Diéz et al. 2001; Moon–Van Der Staay et al. 2001).

In the Haptophyta, the genus *Chrysochromulina* Lackey includes the greatest number of species, c. 60 spp. (Eikrem & Moestrup 1998; Eikrem & Thronsden 1999; Puigserver et al. 2003). Species of *Chrysochromulina* are unicellular flagellates covered by organic scales but no coccoliths. Although members of *Chrysochromulina* have two flagella and a conspicuous haptonema as the common feature, they show notable diversity in cell shape, scale morphology, and ultrastructure (e.g. Manton & Leadbeater 1974; Green & Hori 1994; Birkhead & Pienaar 1995; Eikrem & Moestrup 1998; Puigserver et al. 2003). Furthermore, recent molecular phylogenetic studies have shown the polyphyletic nature of the genus *Chrysochromulina* (Edvardsen et al. 2000; Fujiwara et al. 2001). This evidence suggests that the genus *Chrysochromulina* is the ancestral stock of some haptophyte lineages, and so the phylogenetic study of *Chrysochromulina* is important to clarify the evolutionary history of the Haptophyta.

We isolated from Japanese coastal waters an enigmatic haptophyte alga with two flagella, a haptonema, and organic scales. In this paper, we report on morphological and ultrastructural characteristics of this alga. The phylogenetic position of the alga was also analysed, based on 18S rDNA and *rbcL* sequences. In a traditional taxonomical sense, the alga studied in this paper can be classified as a species of *Chrysochromulina*. However, we propose a new genus and species, *Chrysoculter rhomboideus* gen. et sp. nov., based on its ultrastructural and molecular characters.
Table 1. Nucleotide sequences used in this study.

| Species                                | 18S rDNA Accession no. | rbcL Accession no. |
|----------------------------------------|------------------------|--------------------|
| **Prymnesiophyceae**                   |                        |                    |
| *Chrysochromulina rhomboideus*         | AB158370               | AB158371           |
| **Coccolithales**                      |                        |                    |
| *Calciocystis leptopora* (Murray & Blackman) Loeblich Jr. & Tappan |                     |                    |
| *Calypsothecium phaeoideum* Schiller   |                       |                    |
| *Coccolithus pelagicus* (Wallich) Schiller |                     |                    |
| *Coccolithus neohelis* (McIntyre & Be) Reinhardt | A246261 | AB043690           |
| *Helicosphaera carteri* (Wallich) Kamptner | A246262               | AB043689           |
| *Pleurochrysis carterae* (Braarud & Fagerland) Christensen | A246263 | D11140             |
| *Pleurochrysis elongata* (Droop) Jordan | A246264               |                    |
| *Pleurochrysis haptonegosa* (Inouye & Chihara) Gayral & Fresnel |                     |                    |
| *Pleurochrysis sp. CCMP875*            | A246265               | AB043688           |
| *Reticulosphaera japonensis* Grill     | X09992                 | AB043691           |
| **Isochrysidales**                     |                        |                    |
| *Euglenicia huxleyi* (Lohmann) Hay Mohlaer |                     |                    |
| *Isochrysis galbana* Parke             | A246266               | AB043693           |
| **Prymnesiales**                       |                        |                    |
| *Chrysochromulina spinifera* Fourrier  |                       |                    |
| *Chrysochromulina alifera* Parke & Manton |                     |                    |
| *Chrysochromulina campanulifera* Manton & Leadbeater | A246273 | AB043695           |
| *Chrysochromulina hirta* Manton        | A246272               | AB043632           |
| *Chrysochromulina kappu* Parke & Manton | A246271              | AB043632           |
| *Chrysochromulina parva* Lackey        |                       |                    |
| *Chrysochromulina polyplepis* Manton & Parke |                     |                    |
| *Chrysochromulina scutellum* Eikrem & Moestrup | A246274 |                |
| *Chrysochromulina thronsdenii* Eikrem   | A246277               |                    |
| *Chrysochromulina sp. (‘eyelash’ Chrysochromulina) |                     |                    |
| *Imantonia rotunda* Raynolds           | A246267               | AB043696           |
| *Platychrysis* sp.                     |                       |                    |
| *Prymnesium calathiferum* Chang & Ryan |                       |                    |
| *Prymnesium parvum* N. Carter          | A246269               | AB043698           |
| Symbiont of Globigeneriella            |                       |                    |
| **Phaeocystidales**                    |                        |                    |
| *Phaeocystis antarctica* Karsten       | X77481                 |                    |
| *Phaeocystis cordata* Zingone & Chrétiennot-Dinet | AF163147 |                |
| *Phaeocystis globosa* Scherrfel        | X77476                 |                    |
| *Phaeocystis jahnii* Zingone           | A014184                |                    |
| *Phaeocystis pouchetii* (Hariot) Lagerheim | X77475              |                    |
| Uncultured haptophytes                 |                        |                    |
| OLI16010                               | AF107081               |                    |
| OLI16029                               | AF107080               |                    |
| OLI16108                               | AF107082               |                    |
| OLI26017                               | AF107083               |                    |
| OLI26041                               | AF107084               |                    |
| OLI26047                               | AF107085               |                    |
| OLI51004                               | AF107086               |                    |
| OLI51033                               | AF107087               |                    |
| OLI51050                               | AF107088               |                    |
| OLI51059                               | AF107089               |                    |
| OLI51076                               | AF107090               |                    |
| OLI51102                               | AF107092               |                    |
| **Pavlovophyceae**                     |                        |                    |
| *Exanthemachrysis gayraliae* Lepailleur |                       |                    |
| *Pavlova gyraws Butcher*               | U09222                 | AB043701           |
| *Pavlova sp. CCMP 1416*                | A243369                |                    |
| *Rebecca salina* (N. Carter) Green     | L34669                 |                    |
|                                           | AF102987               | AB043633           |
Abbreviations used in the figures: 1, basal body 1; 2, basal body 2; C, chloroplast; DF, distal fibre; el, electron-dense lump; em, electron-dense mass; ep, electron-dense plate; F, flagellum; f, fibrous root; G, Golgi body; H, haptonema; hf1, haptonema fibre 1; hf2, haptonema fibre 2; hf3, haptonema fibre 3; M, mitochondrion; N, nucleus; P, pyrenoid; PF, proximal fibre; R1, root 1; R1a, anterior component of root 1; R1p, posterior component of root 1; R2, root 2; R3, root 3; R4, root 4; T, teeth-like structure.

Figs 1–4. Chrysoculter rhomboideus, light microscopy (LM) (DIC). Scale bars = 10 μm.  
Figs 1, 2. Typical cells showing asymmetrical cell shape and chloroplasts.  
Fig. 3. Small cell with posterior extension presumably composed of scales (arrowhead).  
Fig. 4. Dividing cell.  
Fig. 5. Drawing of C. rhomboideus. Scale bar = 5 μm.

MATERIAL AND METHODS

Chrysoculter rhomboideus occurred in an enriched culture of a sample collected from the coastal water of Okumatsushima, Miyagi, Japan, in October 1998. Unialgal cultures were established by micropipetting and maintained in Erd-Schreiber medium (ESM) (Watanabe et al. 2000). Cultures were grown at 15–20°C under white fluorescent light of 20–50 μmol photons m⁻² s⁻¹ and a 14:10 h light:dark cycle. To survey the occurrence of different phases of the life cycle, the cells were cultivated under various conditions according to Noël et al. (2004).

For light microscopical observations, a Nikon Optiphot microscope (Nikon, Tokyo, Japan) equipped with differential interference contrast (DIC) optics was used. Whole mount preparations for transmission electron microscopy (TEM) were prepared using the method of Marin & Melkonian (1994). Material for thin sections was fixed in equal volumes of fixative (5% glutaraldehyde, a few drops of 4% OsO₄, 0.5 M sucrose, in 0.1 M cacodylate buffer) for 1 h. After rinsing with 0.05 M cacodylate buffer, cells were postfixed in 2% OsO₄ at 4°C for 1 h, then rinsed once with the same buffer. Cells were embedded in Spurr’s resin (Spurr 1969) after dehydration in a graded ethanol series. Sections were cut with a diamond knife and double stained with 2% uranyl acetate and lead citrate (Reynolds 1963). Observations were carried out with a Jeol JEM 100CXII TEM (Jeol, Tokyo, Japan). Material for scanning electron microscopy (SEM) was fixed with 5% glutaraldehyde for 2 h and adhered to a SEM plate treated with 0.1% poly L-lysine. After rinsing thrice with 0.2 M cacodylate buffer, cells were postfixed in 1% OsO₄ for 30 min, then rinsed once with the same buffer. After dehydration in a graded ethanol series, absolute ethanol was replaced with t-butyl alcohol. The samples were dried in a VFD-21S (Shinku-Device, Ibaraki, Japan) and coated with platinum-palladium using an ion sputter E-102 (Hitachi, Tokyo, Japan). For SEM observations, a JEOL JSM-6330F (Jeol) was used.

Total DNA was extracted using Unset buffer and phenol/chloroform (Garriga et al. 1984) and precipitated with ethanol. The 18S rDNA and partial rbcL were amplified using polymerase chain reaction (PCR) with the reported primers (Nakayama et al. 1998; Fujiwara et al. 2001). PCR products were directly sequenced using an autosequencer ABI 377 and the dye terminator method according to the manufacturer’s in-
Fig. 6. *Chrysoculter rhomboideus*, SEM. Scale bar = 5 μm.

Figs 7–9. *Chrysoculter rhomboideus*, TEM, whole mounts stained with uranyl acetate.

Fig. 7. Cell showing two nearly equal flagella, a haptonema, and scaly covering. Scale bar = 5 μm.

Fig. 8. Scale casing of a cell. Note the large rhomboidal scales in a regular arrangement. Scale bar = 5 μm.

Fig. 9. Field of scales showing large rhomboidal scales with projections and small underlayer elliptical scales. Scale bar = 1 μm.

Figs 10, 11. Scales of *C. rhomboideus*, TEM, thin sections. Both large rhomboidal and small elliptical scales have radial ribs and rims (arrows). Note the tubular projections on the longitudinal poles of the rhomboidal scales (arrowheads). Scale bars = 0.5 μm.

RESULTS

*Chrysoculter rhomboideus* Nakayama, Yoshida, Noël, Kawachi & Inouye, gen. nov. and sp. nov.

Cellulæ solitariae, plerumque asymmetricæ cultiformes, 10–16 μm longæ, 3–6 μm latae. Flagella duo, aequalia vel subaequalia, 14–26 μm longa, ab apice cellulæ orta. Haptonema 4–10 μm longum, spiram non formans. Cellulæ duobus typis squama tectae. Squama stati interioris ellipsoidea, 0.35 × 0.18 μm, cum marginibus
Cells solitary, mostly asymmetric knife-shaped, 10–16 μm long, 3–6 μm wide. Two equal to subequal flagella, 14–26 μm, inserted at the cell apex. Haptonema 4–10 μm, noncoiling. Cells covered by two types of scales. Scales of the inner layer elliptical, 0.35 × 0.18 μm, with a narrow inflexed rim, and a radial pattern. Scales of the outer layer rhomboidal, 1.1 × 0.6 μm, with upright rim (50–80 nm), two projections, and a radial pattern. Rhomboidal scales do not overlap. Two pale yellow chloroplasts, lateral and parietal, are displaced horizontally, each with an immersed pyrenoid. The flagellar transitional region with a terminal transitional plate.

**HOLOTYPE**: Fig. 7.

A unialgal culture used in this study is deposited at the National Institute of Environmental Studies, Japan as P1544.

**TYPE LOCALITY**: Okumatsushima, Miyagi, Japan (42°30’N; 154°10’E).

**ETYMOLOGY**: The generic name refers to the colour and unique asymmetrical cell shape (*chryso* = golden, *culter* = knife), and the specific epithet refers to the shape of the large organic scales (*rhomboideus* = rhomboidal).

**Cell structure**

Living cells of *C. rhomboideus* are usually slender spindle- to knife-shaped, 10–16 μm long, and 3–6 μm wide (Figs 1, 2, 5–7, 12). Cells are asymmetric in outline; one lateral side is somewhat swollen, and the opposite lateral side is flattened (Figs 2, 5, 12). Small cells with an asymmetrical ovoidal outline were sometimes observed (Fig. 3). The posterior end of the cell sometimes protruded as a short hyaline tail (Fig. 5). Two equal to subequal flagella (14–26 μm long) and a haptonema (4–10 μm long) emerged from the anterior tip of the cell (Figs 1–7, 12, 13). We did not observe a coiling haptonema. This alga was not an active swimmer, but it sometimes attached to the substratum by the anterior side of the cell or flagella. Two yellowish parietal chloroplasts were situated asymmetrically. One was situated at the anterior–lateral side of the cell, and the other was positioned at the posterior, op-
旗体和触手的复合侧（Figs 1, 2, 5, 12, 13）。前部的叶绿体通常位于肿胀（Figs 2, 5, 12）。叶绿体包含三个类囊体层，但没有环状间隔，它被盖于被膜端内质网，并与核膜融合（Figs 12, 14）。每个叶绿体包含一个浸入的类囊体，被两条叶绿体层（Fig. 14）。核位于细胞的中心（Figs 1, 5, 12）。切片显示了与类囊体相关的线粒体横切面，可能代表一个大型网状线粒体（Figs 12, 13, 15）。一个典型的高尔基体，典型的稀释的囊泡位于核和基体之间，成熟的一面在基体和后部叶绿体之间（Figs 12, 13, 52）。后部的大型真空泡，没有明显的内含物，位于细胞的后部（Fig. 13）。较小的真空泡，含有一些物质，分布于细胞的后部到中心部分（Figs 13）。较小的真空泡含有物质，位于后部叶绿体的中央部分，这种类型的真空泡，与覆盖的细胞膜（Figs 15, 38, 58）。C. rhomboideus 通过二分裂繁殖（Fig. 4），没有观察到不同的性生殖或生殖阶段。

细胞覆盖

C. rhomboideus 有两个层的有机体尺度。内层由散在的椭圆形尺度（0.35 × 0.18 μm）组成，具有狭窄的环（Figs 9–11, 69）。一些图像暗示，椭圆形尺度在细胞膜处分布，形成一串类型的细胞膜（Figs 9–11, 69）。较大的棱镜尺度（1.1 × 0.6 μm）位于细胞膜处（Figs 9–11, 69）。这些棱镜尺度在细胞膜处有突出的环（50–80 nm）（Figs 9–11, 69）。棱镜尺度的表面有纤维状的突起（0.3 μm 长）在长轴的两端（Figs 9–11, 69）。大型棱镜尺度相互重叠，在细胞膜处有一层外壳（Fig. 4），没有观察到生殖或发生不同的阶段。

Figs 16–23. Flagella and haptonema of C. rhomboideus, TEM, thin sections. Scale bars = 0.2 μm.

Fig. 16. Longitudinal section through a flagellum showing fibrous material on the flagellar surface.

Figs 17–20. Transverse sections through flagella and transition region.

Fig. 21. Longitudinal section through a flagellum and basal body. Note the single transition plate and fragile cylinder (arrows). The numbers mark the corresponding figures in Figs 17–20.

Fig. 22. Transverse sections through a haptonema showing five microtubules and ER.

Fig. 23. Longitudinal sections through a haptonema.

Figs 16–23. Flagella and haptonema of C. rhomboideus, TEM, thin sections. Scale bars = 0.2 μm.

Fig. 16. Longitudinal section through a flagellum showing fibrous material on the flagellar surface.

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Fig. 21. Longitudinal section through a flagellum and basal body. Note the single transition plate and fragile cylinder (arrows). The numbers mark the corresponding figures in Figs 17–20.

Fig. 22. Transverse sections through a haptonema showing five microtubules and ER.

Fig. 23. Longitudinal sections through a haptonema.
times seemed to connect BB1 and BB2 directly (Fig. 37). Between the distal fibre and hf1, another fibre (hf3) connected the haptonema base and BB2 (Figs 26, 33, 34, 62, 70, 71). The free part of the haptonema included five microtubules encircled by the haptonematal endoplasmic reticulum (ER) (Figs 22, 23).

The most conspicuous microtubular root, root 1 (R1), originated from the region of BB1 adjacent to the haptonema base (Figs 24–26, 31–33, 47–49, 71) and was divided into two components from its proximal region. At the proximal end, R1 was composed of c. nine microtubules (MTs) closely aligned in a single row and overlain by a thin plate-like structure (Figs 25, 31, 39, 40, 43, 44, 70). Electron-dense material from BB1 attached to R1 MTs, especially to the most posterior microtubules of R1 (Figs 39–41, 43, 44, 64, 65, 70, 71). A teeth-like structure was situated between the electron-dense material and the plate-like structure (Figs 25, 39, 43, 70). Anteriorly c. five microtubules of R1 extended along the anterior portion of the cell (Figs 24, 41, 42, 46, 51, 56–58, 70) and bent posteriorly to join the posterior component of R1 (Figs 25–30, 51–53, 70). The posterior component of R1 (c. four MTs) split from the anterior component immediately and extended to the posterior part of the cell (Figs 26–30, 49–52, 56–58, 70). A fibrous root from the opposite side of the haptonema connected BB1 and the posterior component of R1, and an electron-dense mass was located between the fibrous root and R1 (Figs 25–27, 31–33, 42, 46, 49–51, 70, 71). As the posterior component of R1 extended posteriorly, it became associated with the anterior component of R1 and an extension of the PER (Figs 25–30, 50–53, 57, 58). The R1 and the
extension of the PER formed a cytoplasmic tongue in the lateral to posterior part of the cell (Figs 52, 54, 55, 58). Some sections suggested that MTs of R1 extend to the posterior end of the cell and support the hyaline tail of the cell (Fig. 55). No close relationship between R1 and mitochondrial profiles was observed around the flagellar apparatus. The root 2 (R2) comprised four MTs arranged in an arc-shaped structure at the proximal end (Figs 39, 40, 43–45, 64, 65). Each microtubule of R2 possessed a short appendage extending to the cavity of the R2 arc (Fig. 39). The R2 originated from the area between the two basal bodies and was closely related (but never connected directly) to the electron-dense material, which is probably an extension of the distal fibre (Figs 39, 43, 64). The arc shape of R2 collapses very soon and the root terminates (Figs 31, 41, 42). Root 3 (R3) originates from the side of BB2 away from the haptonema and at first comprises two MTs attached to BB2 by electron-dense material (Figs 63, 64, 66, 71). The R3 extended along the inner surface of the anterior chloroplast, and an extra microtubule was soon added (Figs 62, 67, 70). Near the proximal end of R3, an electron-dense lump was situated on BB2 (Figs 27, 33, 35, 36, 39, 43, 59, 60, 64, 70, 71). Part of this lump extended under R2, but never attached directly (Figs 39, 43, 64). One section showed an opaque connection between the electron-dense lump and BB1 (Fig. 60). Root 4 (R4) was single-stranded and emanated from the area between hf1 and hf3 (Figs 62, 63, 68). It did not join up with R3 (Figs 59–61). No crystalline root or its homologue associated with the microtubular roots was observed.

Molecular phylogenetic analyses

Phylogenetic analyses based on the 18S rDNA sequences resulted in a tree similar to that of Edvardsen et al. (2000) (Fig. 72). In the Prymnesiophyceae, clades A, B2, C, D, E (sensu Edvardsen et al. 2000) were supported well or moderately in every analysis. The MP analysis resulted in 18 most parsimonious trees (length = 1223, consistency index = 0.55, retention index = 0.73) (not shown). The monophyly of B1 clade sensu Edvardsen et al. (2000) (including Imantonia rotunda) was not recovered in the some MP trees. In the strict
Figs 47–58. Flagellar apparatus and cytoplasmic tongue of *C. rhomboideus*, TEM, thin sections. Scale bars = 0.5 μm.

Figs 47–53. Nonconsecutive serial longitudinal sections of the cell. The posterior component of R1 extends posteriorly (Fig. 49) and is associated with the fibrous root F1 and an extension of the PER (Figs 50, 51). After some distance, the anterior component of R1 bends posteriorly (Fig. 52) and joins the posterior component of R1. At the posterior end of the cell, R1 and PER form a cytoplasmic tongue (Fig. 52). Note the two chloroplasts situated asymmetrically (Figs 47, 48, 51–53).

Fig. 54. Oblique section of the cell showing the cytoplasmic tongue supported by microtubules (arrow).

Fig. 55. Longitudinal section of the posterior end of the cell showing the cytoplasmic tongue extended posteriorly.

Figs 56–58. Nonconsecutive serial oblique sections of the cell showing the cytoplasmic tongue supported by the posterior component of R1.

Consensus tree of the MP trees (not shown), the clade of unidentified haptophytes from the equatorial Pacific Ocean (OLI51033 and OLI51059; Moon–Van Der Staay *et al.* 2001) diverged at first, and the topology (grade B1 (clade B2 ((clade A, clade D), (clade E, clade C))))) was shown. The hierarchical likelihood ratio test selected the Tamura–Nei model (Tamura & Nei 1993) with an estimated proportion of invariant sites (0.5749) and rate heterogeneity among variable sites approximated as a discrete gamma distribution (0.6462) (TrN + I + G). Base frequencies were A = 0.2362, C = 0.2163, G = 0.2889, T = 0.2585; rate matrix was A–G = 1.4639, C–T = 3.6597, others = 1.0000. The NJ tree was similar to the MP trees except for the monophyly of the B1 clade (not shown). In the ML tree (Fig. 72), clade A + D diverged at first, and clades B1 (including OLI51033 and OLI51059) and B2 formed a clade. Bootstrap supports for the relationship between clades and monophyly of B1 clade were weak in every analysis. In all trees, *C. rhomboideus* formed a clade with the clade E including unidentified haptophytes (OLI26041 and OLI51050; Moon–Van Der Staay *et al.* 2001), and this clade was sister to the coccolithophorids (clade C) including the Isochrysidales and Coccolithales. However, bootstrap values
for these relationships were relatively low (> 77%). Phylogenetic analysis including partial 18S rDNA sequences reported by Diéz et al. (2001) also showed no close relatives of Chrysoculter (not shown).

The MP analysis based on the partial sequences of rbcL resulted in a single most parsimonious tree (length = 1686, consistency index = 0.4075, retention index = 0.4165) (not shown). The hierarchical likelihood ratio test selected the general time reversible model (Rodríguez et al. 1990) with an estimated proportion of invariant sites (0.5788) and rate heterogeneity among variable sites approximated as a discrete gamma distribution (2.7228) (GTR + I + G). Base frequencies were A = 0.2706, C = 0.1871, G = 0.2031, T = 0.3391; rate matrix was A–C = 0.3242, A–G = 6.4582, A–T = 8.8998, C–G = 0.8879, C–T = 7.2952, G–T = 1.0000. The ML tree is shown Fig. 73. All analyses generated similar trees, in which Chrysoculter was distantly related to Chrysochromulina species and formed a clade with the Coccolithales and Isochrysidales (bootstrap values less than 50%). In the rbcL trees, the monophyly of Chrysochromulina sensu stricto (C. alifera + C. parva), position of Imantonia, and relationship within the clade coccolithophorids + Chrysoculter were not settled.

**DISCUSSION**

Chrysoculter rhomboideus is apparently a member of the Haptophyta as it possesses (1) a haptonema, (2) two yellowish chloroplasts with periplastidal endoplasmic reticulum and no girdle lamella, and (3) a system of PER. Two nearly equal flagella with no appendages and organic scales with radial ribs covering the cell indicate that it is a member of the Prymnesiophyceae (sensu Edvardsen et al. 2000). Some ultrastructural features, such as splitting R1 (Beech & Wetherbee 1988; Birkhead & Pienaar 1995; Eikrem & Moestrup 1998), the arc-shaped R2 (Eikrem & Moestrup 1998), and the additional microtubule on R3 (Green & Hori 1994) also support inclusion of Chrysoculter in the Prymnesiophyceae. Flagellate Prymnesiophycean algae possessing a conspicuous haptonema and various types of organic scales but no coccoliths have been classified in the genus Chrysochromulina. Chrysoculter rhom-
boideus would be classified in the genus Chrysochromulina in this sense. However, the genus Chrysochromulina is very diverse morphologically (e.g. Green & Hori 1994; Birkhead & Pienaar 1995; Eikrem & Moestrup 1998; Eikrem & Edwardsen 1999). Recent molecular phylogenetic studies support the polyphyletic nature of the genus Chrysochromulina (Edwardsen et al. 2000; Fujiwara et al. 2001) and indicate that the genus should be recircumscribed and divided into several genera (Edwardsen et al. 2000). Because the type species, C. parva Lackey, has a saddle-shaped cell with a long haptonema, Chrysochromulina sensu stricto should be limited to the saddle-shaped species such as C. acantha Leadbeater & Manton, C. campanulifera Manton & Leadbeater, C. scutellum Eikrem & Moestrup, and C. throndsenii Eikrem. In the 18S rDNA tree, these species (C. parva has not been studied yet) form a robust clade distantly related to C. rhomboideus. Furthermore, we found no ultrastructural apomorphic feature common to the saddle-shaped Chrysochromulina and Chrysoculter. The alga therefore cannot be included in the genus Chrysochromulina. In the molecular phylogenetic trees, most of other ‘Chrysochromulina’ species (e.g. C. hirta Manton, C. kappa Parke & Manton, C. polylepis Manton & Parke, Chrysochromulina sp. in Birkhead & Pienaar 1995) form a clade (clade B1 sensu Edwardsen et al. 2000, see also Fujiwara et al. 2001) with Prymnesium Massart. Although the apomorphic character of this clade is uncertain, there is no significant ultrastructural similarity between Chrysochromulina and the algae in clade B1. The 18S rDNA analysis also places C. rhomboideus distantly from the clade B1 and from any other Prymnesiophyceae analysed. Based on this evidence, we propose the new genus, Chrysoculter. Most species of the genus Chrysochromulina are characterized by their scale morphology, and some species were described based on only the scales. The large rhomboidal scale of C. rhomboideus is a very distinctive character, and there is no previous report of such a unique scale. The asymmetrical knife-shaped cell is also a characteristic feature that has not been reported previously.

In addition to the characters mentioned above (scale and cell morphology, gene sequences), certain ultrastructural characters also support the isolated phylogenetic position of Chrysoculter in the Prymnesiophyceae. A single transitional plate in the transition region of the flagellum is one of the most distinctive characters of Chrysoculter. Most members of the Prymnesiophyceae have two, a distal and proximal, transitional plates (Moestrup 1982; Preissig 1989; Green & Hori 1994). Coccolithophorid species have been reported to have a single transitional plate with or without the helical bands (Beech & Wetherbee 1988; Hori & Green 1991; Kawachi & Inouye 1994; Sym & Kawachi 2000). Because of its position in the flagellum and its morphology (i.e. a perforated septum with an axosome), the single transitional plate of the coccolithophors is probably homologous with the proximal transitional plate of other Prymnesiophyceae. On the other hand, the single transitional plate of Chrysoculter is apparently homologous with the distal transitional plate of other Prymnesiophyceae. The loss of the proximal transitional plate is unique among members of the Prymnesiophyceae, and it is an autapomorphic characteristic of Chrysoculter. Although the fragile cylindrical structure found in the transition region of Chrysoculter has not been reported previously, a similar struc-

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Fig. 69. Diagrammatic reconstruction of the rhomboidal overlayer and elliptical underlayer scales of C. rhomboideus. Scale bar = 0.5 μm.

Figs 70, 71. Diagrammatic reconstructions of the flagellar apparatus of C. rhomboideus. Viewed from the lateral (Fig. 70) and anterior (Fig. 71) side of the cell. Not to scale.
Fig. 72. Phylogenetic tree based on 18S rDNA sequences using the maximum likelihood method ($-\ln L = 9124.012$). The tree is rooted on the branch between the Pavlovophyceae and Prymnesiophyceae. Asterisks refer to the names of the clades in Edvardsen et al. (2000). Numbers around the internodes indicate bootstrap values (> 50%) in the MP, NJ, and ML analyses (1000, 1000, 100 replications, respectively).

The structure is found in *Prymnesium nemamethecum* Pienaar & Birkhead (Birkhead & Pienaar 1994a), *Chrysochromulina brevisilium* Parke & Manton (Birkhead & Pienaar 1994b), and *Phaeocystis globosa* Scherffel (figs 26, 27 in Parke et al. 1971). Interestingly, the position of this structure corresponds to the place where flagellar shedding takes place in the Prymnesiophyceae (Eikrem & Moestrup 1998) and to the place where the stellate structure is situated in the Viridiplantae (Sanders & Salisbury 1989). This structure may be more common, although it is difficult to detect because of its tenuous nature. The teeth-like structure on the electron-dense plate overlying the R1 microtubular sheet is another distinctive feature of *Chrysoculter*. It has not been reported in other haptophytes. However, the thinness of the structure (it appears in only one or two sections) could cause it to be overlooked in other species.

Billard (1994) proposed that the haptophyte life cycle includes a haploid cell with dimorphic scale faces and a diploid cell with monomorphic scale faces. Recent findings on *Chrysochromulina polypleta* and *Prymnesium parvum* N. Carter reinforce this idea (Edvardsen & Paasche 1992; Edvardsen et al. 1996; Edvardsen & Medlin 1998; Larsen 1999), and morphologically distinct haptophytes sometimes represent different generations of the same species (Parke & Adams 1960; Gayral & Fresnel 1983a; Green et al. 1996; Noël et al. 2004). Thus, *C. rhomboideus* may represent a generation of a known species. There is superficial similarity between the organic scales of *C. rhomboideus* and the coccoliths of some heterococcolithophorids such as *Anoplotosolenia* Deflandre and *Calcosolenia* Gran. However, the monomorphic scale face of *C. rhomboideus* indicates that this is a diploid generation. This is the same ploidy stage as a heterococcolithophorid, and it is therefore unlikely that *Chrysoculter* is a stage in the life cycle of a heterococcolithophorid. Neither the production of the coc-
coliths nor morphologically different phases were observed in this study. We therefore consider *C. rhomboideus* to be a distinct, new species.

Interestingly, some ultrastructural characters suggest phylogenetic affinity between *Chrysoculter* and coccolithophorids. The fibrous root connecting BB1 and the posterior component of R1 is a notable feature of *Chrysoculter*. This structure, called F1 by Roberts & Mills (1992), was described also from the Coccolithales (Inouye & Chihara 1983; Inouye & Pienaar 1985; Beech & Wetherbee 1988; Roberts & Mills 1992; Kawachi & Inouye 1994) and the Isochrysidales (Hori & Green 1991). Although Green & Hori (1990, 1994) noted the presence of F1 in *P. parvum* (as *P. patellifera* Green, Hibberd & Pienaar), the published micrograph (fig. 6h in Green & Hori 1990) shows that this fibrous structure originates from the side of BB1 facing the microtubular sheet of R1. In the coccolithophorids, F1 emerges from the side of BB1 away from the haptonema. So, the true F1 is probably a synapomorphic character of the coccolithophorids (Coccolithales and Isochrysidales). The occurrence of F1 in *Chrysoculter*, although it is less developed, suggests a close relationship to the coccolithophorids. Although the bootstrap values were low, this relationship was also shown in the molecular analyses. The evidence suggests that *Chrysoculter* is derived from the common ancestor of the coccolithophorids before the ability to form cololiths was developed. Other ultrastructural characters also support this hypothesis. Thus both *Chrysoculter* and some members of the Coccolithales and Isochrysidales have an electron-dense plate located next to the R1 microtubular sheet on the side facing BB1 (Inouye & Pienaar 1985, 1988; Beech & Wetherbee 1988; Hori & Green 1991). Other prymnesiophyceans have no such a structure. However, it should be noted that some prymnesialean algae possess a flange-like structure on the R1 microtubule, and longitudinal sections of R1 sometimes show structure of similar appearance to the electron-dense plate in the coccolithophorids (Green & Hori 1990; Birkhead & Pienaar 1994a, 1995; Eikrem & Moestrup 1998). The number of microtubules in R2 is usually one to three in prymnesiophyceans except in the Coccolithales (Green & Hori 1994; Eikrem & Moestrup 1998). In most coccolithophorids studied R2 comprises four microtubules as in *Chrysoculter* (Inouye & Pienaar 1984, 1985, 1988; Kawachi & Inouye 1994; Sym & Kawachi 2000). Green & Hori (1990) reported appendages on the R2 microtubules of *P. parvum*. However, the appendages of *P. parvum* and *Chrysoculter* are situated on opposite sides. Interestingly, published micrographs of some coccolithophorids suggest that appendages are present on R2 as in *Chrysoculter* (fig. 21 in Inouye & Chihara 1983, fig. 23 in Inouye & Pienaar 1984, fig. 18 in Inouye & Pienaar 1985). The numbers of microtubules in the free part of the haptonema vary in the Haptophysya. Most prymnesialean, phaeocystidalean, and pavlovophycean species investigated have six or seven microtubules in the haptonema (reviewed by Edvardsen et al. 2000). No more than five microtubules have been reported in some coccolithophorids (Inouye & Chihara 1983; Hori & Green 1991; Roberts & Mills 1992; Kawachi & Inouye 1994; Sym & Kawachi 2000), and in this respect *Chrysoculter* resembles the coccolithophorids. However, some species of the Coccolithales also have haptonema with six or seven microtubules (Inouye &
Pienaar 1985, 1988). In conclusion, the fibrous root (F1), the electron-dense plate on the R1 microtubular sheet, R2 of four microtubules with appendages, and the low number of haptonema microtubules suggest a phylogenetic relationship between Chrysocluter and coccolithophorids. Some (or all) of these characters are synapomorphies of the clade that includes Chrysocluter and coccolithophorids.

A compound structure composed of R1 microtubules and PER, the cytoplasmic tongue, has been considered a characteristic feature of the coccolithophorids (Gayral & Fresnel 1983a; Beech & Wetherbee 1988). This structure is also present in Chrysocluter. However, the cytoplasmic tongue has now been reported from some members of the B1 clade, such as P. nemamethecum (Birkhead & Pienaar 1994a; Pienaar & Birkhead 1994) and ‘eyelash’ Chrysochromulina (Birkhead & Pienaar 1995). This structure may be a synapomorphy of the lineage including coccolithophorids, Chrysocluter, and the B1 clade, although some species have subsequently lost it again. Another character shared with the coccolithophorids and B1 clade is the compound R1. An R1 with accessory bundles of microtubules has been reported from some species of Prymnesium (Birkhead & Pienaar 1994a), Pleurochrysis (Gayral & Fresnel 1983b), Chrysochromulina (except Chrysochromulina sensu stricto; Birkhead & Pienaar 1995), and many coccolithophorids (e.g. Gayral & Fresnel 1983a; Inouye & Chihira 1983; Inouye & Pienaar 1984, 1985; Beech & Wetherbee 1988; Roberts & Mills 1992; Kawachi & Inouye 1994), and it may be another apomorphy shared between the coccolithophorids and the B1 clade. Chrysocluter lacks a compound R1, but some coccolithophorids and the B1 clade also have very reduced or no compound root (e.g. Green & Hor 1986, 1990; Inouye & Pienaar 1988; Sym & Kawachi 2000). The frequent loss of the compound root has occurred also in the Prymnesiophyceae. A close relationship between the cell cycle and the development of the compound R1 also makes it difficult to judge the phylogenetic relevance of this characteristic (Beech et al. 1988; Birkhead & Pienaar 1995).

The phylogenetic considerations based on ultrastructural and molecular evidence have induced us to propose a new monotypic family, Chrysocluteraceae, for C. rhomboideus. A new order may also have to be created for this alga, but we prefer to postpone the erection of a new order until the relationships and taxonomic relevance of the Prymnesiophyceae orders have been clarified better.

Prymnesiophyceae, incertae sedis

Chrysocluteraceae Nakayama, Yoshida, Noël, Kawachi & Inouye fam. nov.

Cellula natans cum flagellis duobus et haptonemate. Choroplasti duo, luteoli, parietales, pyrenoide thalakoidibus bini perducta. Corpus cellularae squamis typorum duorum vestita. R1 radicis fibroseae (F1), structurae tabularis exilis et structurae dentatae. Regio tarm-sitiva tabula transversa terminali una.

Swimming cell with two equal flagella and a haptonema. Chloroplasts two, parietal, with pyrenoid traversed by a pair of thylakoids. Cell body covered with scales of two types. R1 with fibrous root (F1), thin plate, and teeth-like structure. Transitional region includes a terminal transitional plate.

TYPE GENUS: Chrysocluter Nakayama, Yoshida, Noël, Kawachi & Inouye gen. nov.

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