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Morphology and genetic affinities of a novel *Chattonella* isolate (Raphidophyceae) isolated from Iran's south coast (Oman Sea)

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Abstract: The morphology and genetic affinity of a novel raphidophyte belonging to the genus *Chattonella* Biecheler is described for the first time from the Oman Sea along the south-east coast of Iran. While morphologically very similar to *Chattonella subsalsa* Biecheler, the Iranian isolates contain a distinct red eyespot. A comparison of LSU-rDNA and rDNA-ITS show that the Iranian isolate is genetically distinct from other *Chattonella subsalsa* strains isolated from across a wide global range and indicates that the Iranian isolates represent a distinct species related to *Chattonella subsalsa*.

Key words: *Chattonella*, phylogeny, morphology, raphidophyte, cyst, LSU-rDNA, rDNA-ITS

1. Introduction

The genus *Chattonella* Biecheler, belonging to the class Raphidophyceae, consists of small golden-brown flagellates. Taxonomy of the class is based mainly on cell shape and size and the ultrastructure of the chloroplasts, mucocysts, trichocysts, and ejectosomes (Marshall et al., 2002). However, the existing taxonomy is still controversial (Hosini-Tanabe et al., 2006). Previously, 7 species in the genus *Chattonella* have been described: *C. subsalsa* Biecheler, *C. antiqua* (Hada) Ono, *C. marina* (Subrahmanyan) Hara & Chihara, *C. minima* Hara & Chihara, *C. ovata* Hara & Chihara, *C. globosa* Hara & Chihara, and *C. verruculosa* Hara & Chihara (Hara et al., 1994; Hallegraeff and Hara, 1995). Recently, 2 *Chattonella* species (*C. globosa* and *C. verruculosa*) have been separated from the class Raphidophyceae based on molecular analysis. They now belong to the class Dictyochophyceae and are currently regarded as a taxonomic synonym of *Vicicitus globosus* (Hara & Chihara) Chang and *Pseudochattonella verruculosa* (Hara & Chihara) Hosoi-Tanabe, Honda, Fukaya, Inagaki & Sako (Edvardsen et al., 2007; Hosoi-Tanabe et al., 2007; Takano et al., 2007; Cheng et al., 2012). Therefore, the genus *Chattonella* has 5 species. Demura et al. (2009) suggested a taxonomic revision of *C. marina*, *C. ovate*, and *C. antiqua* based on morphological characteristics and genetic diversity and considered these species a variety of *C. marina* and offered a new status: *C. marina* var. *ovata* (Hara & Chihara) Demura & Kawachi and *C. marina* var. *antiqua* (Hada) Demura & Kawachi.

Wild resting cysts from sediment have been reported in some species of the genus including *C. marina* var. *antiqua*, *C. marina* (Yamaguchi and Imai, 1994), *C. marina* var. *ovata* (Yamaguchi et al., 2008), and *C. subsalsa* (Steidinger and Penta, 1999). These cysts may have a role in bloom initiation in coastal areas (Peperzak, 2001; Blanco et al., 2009; Cucchiari et al., 2010; Imai and Yamaguchi, 2012).

Noxious blooms of *C. marina* var. *antiqua*, *C. marina*, *C. subsalsa, Fibrocapsa japonica* Toriumi & Takano, and *Heterosigma akashiwo* (Hada) Hada ex Hara & Chihara have often been associated with mortalities of both cultured and wild fish and shellfish (Oda et al., 1997; Hard et al., 2000; Imai et al., 2001; Landsberg, 2002; Hiroishi et al., 2005; Matsubara et al., 2007; Shen et al., 2011). *C. marina* var. *ovata* has also been reported to form a harmful bloom (Imai and Yamaguchi, 2012). In the north part of the Oman and Arabian seas phytoplankton blooms occur during and after the north-east monsoon every year (Attaran-Fariman and Javid, 2013; Latif et al., 2013). In autumn 2010 a massive bloom of *Chattonella* sp. occurred along the south-east coast of Iran (Pasabandar, Bris) in the north part of the Oman sea, causing massive mortality of fish and shellfish and 4 green turtle species (Nabavi, 2010). Identification and characterisation of Iranian *Chattonella* is an important step towards understanding

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the potential harmful consequences of future blooms in the area. Identification of raphidophytes species based solely on morphology is problematic due to their fragile nature and the pleomorphic morphology of some species (Aizdaicher, 1993; Tyrrell et al., 2001; Demura et al., 2009), leading to difficulties distinguishing one species from another, especially within the varieties of *C. marina* (Bowers et al., 2006; Demura et al., 2009). The fragile cells of this group are also difficult to identify by transmission electron microscopy after fixation due to a range of fixation artefacts (Throndsen, 1993; Marshall et al., 2002).

Utilisation of internal transcribed spacer sequence comparison has been well established in plants and marine algae (Hosoi-Tanabe et al., 2007; Terzioğlu et al., 2012; Dündar et al., 2013). rDNA sequencing has been used to examine relationships among marine microalgae including Raphidophyceae taxa, both at the population and species level (Connell, 2000, 2002; Hosoi-Tanabe et al., 2007). Several studies of raphidophytes have successfully used the rDNA-ITS region to examine raphidophyte relationships, and there is relatively broad coverage of rDNA-ITS sequences available in public databases. These studies have shown that, while there is a variation among different species, there is little or no rDNA-ITS sequence variation within species, even among isolates from across the globe (Kooistra et al., 2001; Bowers et al., 2006; Edvardsen et al., 2007). Therefore, this region is a potentially useful means to distinguish distinct species of raphidophytes. In this study we describe motile cells of a novel *Chattonella* isolate from the south coast of Iran (Oman Sea) by light and scanning electron microscopy and phylogenetic analyses carried out based on LSU-rDNA and rDNA-ITS sequences.

2. Materials and methods

2.1. Cell culture and microscopy

Single flagellated raphidophyte cells were isolated from incubated mixed sediment collected from the south-east coast of Iran using a micropipette under a Leica stereomicroscope. Isolated cells were placed into 55-mm polystyrene petri dishes containing 15 mL of GSe medium and incubated at 26 °C ± 0.5 °C under cool white fluorescent light (70–90 µmol photon $m^{-1}s^{-1}$) with a 12 h light/12 h dark cycle. Successfully established cultures were subsequently transferred to 100-mL Erlenmeyer flasks containing 50 mL of GSe and sub-cultured every 3 weeks under the conditions described above.

Encystment in cultures was examined by transfer to nitrate/phosphate-deficient GSe medium and incubation under the conditions above (Yoshimatsu, 1987; Imai and Itakura, 1999). Cells were photographed with an Olympus BH-2 microscope equipped with a Leica DC300F digital imaging system and a Zeiss Axioplan 2-Plus microscope (Zeiss, Gottingen, Germany) equipped with a Zeiss AxioCam HR digital camera using bright field and differential interference contrast illumination. For SEM, 10 mL of mid-logarithmic growth-phase cultures were concentrated by centrifuge, fixed with 4% osmium tetroxide (OsO_4), and adhered to polylysine-coated coverslips (Marchant and Thomas, 1983). Coverslips were then critical-point dried via liquid CO_2, mounted on SEM stubs, sputter coated with gold, and examined with a JEOL JSM-840 scanning electron microscope.

2.2. DNA extraction, PCR, and DNA sequencing

DNA was extracted by a phenol:chloroform:isoamyl alcohol gentle-lysis method (Bolch et al., 1998), and the internal transcribed spacer 1 (ITS1), 5.8S rRNA gene, and internal transcribed spacer2 (ITS2) were amplified. For amplification of the rDNA-ITS, primers ITSA and ITSB (Adachi et al., 1994) were used, and for partial large subunit (LSU) rRNA gene, D1R-F and 1483-R primers (Daugbjerg et al., 2000) were used. Amplified PCR products were purified using Montage PCR clean-up columns (Millipore, USA), and 60 ng of purified product was used as template in DNA sequencing reactions. PCR products were sequenced using a Beckman-Coulter Dye Terminator Sequencing Kit, according to standard protocols. Sequence base-calling errors were corrected by manual inspection of electropherograms using the software program BioEdit (Hall, 1999). DNA sequence data from Iranian *Chattonella* sp. isolate CHPI36 was aligned to comparable nucleotide sequences of other raphidophytes available from GenBank using Clustal-X software v.1.83 (Jeanmougin et al., 1998), and alignments were improved by manual inspection. Details of the taxa included in the analyses are summarised in Tables 1 and 2.

2.3. Alignment and phylogenetic analyses

Two sequence alignments were used to infer relationships among *Chattonella* spp. and the phylogenetic position of the *Chattonella* sp. CHPI36. The rDNA-ITS dataset contained 27 taxa and 730 characters in the sequence alignment. *Olisthodiscus luteus* Carter, 1937 was used as outgroup for the analysis. The large subunit rDNA-LSU rRNA gene dataset contained 16 taxa and 1361 characters. The diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin was used as an outgroup for the analysis. The LSU-rDNA sequences *C. subsalsa* CCMP217 (AF409129) and *Chattonella* sp. (CHPI36) were approximately 1360 bp in length, whereas all other sequences corresponded to approximately the first 680–700 bp of the alignment. Analyses were repeated with all sequences truncated to the first 700 bp of the alignment, and any changes in branching order were noted. PAUP* version 4.0b10 for Macintosh (PPC) was used (Swofford, 2002) for all phylogenetic analysis of rDNA-ITS region and partial LSU-rDNA. Phylogenetic structure was examined and tested by the randomisation tests and probability tables

Table 1. Details of species used in the phylogenetic analysis of partial LSU-rDNA sequences.

Species	GenBank accession no.	Strain code	Geographical locations
***Chattonella* sp.**	**JF896100**	**CHPI36**	Iran
Chattonella subsalsa	AF409126	CCMP217	Gulf of Mexico
Chattonella subsalsa	AF210736	CCMP217	Gulf of Mexico
Chattonella marina	AY704162		Hong Kong
Chattonella marina	AF210739	CCMP-217	Gulf of Mexico
Heterosigma akashiwo	AY704161		Hong Kong
Heterosigma akashiwo	AF086948	CCMP-452	Long Island Sound, USA
Heterosigma akashiwo	AF042820		Masan Bay, Korea
Heterosigma akashiwo	AF210741	CAWR05	
Vacuolaria virescens	AF210742	LB2236	
Vacuolaria virescens	AF409125	SAG1195.1	Wirral, Cheshire, England
Chattonella ovata	AF210738	NIES-603	Seto Inland Sea, Japan
Chattonella ovata	AY704163		Hong Kong
Chattonella antiqua	AF210737	NIES-1	Seto Inland Sea, Japan
Olisthodiscus luteus	AF210743	NIES-15	Seto Inland Sea, Japan
Cylindrotheca closterium	AF417666	K-520	

Table 2. List of species included in the phylogenetic analysis of ITS region of rDNA.

Species	GenBank accession no.	Strain code	Geographical locations
***Chattonella* sp.**	**JF896101**	**CHPI36**	Iran
Chattonella subsalsa	AF409126	CCMP217	Gulf of Mexico
Chattonella subsalsa	AY858871	C. Tomas	Texas
Chattonella subsalsa	AY858870	C. Tomas	Singapore
Chattonella subsalsa	AY858869	C. Tomas	Sardinia
Chattonella subsalsa	AY858867	C. Tomas	Delaware
Chattonella subsalsa	AY858866	C. Tomas	California
Chattonella subsalsa	AY858864	C. Tomas	Japan
Chattonella subsalsa	AY858868	C. Tomas	North Carolina
Chattonella marina	AY858862	C. Tomas	North Carolina
Chattonella marina	AY858861	C. Tomas	Maryland
Chattonella marina	AY858860	C. Tomas	Japan
Chattonella marina	AY865604	CCMP 2049	Kagoshima Bay, Japan
Chattonella marina	AY704165		Hong Kong
Chattonella marina	AF137074	NIES 3	Osaka Bay, Japan
Heterosigma akashiwo	AY858874	CCMP 1680	Sandy Hook Bay, USA
Heterosigma akashiwo	AY858875	CCMP 1912	Kalaloch, USA
Vacuolaria virescens	AF409125	SAG1195.1	Wirral, Cheshire, England
Chattonella antiqua	AY858858	C. Tomas	Japan
Chattonella antiqua	AY858857	CCMP 2052	Mikawa Bay, Japan
Chattonella antiqua	AY858856	CCMP 2050	Seto Inland Sea, Japan
Chattonella antiqua	AF136761	NIES 1	Seto Inland Sea, Japan
Chattonella ovata	AY858872	CCMP 216	Japan
Chattonella ovata	AY858863	C. Tomas	Japan
Chattonella ovata	AY704166		Hong Kong
Fibrocapsa japonica	AF112991	LB 2162	
Olisthodiscus luteus	AF112992	NIES-15	Seto Inland Sea, Japan

of critical values of g_1 (Hillis and Huelsenbeck, 1992). Neighbour-joining (NJ) trees were constructed with the minimum evolutionary (ME) model using logdet distances (ME-LgD) and the mean distance metric (Bolch and Campbell, 2004). Maximum parsimony (MP) analyses used the branch and bound search algorithm to find the most parsimonious trees. All characters were equally weighted and gaps were treated as missing data; multistate characters were interpreted as uncertainty. To estimate the reliability of the MP trees and the NJ tree, bootstrap analyses were carried out utilising 1000 replicates of the full heuristic search algorithm.

3. Results

3.1. Morphology

Cells of *Chattonella* sp. are 24–43 µm long and 17–23 µm wide, slightly compressed, and tear-shaped to lanceolate in lateral view (Figure 1). The large oval-shaped nucleus

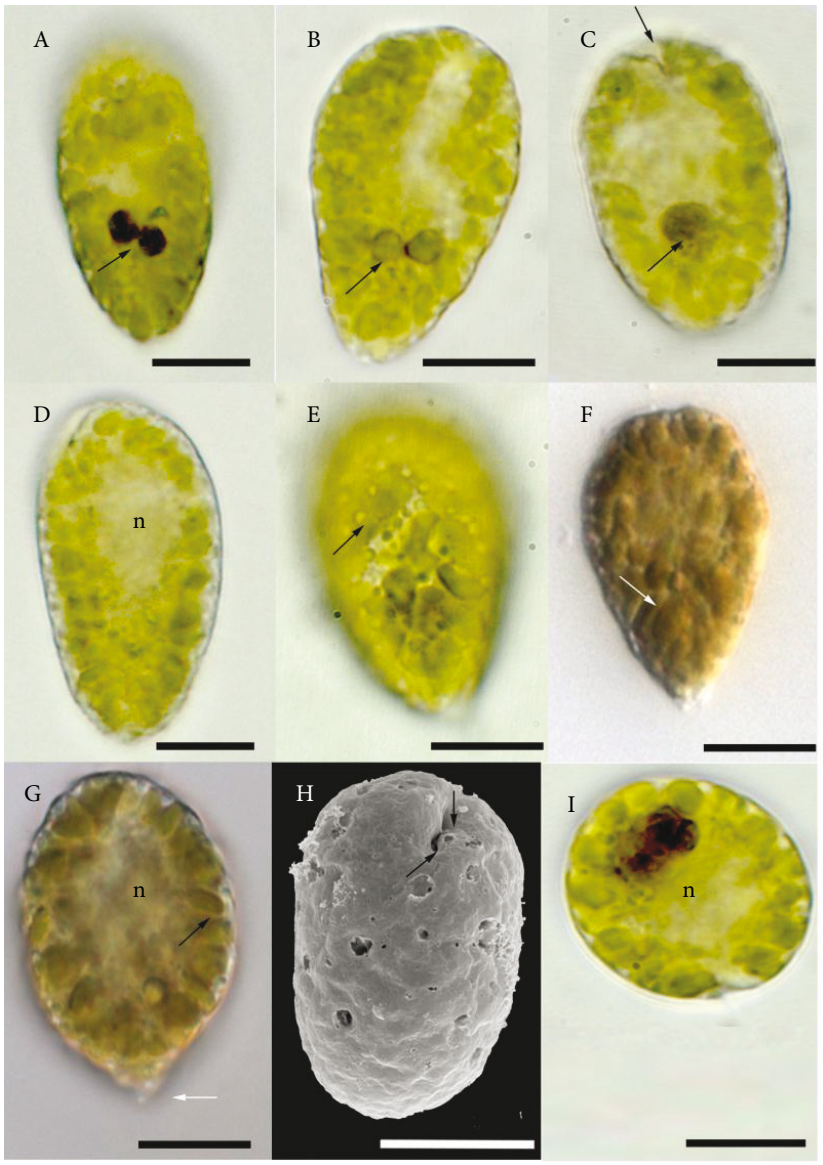

Figure 1. Motile cells of *Chattonella* sp. CHPI36 germinated from mixed incubated sediment. A- cell with 2 dark brown eyespots; B- same cell as Figure A (note the eyespot colour changes to a lighter colour during observation); C- cell in lateral view, showing 1 visible eyespot (bottom arrow). Note anterior depression (top arrow); D- cell showing a large oval nucleus; E- mucocysts on the surface of the cell (arrow); F- tear-shaped cell showing the densely-packed chloroplasts (arrow); G- cell showing posterior tail (bottom arrow). Note the nucleus; H- SEM. Anterior depression of the cell and 2 flagella grooves (arrows); I- polar view of cell showing the nucleus (n) and eyespot. All scale bars = 10 µm, except Figure G = 5 µm.

extends from beneath the anterior depression of the cell toward the cell centre (Figure 1). Numerous mucocysts are present on the cell surface. The numerous, densely packed chloroplasts are green, peripherally placed, and ellipsoid-to-ribbon shaped (Figure 1). Some cells show a posterior projection. Two sub-equal flagella project from 2 flagella grooves that arise from a clearly defined anterior depression (Figure 1). The cells are golden yellow in colour under bright field illumination. Two dark brown-red eyespots are present in the posterior part of the cell (Figure 1). Examination of live cells under the light microscope causes cells to quickly lose motility, and the eyespot colour fades to a lighter brown. The eyespot is not visible in lateral view (Figure 1).

Presumed encysted cells were observed only in senescent, late stationary-phase cultures approximately 6 months after transfer into nutrient replete medium. Cysts were not produced in cultures transferred into nutrient-deficient media. When grown to senescence in nutrient-replete medium, non-motile vegetative cells ranged from 9 to 12 µm in diameter (Figure 2). Putative resting cysts were pale-brown, spherical, and ranged from 17–21 µm in diameter and contained a brown accumulation body (Figure 2).

3.2. Phylogenetic analyses

Based on partial LSU-rDNA data, both NJ (figures not shown) and MP analyses generated a tree with the same primary branching order (Figure 3). All analyses, including raphidophyte taxa, formed a monophyletic group with 100% bootstrap support. In both trees, *O. luteus* branched first followed by *Vacuolaria* Cienkowski, 1870 and *Chattonella*. The genus *Chattonella* was monophyletic in both trees. Within *Chattonella*, 2 monophyletic groups were formed, 1 comprising all strains of *C. marina*, *C. ovata*, and *C. antiqua* with 100% bootstrap support and near identical sequences (referred to as *C. marina* group hereafter) and differing by only 1–2 nucleotide substitutions. The second group included all *C. subsalsa* strains. *Chattonella* sp. CHPI36 clustered with *C. subsalsa* but was clearly distinct, differing by 17 base-pairs over the 1372 bp of LSU-rDNA compared.

The trees derived from analysis of rDNA-ITS sequences supported the analyses of the LSU-rDNA data. Both NJ and MP analyses of the rDNA-ITS resulted in trees with similar branch order (Figures 4 and 5). The MP analysis resulted in 2 most parsimonious trees with identical branching order; therefore, only 1 is presented in Figure 5. In this tree the branch orders are *F. japonica*, *V. virescens* (freshwater species), and *H. akashiwo*, followed by *Chattonella* spp., respectively. Within *Chattonella*, 2 groups were evident. The first group comprised the *C. marina* group, differing by 1–4 nucleotides across the rDNA-ITS. The second group contained all *C. subsalsa* strains and *Chattonella* sp. CHPI36, which was clearly distinct from *C. subsalsa*, differing by 12 base pairs over the ITS region.

4. Discussion

4.1. Morphology of *Chattonella* sp. CHPI36

Due to the morphological similarity between *C. subsalsa* and *C. marina,* identification based on light microscopy is often difficult (Figure 6). *C. subsalsa* is the type species for the genus and morphologically related to *C. marina.* Hara and Chihara (1982) separated these 2 species based on 2 ultrastructure characteristics: the presence of oboe-shaped mucocysts in *C. subsalsa* and the relationship between the thylakoid membranes and chloroplast pyrenoid matrix. In *C. subsalsa*, the thylakoids do not penetrate the pyrenoid, but in *C. marina* the thylakoids are in the pyrenoid matrix, and the cells have distinctive mucocysts. However, there are a number of unresolved questions regarding the type

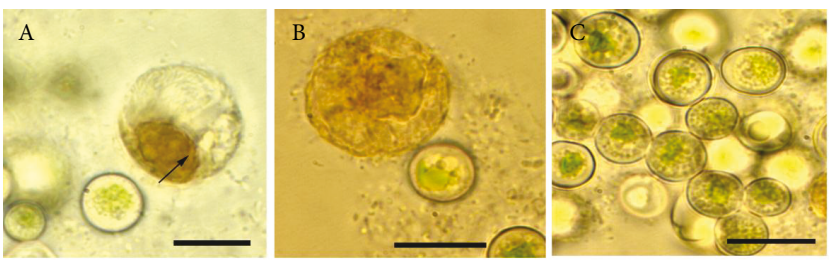

Figure 2. *Chattonella* sp. putative resting cysts and non-motile cells. A- spherical cyst showing large accumulation body (arrow). Note non-motile cells produced in old cultures; B- cysts of *Chattonella* sp. surrounded with a mucilaginous layer; C- non-motile spherical cells produced in nutrient-depleted medium. All scale bars = 10 µm.

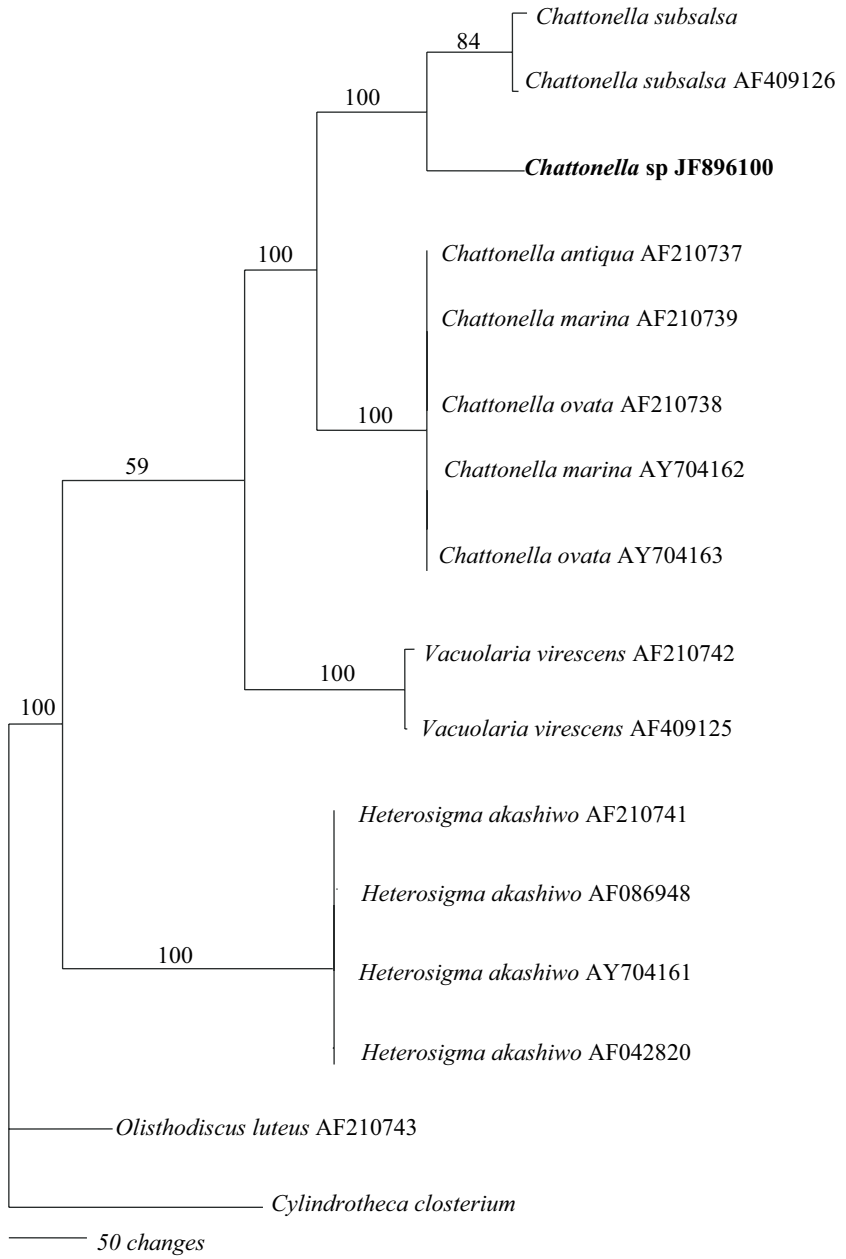

Figure 3. Phylogenetic relationship among *Chattonella*-like species inferred from phylogenetic of partial LSU of rDNA gene. Most parsimonious tree obtained using branch and bound search. Bootstrap values from 100 replicates are shown above the nodes. *Cylindrotheca closterium* is the outgroup taxon.

material of *C. subsalsa,* and it has been suggested that re-examination of cells from the type locality (from India) is necessary to clarify the identity and circumscription of both *C. subsalsa* and *C. marina* (Imai and Yamaguchi, 2012).

In the present study, the cell shape of *Chattonella* sp. CHPI36 resembles *C. marina* more than *C. subsalsa*, sometimes possessing a posterior tail similar to *C. marina*. Comparing the Iranian isolate with *C. subsalsa* CCMP217 (Figure 7), the 2 strains have quite different cell outlines; however, cell shape is known to be pleomorphic in most *Chattonella* Raphidophytes and varies with the age of the cells (Hara and Chihara, 1987; Aizdaicher, 1993; Tomas, 1998; Demura et al., 2009). In older cultures, the posterior tail of *C. marina* cells become narrower and longer, similar to those of mature cells of *C. antiqua* (Band-Schmidt et al., 2004; Hosoi-Tanabe et al., 2006), indicating that cell morphology alone is an unreliable taxonomic character

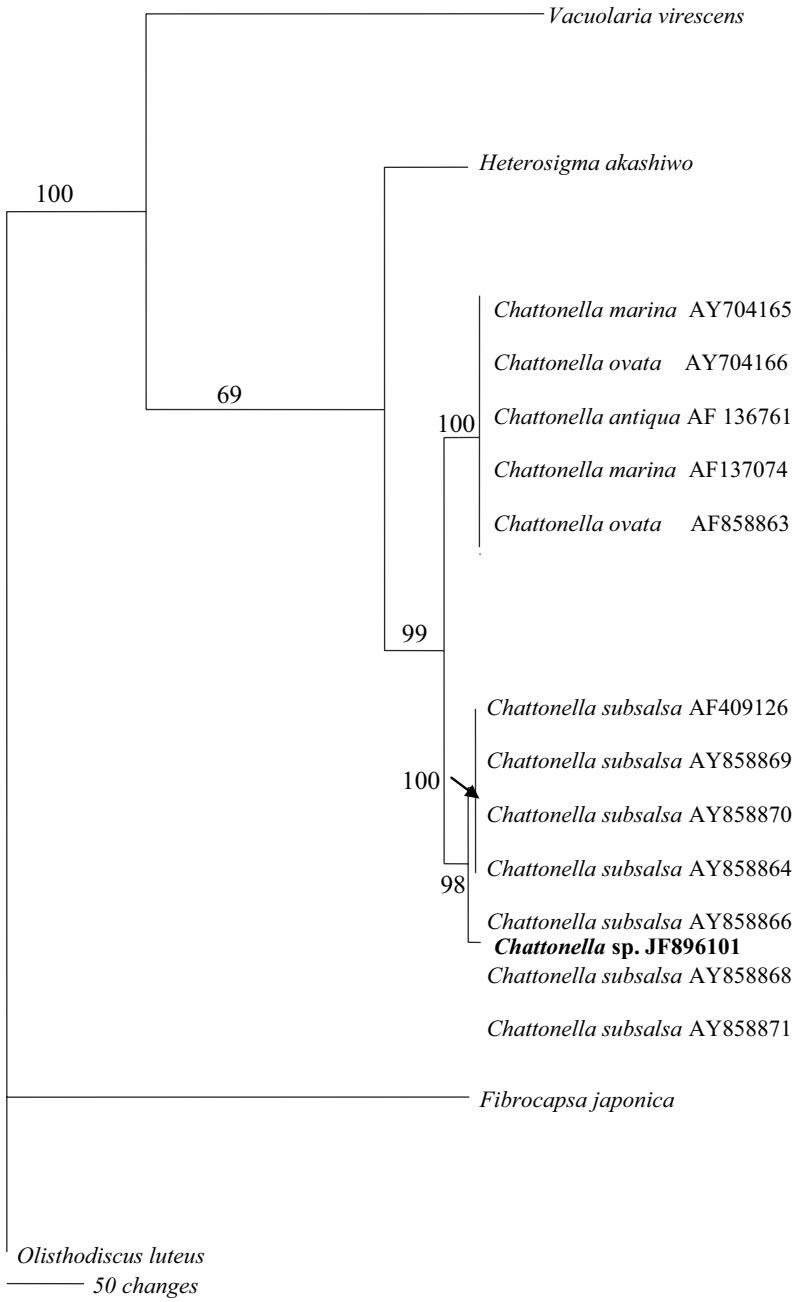

Figure 4. Molecular analysis of ITS regions of rDNA gene of *Chattonella* sp. CHPI36. Most parsimonious tree obtained using branch and bound search. Values above nodes represent bootstrap values (100 replicates). *O. luteus* is the outgroup taxon.

for field samples containing multiple species with cells of different ages.

Chattonella sp. CHPI36 clearly differs from *C. antiqua* due to its smaller size (*C. antiqua*, 70–130 µm long), lack of a long posterior tail, and presence of mucocysts in the cell surface (Table 3). Strain CHPI36 more closely resembles *C. subsalsa* in many features. Both possess a tear-shaped nucleus that is centrally positioned, both have oval-shaped chloroplasts that are peripherally placed, both possess many mucocysts on the cell surface, and neither possesses contractile vacuoles. The anterior depression of strain CHPI36 where flagella arise is deep and clear; however, this feature is not clearly documented for *C. subsalsa* (Hallegraeff and Hara, 2003).

There is little published information describing the resting cysts of *C. subsalsa*, and those that refer to the

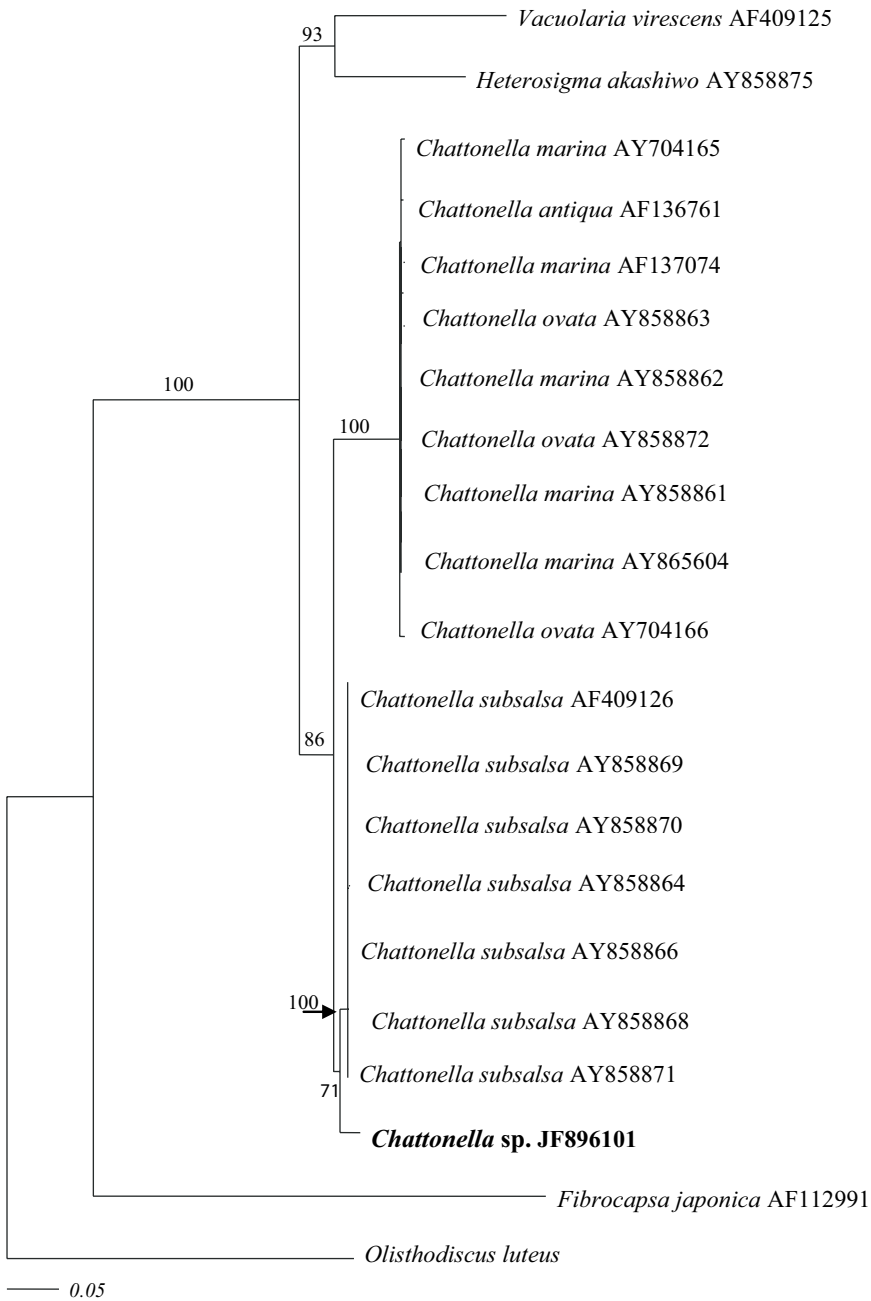

Figure 5. Phylogenetic analysis of the ITS-rDNA of Iranian *Chattonella* sp. isolate CHPI36 with other *Chattonella*-like species. The tree was constructed by neighbour-joining from logdet genetic distance (ME-LgD analysis). Numbers above branches represent bootstrap support values (100 replicates). *O. luteus* was used as an outgroup taxon.

production of a resting stage do not give morphological descriptions (Steidinger and Penta, 1999). The putative cyst stages described here for strain CHPI36 are smaller than the yellow-greenish-to-brownish, hemispherical cysts described for *C. marina* (20–30 µm diameter; Imai, 1989) and *C. ovata* (30 µm diameter; Yamaguchi et al., 2008). The large dark brown accumulation body in cysts of Iranian isolate CHPI36 also differs from the several dark brown spots or black material in the cysts of *C. marina* (Imai, 1989). The appearance of small cells (before encystment) in N-limited medium has been reported for *C. marina* (Imai et al., 1998), and Band-Schmidt et al. (2004) noticed that the morphology of *C. marina* is affected by the age of the culture. In older and N-limited cultures, cells become

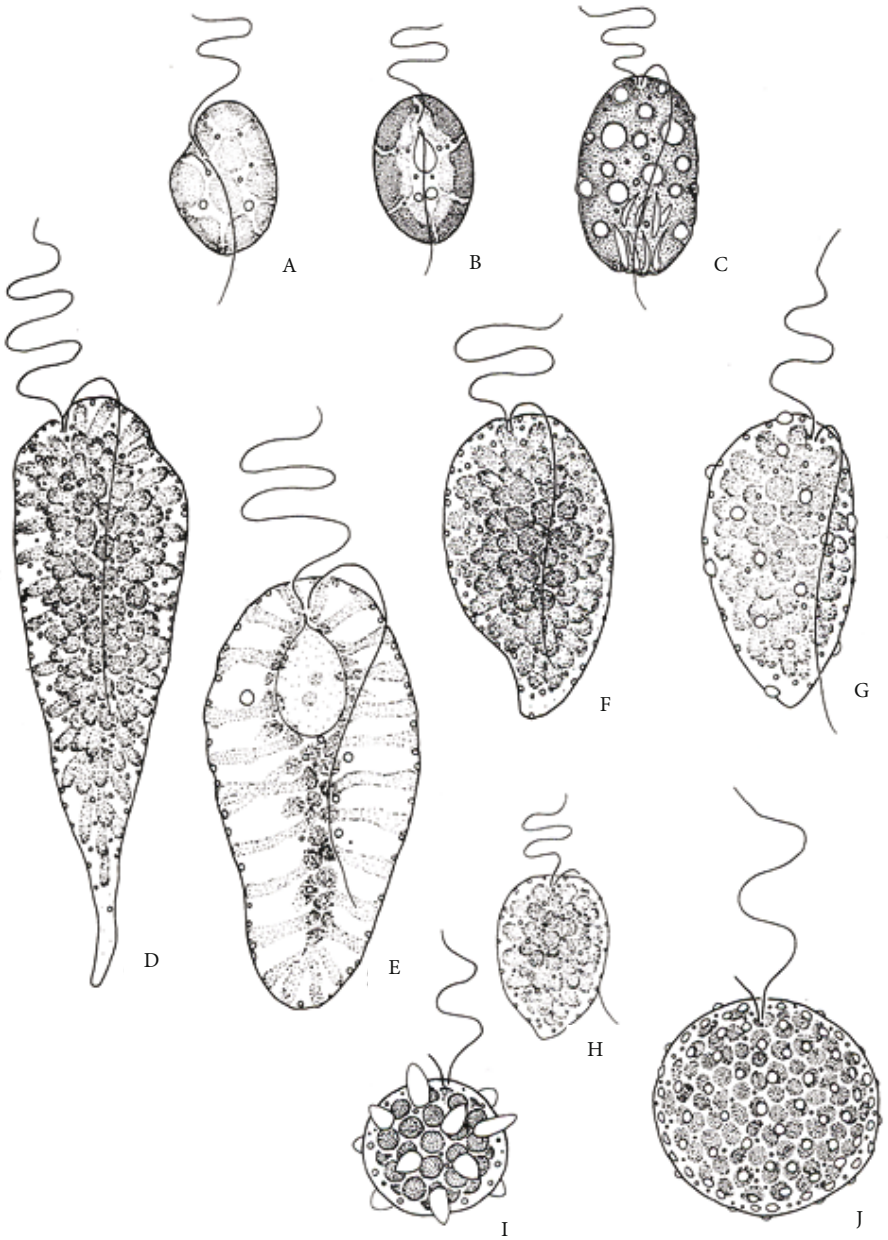

Figure 6. Comparison of different Raphidophyceae species (A–H), Dictyochophyceae (I–J; previously class Raphidophyceae). A- *Heterosigma carterae*; B- *Olisthodiscus luteus*; C- *Fibrocapsa japonica*; D- *Chattonella. antiqua*; E- *Chattonella ovata*; F- *Chattonella marina*; G- *Chattonella subsalsa*; H- *Chattonella. minima*; I- *Pseudochattonella verruculosa*; J- *Dictyocha fibula* var. *stapedia* (after Hara and Chihara, 1987).

smaller, more ovoid or spherical and non-motile. Similar changes were noted in nutrient-limited cultures of Iranian strain CHPI36.

Strain CHPI36 is similar in general morphology to *Chattonella subsalsa* CCMP217, as both have similar chloroplast arrangement, cell shapes, and mucocysts. Despite these similarities, strain CHPI36 and *C. subsalsa* show distinct differences. The cell shape/outline of strain CHPI36 is different (Figure 7), and it is slightly smaller than *C. subsalsa*, although there is considerable overlap in the size ranges (Hallegraeff and Hara, 1995) (Table 3). CHPI36 isolate also has an obvious eyespot, whereas *C. subsalsa*, and most other members of *Chattonella*, do not possess an eyespot (e.g., *C. ovata, C. antiqua*, and *C. marina*; Hara and Chihara, 1982; Yamaguchi et al., 2008; Demura et al., 2009). Colour is also considered one of the

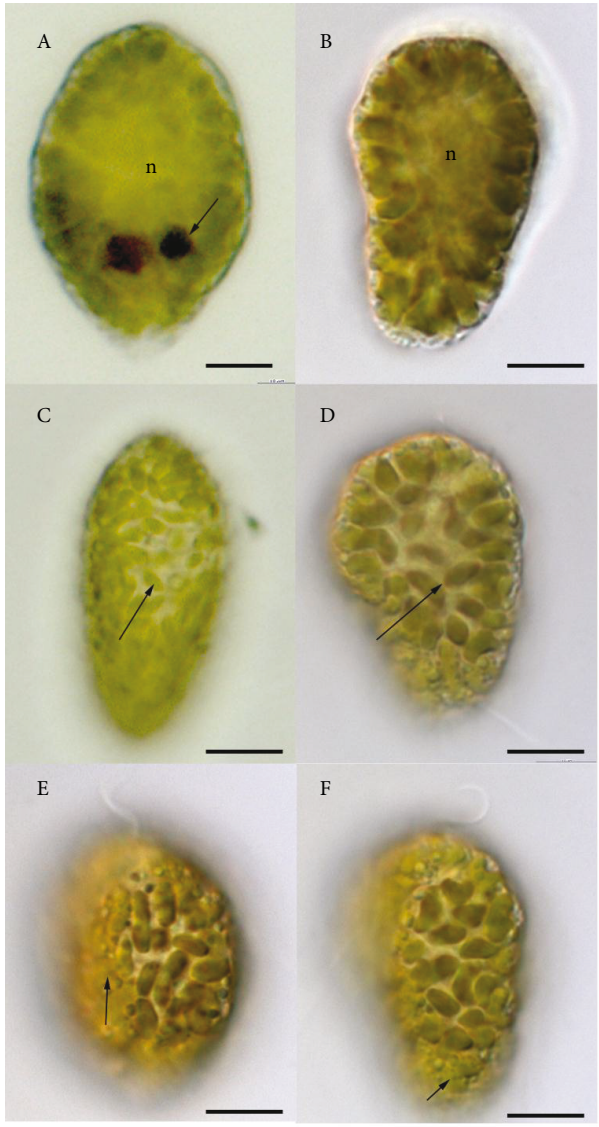

Figure 7. Comparing the morphology of vegetative cells of *Chattonella* sp. CHPI36 with *C. subsalsa* CCMP217. A- strain CHPI36 in deep focus, showing 2 eyespots; B- CCMP217 in deep focus, note the lack of eyespot; C- and D- strains CHPI36 (left) and CCMP217 (right) in surface focus showing chloroplasts shape and arrangement; E- and F- strains CHPI36 (left) and CCMP217 (right). Note the presence of mucocysts (arrow). All scale bars = 10 µm.

Table 3. Comparison of *Chattonella* sp. CHPI36 with similar *Chattonella* spp.

Vegetative cell	*Chattonella* sp. CHPI36	[a]*C. subsalsa*	[b]*C. marina*	[c]*C. antiqua*
Size: length (µm)	24–43	30–50	30–70	70–130
width (µm)	17–23	15–25	20–30	30–70
Chloroplast: colour	Green-brown	Green-brown	Yellowish-green-brown	Green-brown
Shape	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid-to- tear-shaped
Eyespot	Present	Absent	Absent	Absent

[(a,b,c)]: described in Hara and Chihara (1987) and Hallegraeff and Hara (1995).

important features for distinguishing *C. subsalsa* from *C. marina* (Hallegraeff and Hara, 2003). *C. subsalsa* possesses a green-brown colour, whereas *C. marina* possesses a green yellowish-brown colour. Although colour can be a somewhat subjective character, under identical culture conditions isolate CHPI36 differs from *C. subsalsa* by having a greener colour.

4.2. Molecular analyses

The analyses presented here show that the raphidophytes included form a monophyletic group with high bootstrap support that includes both the marine and freshwater *Vacuolaria* genera. This agrees with previous studies (Potter et al., 1997; Ben Ali et al., 2001; Ben Ali et al., 2002; Hosoi-Tanabe et al., 2006). Members of the 4 different genera are quite distinct from each other. Connell (2000) suggested that the *H. akashiwo* ITS sequence was quite divergent from both *C. antiqua* and *C. subsalsa* (20% and 18% divergence, respectively), and the present study supports this result. The 2 strains of *H. akashiwo* were distinct from all members of other genera, with sequence divergences of 22% and 19% in ITS sequence from the 2 later species. In addition, *F. japonica* and *O. luteus* showed high ITS sequence divergence by pairwise comparison (47%, Connell, 2000; 50%, present study). The amount of either ITS sequence or partial LSU sequence divergence and nucleotide base difference between *Chattonella* species and other species of different genera is high.

Many algal species show intra-specific sequence variation in the rDNA-ITS and LSU-rDNA genes between different geographical isolates (Chopin et al., 1996; Bolch et al., 1998; Hirashita et al., 2000). For example, Atlantic and Pacific isolates of *Cladophora albida* (Nees) Kutzing, 1843 showed up to 1% sequence divergence across the ITS region within each oceanic basin and as much as 21% between the 2 oceanic basins (Bakker et al., 1992). In contrast, past studies on raphidophytes have demonstrated little or no variation in LSU-rDNA and rDNA-ITS sequences within each species (Connell, 2000; Hirashita et al., 2000; Connell, 2002). For example, 20 strains of *H. akashiwo* from across the globe have almost 100% ITS sequence identity, indicating that populations of this species represent only 1 worldwide species (Connell, 2000). Similar results have been reported for 16 isolates of *F. japonica* (Kooistra et al., 2001). The sequences from the 4 *H. akashiwo* used in the present study were also virtually identical across the 700 bp of LSU examined, with a sequence divergence of <0.6% between strains.

Within the genus *Chattonella*, strains of *C. marina*, *C. ovata*, and *C. antiqua* show remarkable similarity across the rDNA-ITS regions with <1.2% sequence divergence between *C. marina* and *C. ovata* and only a few base (maximum 7 nucleotides) differences in nucleotide sequence. Past studies on *C. marina* and *C. antiqua* have considered these to be one species (Connell, 2000; Hirashita et al., 2000; Sako et al., 2000; Connell, 2002). Hosini-Tanabe et al. (2006) also documented high genetic homogeneity of *C. marina*, *C. antiqua*, and *C. ovata* in the 5.8S rDNA D1/D2 region of the LSU-rDNA and rDNA-ITS1 and ITS2 regions.

From this study and previous work, it is clear that global geographical variation in both the LSU-rDNA and rDNA-ITS is very low within raphidophyte species. Strain CHPI36 from the Oman Sea is clearly distinct from *C. subsalsa* and exhibits small but consistent morphological differences from *C. subsalsa*, indicating that isolate CHPI36 is a distinct species related to *C. subsalsa*.

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