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Florenciella parvula gen. et sp. nov. (Dictyochophyceae, Heterokontophyta), a small flagellate isolated from the English Channel

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Florenciella parvula (Dictyochophyceae) was isolated from the English Channel in December 2000. In general the cells are spherical, measure 3–6 μm and have two markedly unequal flagella as well as two yellow-brown chloroplasts. The long hairy flagellum pulls the cells through the water and the short flagellum is acronematic. Their morphology and fine structure indicate a close relationship with the Heterokonts. Phylogenetic analysis of the small subunit of the ribosomal RNA gene clearly places *F. parvula* within the class Dictyochophyceae and more precisely the order Dictyochales, despite the absence of the external skeleton characteristic of this order. The pigment suite consists of chlorophylls *a*, c_2, c_3, 19′ butanoyloxy-fucoxanthin, fucoxanthin, diadinoxanthin and β-carotene. This pigment composition is typical of the class Pelagophyceae.

INTRODUCTION

During the second half of the 20th century, research has established that the contribution of small (less than 5 μm) algae to the primary production in the sea may be considerable (Saijo 1964; Zeitschel 1970). The taxonomic knowledge of the species composition of this size fraction has been limited (see Thomsen 1986 for a review). Although in recent years several novel taxa have been described from cultures, including not only new species and genera, but also new classes, e.g. Pelagophyceae (Andersen *et al.* 1993), Bolidophyceae (Guillou *et al.* 1999) and Pinguiophyceae (Kawachi *et al.* 2002), the application of molecular methods to the direct analysis of marine samples suggests that the taxa actually present in nature far outnumber those that have been isolated in culture and described formally (Moon-van der Staay *et al.* 2000).

One of the newer classes, the Dictyochophyceae, which was considered as an order within the old and well-established class Chrysophyceae (Deflandre 1950), was erected as a separate class by Hibberd (1986) and Kristiansen (1986, 1990) on the basis of fine structure. No small representative of the order Dictyochales (silicoflagellates) has been described until now. The silicoflagellates are widespread in marine waters and may occasionally reach bloom concentrations (Tangen 1974; Henriksen *et al.* 1993). Because of their silicified skeletons, they are well preserved in the sediments and appear in the fossil records from the lower Cretaceous onwards. The number of genera and species have varied and showed a peak in the Miocene (Tappan 1980). Currently, a single genus, *Dictyocha* Ehrenberg, is recognized (Deflandre 1950; Moestrup & Thomsen 1990; Moestrup 2000).

In this paper, we describe a new species, *Florenciella parvula* gen. et sp. nov. Although no trace of a skeleton has been observed, its fine structure, pigment composition and sequences of the small subunit (SSU) of the ribosomal RNA (rRNA) gene indicate that it should be included in the order Dictyochales.

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MATERIAL AND METHODS

Material and cultivation

Florenciella parvula was isolated by one of us (F.L.G.) from a 3 μm prefiltered surface sample collected in the English Channel (48°45′N, 3°57′W) on 4 December 2000 and purified by serial dilution. It is maintained as strain RCC 446 in the Roscoff Culture Collection (http://www.sb-roscoff.fr/Phyto/collect.html; Vaulot *et al.* 2004) and grown in K-medium (Keller *et al.* 1987) at 15°C under white fluorescent light with a photon flux rate of about 150 μmol photons m^{-2} s^{-1} and a 12 : 12 h light–dark cycle.

Molecular analysis

The culture was harvested by centrifugation and the pellet used directly for DNA extraction. Total nucleic acids were obtained using a 3% CTAB (hexadecyltrimethylammonium bromide) procedure (Doyle & Doyle 1990). DNA was purified with the Geneclean II kit (BIO 101, La Jolla, CA, USA). The SSU rDNA of *F. parvula* was amplified by polymerase chain reaction (PCR) using universal oligonucleotide primers Euk328f (5′-ACC TGG TTG ATC CTG CCA G-3′) and Euk329r (5′-TGA TCC TTC YGC AGG TTC AC-3′), complementary to regions of conserved sequences proximal to the 5′ and 3′ termini of the SSU rRNA gene, as described by Moon-van der Staay *et al.* (2000). The PCR reaction was cycled 34 times. Amplification products were purified with the Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany). The purified PCR products were cloned into the pCR 2.1 vectors using the Invitrogen kit TOPO-TA cloning (Carlsbad, CA, USA) according to the manufacturer's protocol. Nucleotide sequences of the cloned DNA fragment were determined, in

both directions using internal primers (Elwood *et al.* 1985), by the sequencing service of Qiagen. The consensus sequence was subjected to a BLASTN search against sequences available in GenBank. The sequence was added to a database encompassing more than 5000 published and unpublished eukaryotic SSU rDNA sequences running under the programme ARB (available at http://www.arb-home.de/) and aligned using the ARB alignment tool, with 51 other heterokont sequences (Table 1). *Labyrinthuloides minuta* and *Cafeteria roenbergensis* were included in the analyses to form an outgroup. Phylogenetic analyses were performed with the following methods as implemented in ARB: parsimony, neighbour joining (NJ) using the Jukes–Cantor correction and maximum-likelihood (ML). Bootstrapping with 1000 replicates allowed us to evaluate the robustness of both the maximum parsimony and NJ analyses. The sequence of the SSU rDNA of *F. parvula* is deposited in GenBank under the accession number AY254857.

Pigments

For pigment analysis, 30 ml of *F. parvula* culture was filtered on a 25 mm diameter GF/F filter (Whatman, Brentford, UK) that was sent from Roscoff (France) to Barcelona (Spain) on dry ice and kept deep-frozen (−80°C) until analysis. High-performance liquid chromatography (HPLC) pigment analyses were performed using the method of Zapata *et al.* (2000) with minor modifications as described in Latasa *et al.* (2001). Pigments were extracted by soaking the filter into 3 ml of 90% acetone overnight at 4°C. The tube with the filter in acetone was then treated in an ice-cooled Vibrogen IV cell mill (Edmund-Buehler, Hechingen, Germany) with 1 mm diameter glass beads for 10 min. The extract was centrifuged at 4°C and the supernatant transferred to an Eppendorf vial. One millilitre of clear extract was mixed with 0.2 ml of pure H_2O and injected into the chromatographic system. The Thermo Quest chromatograph (Thermo Electron, Waltham, MA, USA) included a P2000 solvent module, an A/S 3000 auto sampler set at 4°C, a UV-3000 absorbance detector in spectral (400–700 nm, 2 nm resolution) recording mode, an FL2000 fluorescence detector (λ_{ex} = 430 ± 40 nm, λ_{em} = 662 nm) and an SN 4000 controller. Chromatograms were processed with Chromquest software (Thermo Electron). Peaks were identified by their retention time and absorption properties compared with those of pure standards obtained from the Danish Hydraulic Institute.

Light microscopy

The cells were studied either fixed with a drop of Lugol's solution or live under a Microphot FX (Nikon, www.nikon.com) and a BX 51 (Olympus, www.olympus.com), both fitted with phase contrast and differential interference contrast optics. Digital pictures were taken with a Spot RT Camera (Diagnostic Instruments, Sterling Heights, MI, USA).

Electron microscopy

Whole mounts were prepared by placing a drop of the culture on a formvar and carbon-coated grid. The drop was allowed to dry on the grid and was subsequently rinsed in distilled water. The whole mounts were stained with saturated aqueous uranyl acetate for 20 min and subsequently rinsed in distilled water. Thin sections were, except for the one shown in Fig. 11, prepared according to the following protocol: fixation in 1% glutaraldehyde for 2 h; rinsing 3× 30 min in growth medium and 2× 10 min in 0.1 M sodium cacodylate buffer (pH 8); postfixation in 1% osmium tetroxide and 1.5% potassium ferricyanide in 0.1 M sodium cacodylate buffer for 3 h; rinsing 3× 15 min in sodium cacodylate buffer and 2× 10 min in distilled water. The cells were stained *en bloc* overnight in a solution of aqueous uranyl acetate. Dehydration was accomplished 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 overnight in a 1 : 1 mixture of propylene oxide and Epon embedding resin (Sigma-Aldrich Fluka, Buchs SG, Switzerland). Finally the cells were treated 3× 1 h in Epon before polymerization at 50°C for 12 h. Thin sections were cut on an ultramicrotome (Reichert, Vienna, Austria) and subsequently soaked 5 min in lead citrate. In the fixation protocol used for the thin section shown in Fig. 11, ferricyanide was omitted in the postfixative and the en bloc uranyl acetate step was left out. Instead the thin sections were contrasted with uranyl acetate and, after a thorough rinse in distilled water, contrasted with lead citrate. Thin sections and whole mounts were viewed in Philips 12 and 100 electron microscopes (Philips, Eindhoven, The Netherlands) at the electron microscopy laboratories for Biosciences, Department of Biology, University of Oslo.

RESULTS

Phylogenetic analysis based on SSU rDNA

The SSU rDNA sequence of *F. parvula* is most closely related to that of *Dictyocha speculum* (class Dictyochophyceae), with which it shares 92.3% identity. Phylogenetic analyses of full length SSU rDNA sequences with distance (Fig. 1) and maximum parsimony methods (data not shown) provide consistent evidence for an affiliation of *F. parvula* with the class Dictyochophyceae. Within this class, three clades are apparent, corresponding to the three orders Pedinellales, Rhizochromulinales, and Dictyochales, and *F. parvula* falls into the last of these. All phylogenetic analyses yield the same tree topology and branching order for major heterokont lineages, except for the Eustigmatophyceae (which is placed as a sister group of the Chrysophyceae lineage in parsimony analysis) and the Pinguiophyceae (which are placed as a sister group of the Bacillariophyceae and Bolidophyceae lineages in parsimony analysis – tree not shown). These sister relationships are not supported by bootstrap values. The Dictyochophyceae always forms a monophyletic clade (Fig. 1) within the phylum Heterokonta with strong bootstrap support (97%), as reported in other studies (e.g. Daugbjerg & Henriksen 2001), and is a sister group of the Pelagophyceae.

Pigment analysis

Florenciella parvula (Fig. 2, Table 2) contains 19′ butanoyloxyfucoxanthin as the most characteristic pigment marker. Fucoxanthin, diadinoxanthin and β-carotene are other carotenoids present, whereas 19′ hexanoyloxyfucoxanthin is ab-

Table 1. List of species included in the phylogenetic analyses with accession numbers of SSU rDNA sequences.

Taxon	Accession no.
Bacillariophyceae	
Skeletonema costatum (Greville) Cleve	X52006
Skeletonema pseudocostatum Medlin	X85394
Thalassiosira eccentrica (Ehrenberg) Cleve	X85396
Thalassiosira rotula Meunier	AF462058
Bolidophyceae	
Bolidomonas mediterranea Guillou & Chrétiennot-Dinet	AF123596
Bolidomonas pacifica Guillou & Chrétiennot-Dinet	AF123595
Chrysophyceae	
Epipyxis aurea (Bourrelly) Hilliard	AF123301
Epipyxis pulchra Hilliard & Asmund	AF123298
Ochromonas sphaerocystis Matvienko	AF123294
Paraphysomonas foraminifera Lucas	AB022864
Paraphysomonas vestita (A.C. Stokes) De Saedeleer	Z28335
Dictyochophyceae	
Apedinella radians (Lohmann) P.H. Campbell	U14384
Ciliophrys infusionum Cienkowski	L37205
Dictyocha speculum Ehrenberg	U14385
Florenciella parvula Eikrem	AY254857
OLI11025	AJ402337
Pteridomonas danica Patterson & Fenchel	L37204
Pseudopedinella elastica Skuja	U14387
Rhizochromulina Hibberd & Chrétiennot-Dinet MBIC 10538	rs10393[1]
Rhizochromulina cf. *marina* Hibberd & Chrétiennot-Dinet	U14388
Eustigmatophyceae	
Eustigmatos magma Hibberd	U41051
Nannochloropsis granulata Karlson & Potter	U41092
Nannochloropsis salina (Bourrelly) Hibberd	AF045046
Pseudocharaciopsis minuta (Braun) Hibberd	U41052
Vischeria helvetica (Vischer & Pascher) Hibberd	AF045051
Hyphochitriomycetes	
Hyphochytrium catenoides Karling	X80344
Rhizidiomyces apophysatus Zopf	AF163295
Oomycetes	
Phytophthora megasperma Drechsler	X54265
Phytophthora undulate (H.E. Pedersen) M.W. Dick	AJ238654
Pythium insidiosum De Cock, Mendoza, Padhye, Ajello & Kaufman	AF289981
Pythium monospermum Pringsheim	AJ238653
Pelagophyceae	
Aureococcus anophagefferens Hargraves & Sieburth	AF117778
coccoid CCMP 1145	U40928
Pelagomonas calceolata Andersen & Saunders	U14389
Phaeophyceae	
Coccophora langsdorfii (Turner) Greville	AB011426
Cystoseira hakodatensis (Yendo) Fensholt	AB011425
Pylaiella littoralis (Linnaeus) Kjellman	AY032606
Nematochrysopsis marina (Feldmann) Billard	AF038005
Pinguiophyceae	
Glossomastix chrysoplasta O'Kelly	AF438325
Phaeomonas parva Honda & Inouye	AF438323
Pinguiococcus pyrenoidosus Honda & Inouye	AF438324
Pinguiochrysis pyriformis Kawachi	AF438326
Raphidophyceae	
Chattonella subsalsa Biecheler	U41649
Heterosigma akashiwo (Hada) Hada *ex* Hara & Chihara	AB001287
Heterosigma carterae (Hulburt) Taylor	U41650
Vacuolaria virescens Cienkowski	U41651
Thraustochytriidae/ Bicosoecida	
Cafeteria roenbergensis Larsen & Patterson	AF174364
Labyrinthuloides minuta (Watson & Raper) Perkins	L27634

Table 1. Continued.

Taxon	Accession no.
Xanthophyceae	
Botrydium stoloniferum Mitra	U41648
Heterococcus caespitosus Vischer	AF083399
Mischococcus sphaerocephalus Vischer	AF083400
Tribonema intermixtum Pascher	AF083397

[1] Sequence is downloaded from the Marine Biotechnology Institute Culture Collection (http://seasquirt.mbio.co.jp/icb/browsedata/bd_icbnumber.php?icbnumber=rs10393).

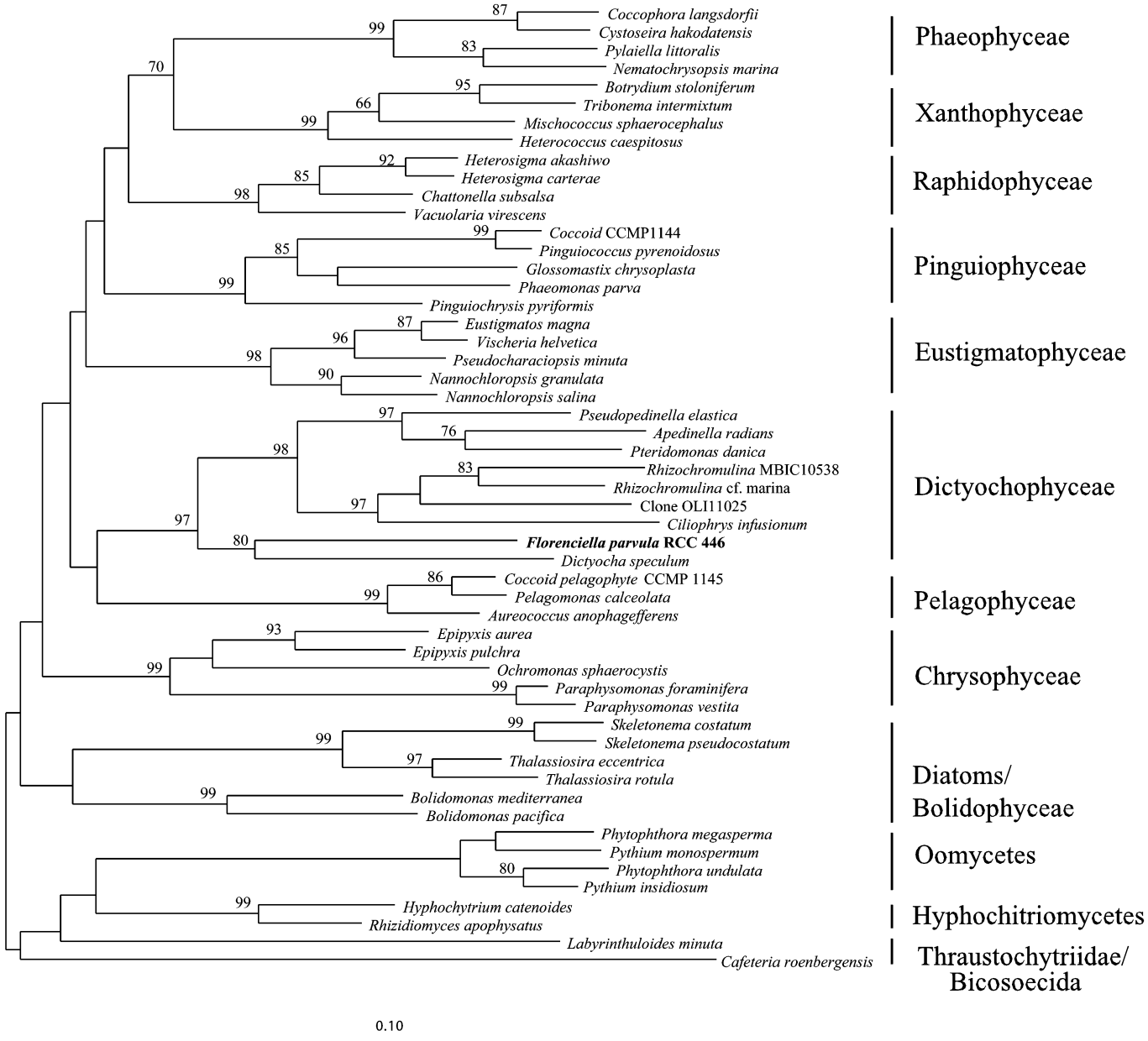


Fig. 1. Phylogenetic position of *Florenciella parvula* inferred from SSU rDNA sequence comparisons of 52 taxa with *Labyrinthuloides minuta* and *Cafeteria roenbergensis* as outgroup, by distance method (NJ). Bootstrap values above 50% (1000 replicates) are given at the internodes in percentages. The scale bar indicates the branch length corresponding to 10 changes per 100 nucleotide positions.

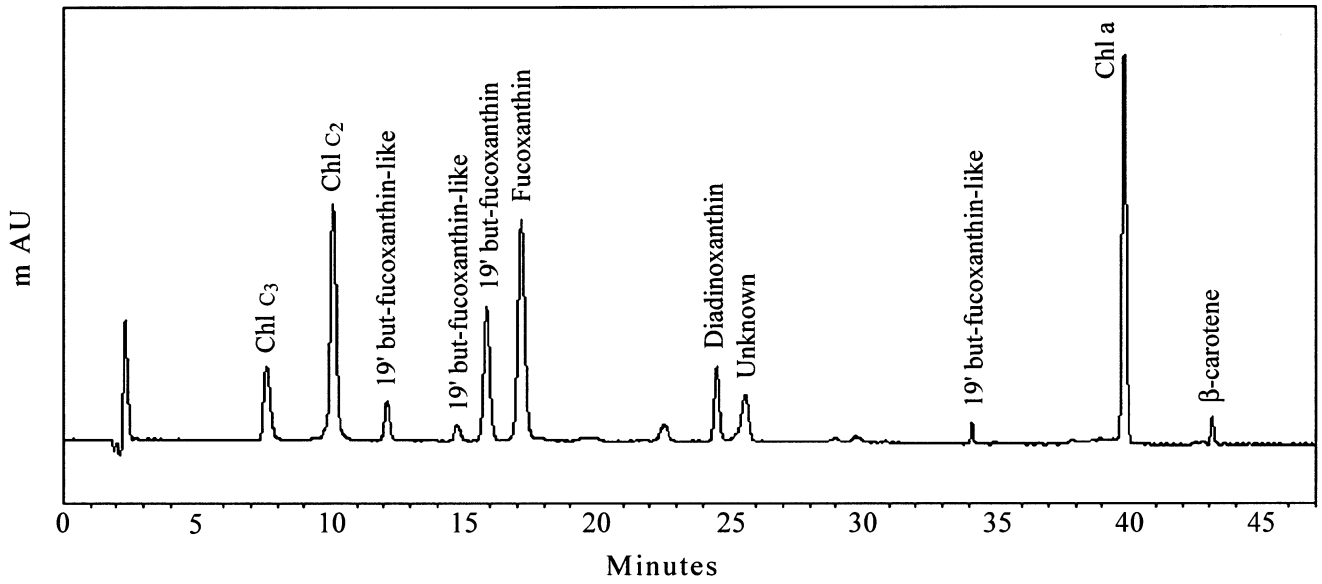


Fig. 2. Absorption chromatogram at 440 nm of *Florenciella parvula* showing the peaks identified in Table 2. Retention time in minutes on the x-axis and milliabsorbance units (mAU) on the y-axis.

sent. Chlorophylls c_2 and c_3 are the only accessory chlorophylls. Some uncharacterized minor peaks present spectral characteristics of 19′ butanoyloxyfucoxanthin or fucoxanthin and are probably degradation products from those main pigments. One of those peaks eluted with a retention time very close to that of 19′ hexanoyloxyfucoxanthin in our system and could have been misidentified. However, coinjection with an authentic 19′ hexanoyloxyfucoxanthin standard clearly showed a mismatch and confirmed the absence of 19′ hexanoyloxyfucoxanthin in *F. parvula.* Another carotenoid that could not be identified possessed absorption maxima at 442 and 462 nm and eluted after diadinoxanthin.

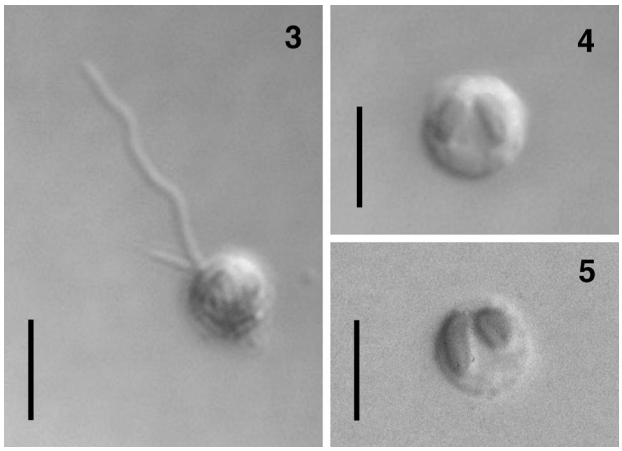
Morphology and ultrastructure

The majority of the cells are more or less spherical (Figs 3–5) and measure 3–6 μm, with two heterokont flagella (Fig. 3) and two chloroplasts (Fig. 4) that may have different sizes (Fig. 5). Some cells are elongated (up to 8 μm long) and irregular in shape, and may lack the short flagellum. On a few occasions spherical cells (6–8 μm) with a single flagellum have been observed. The short flagellum is smooth and the central pair of microtubules extends beyond the flagellum proper (*c.* 1–3 μm), creating a thin terminal extension, which can be one to two times the length of the short flagellum (Figs 6, 15). A flagellar swelling is present on the short flagellum (Fig. 7) *c.* 0.7 μm from the base. The long anteriorly directed flagellum (11–16 μm) has long tubular mastigonemes that are produced in the perinuclear compartment (Fig. 8). The mastigonemes show no partitions and appear as one long tube (Figs 9, 10). They are easily shed during fixation and may break up into small pieces when prepared for whole mounts. Usually, the cells contain two chloroplasts, each with an immersed pyrenoid that may be traversed by tubules and a girdle lamella (Figs 11, 14). The mitochondrion has tubular cristae and is probably single and branched (Figs 11, 13). Vesicles located close to the cell membrane are commonly observed (Fig. 11). The nuclear membrane is continuous with the outer chloroplast membrane. The nucleus is placed centrally (Fig.

Table 2. Pigment identification for *Florenciella parvula*. The suffix '-like' for the unknown carotenoids indicates that spectral properties (in eluent) are similar to those of the named pigment.[1]

Peak	Rt (min)	Pigment	Absorption max (nm)
1	7.6	chlorophyll c_3	460, 590
2	10.1	chlorophyll c_2	454, 586, 634
3	12.1	unknown 19′ butanoyloxyfucoxanthin-like	446, 472
4	14.8	unknown 19′ butanoyloxyfucoxanthin-like	444, 472
5	15.9	19′ butanoyloxyfucoxanthin	446, 472
6	17.2	fucoxanthin	448, 468
7	22.5	unknown	440, 464
8	24.5	diadinoxanthin	446, 462
9	25.2	unknown fucoxanthin-like	446, 468
10	25.6	unknown	442, 462
11	34.1	unknown 19′ butanoyloxyfucoxanthin-like	446, 472
12	39.8	chlorophyll *a*	432, 664
13	43.1	β-carotene	454, 480

[1] RT, retention, time.



Figs 3–5. *Florenciella parvula,* LM. Scale bars = 5 μm.
Fig 3. Cell fixed in Lugol's solution with one long and one short flagellum.
Fig. 4. Live cell with two chloroplasts.
Fig. 5. Live cell with two chloroplasts of different size.

11), at the anterior end of the cell just below the flagellar basal bodies (Fig. 12). The nucleus has a depression where the basal bodies fit (Fig. 13). The dictyosome is situated at the anterior end of the cell in the vicinity of the flagellar bases (Figs 12, 15, 16). No microtubular roots have been observed. The transition region of the flagella contains a transitional plate and two proximal rings (Fig. 12). On some occasions, a distal helix has been observed (Fig. 12).

Florenciella Eikrem, *gen. nov.*

Cellulae heterocontae, phototrophicae, cristis tubularibus mitochondrialibus praeditae. Flagellum longius pilis tubularibus instructum, flagellum brevius leve et acronematicum. Dictyosoma in parte cellulae anteriore prope nucleum et bases flagellorum positum. Bases flagellorum depressioni nuclei aptatae. Chloroplasti lamellas cingulares continentes. Pigmenta chloroplasti: chlorophyllum *a,* c_2 et c_3, 19′ butanoyloxyfucoxanthinum, fucoxanthinum, diadinoxanthinum et β-carotenum.

Cells heterokont, phototrophic, with tubular mitochondrial cristae. Long flagellum with tubular hairs, short flagellum smooth and acronematic. Dictyosome located in anterior part of cell in proximity of nucleus and flagellar bases. Flagellar bases fit into depression of nucleus. Chloroplasts with girdle lamellae. Chloroplast pigments: chlorophyll *a,* c_2 and c_3, 19′ butanoyloxyfucoxanthin, fucoxanthin, diadinoxanthin and β-carotene.

TYPE OF GENUS: *Florenciella parvula* Eikrem, *sp. nov.,* designated here.

Florenciella parvula Eikrem, *sp. nov.*

Figs 3–16

Cellulae (3–6 μm) duobus flagellis apicaliter infixis praeditae. Flagellum longius (11–16 μm) pilis tubularibus longis vestitum sine partitionibus; flagellum brevius (1–3 μm) acronematicum, leve, extensione terminali ad longitudinem flagelli proprii duplicem. Corpora basalia radicibus fibrosis connexa. Chloroplasti duo, pyrenoidibus immersis tubulis interdum perductis. Vesiculae vix sub membrano cellulae positae. Sequentia SSU rDNA (AY254857) haec species classi Dictyochophycearum attribuenda est.

Cells (3–6 μm) with two apically inserted flagella. Long flagellum (11–16 μm) with long tubular hairs without partitions; short flagellum (1–3 μm) acronematic, smooth with terminal extension up to twice the length of the flagellum proper. Fibrous roots connect basal bodies. Chloroplasts two, with immersed pyrenoids that may be traversed by tubules. Vesicles are situated just beneath cell membrane. Sequence of SSU rDNA (AY254857) places species within Dictyochophyceae.

HOLOTYPE (designated here): Figs 3, 11. Whole mount preparations and inclusions of the authentic culture are deposited at the Department of Biology, University of Oslo, PO Box 1069, Blindern, 0316 Oslo, Norway.

AUTHENTIC CULTURE: RCC 446 of the Roscoff Culture Collection at Station Biologique, UMR 7127 CNRS et Université Pierre et Marie Curie, 29682 Roscoff Cx, France.

TYPE LOCALITY: English Channel (48°45′N, 3°57′W).

HABITAT: Marine.

ETYMOLOGY: The genus is named after one of us (F.L.G.) who isolated the type culture. The species epithet *parvula* refers to its very small size.

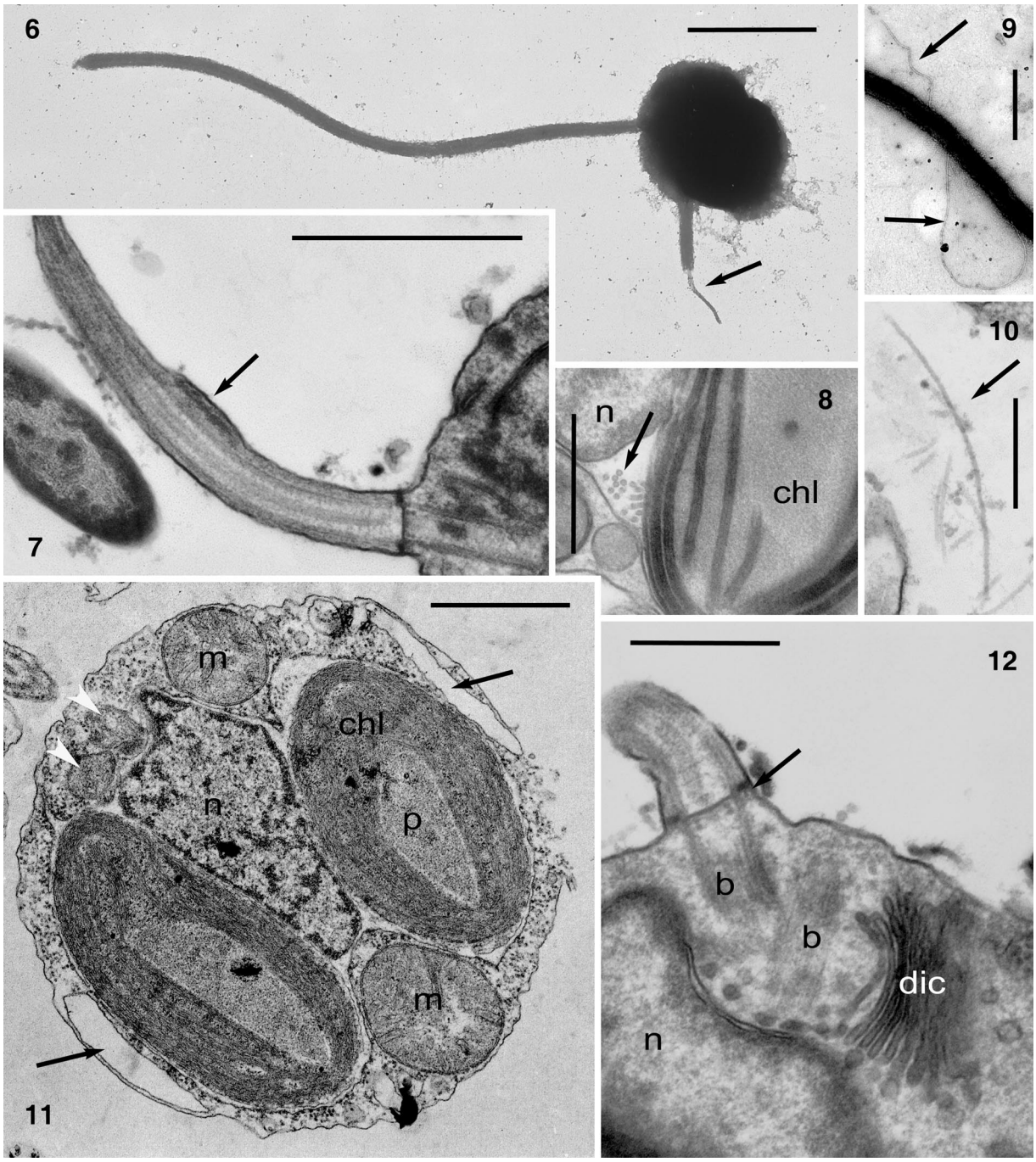
DISCUSSION

The appearance and behaviour of *F. parvula* in light microscopy (LM) is reminiscent of a small ochromonad with a prominent flagellum pointing forward in the swimming direction. This anteriorly directed hairy flagellum is pulling the cell and is a distinguishing characteristic of the division Heterokontophyta. To establish affinities to class and order, it was necessary to obtain detailed information on the fine structure, pigment composition and SSU rDNA sequence of *F. parvula.* The fine structural details did not conclusively point to any of the heterokont classes in particular. The pigment analysis revealed a firm relationship with the Pelagophyceae and the SSU rDNA sequence of *F. parvula* is most closely related (92.3% similarity) to that of *D. speculum,* order Dictyochales of the class Dictyochophyceae.

The members of the Dictyochophyceae were initially classified within the class Chrysophyceae (Deflandre 1950; Christensen 1980), but were later separated into their own class (Hibberd 1986; Kristiansen 1986, 1990). Whereas Cavalier-Smith (1993) and Saunders *et al.* (1995) suggested separate classes for the silicoflagellates and the pedinellids, Moestrup (1995) proposed to retain the class Dictyochophyceae with three orders: Pedinellales (Zimmermann *et al.* 1984), Rhizochromulinales (O'Kelly & Wujek 1995) and Dictyochales (Haeckel 1894).

The order Pedinellales is commonly referred to as the pedinellids. It comprises nine genera and more than 25 species and contains both heterotrophic (e.g. *Ciliophrys* Cienkowski and *Actinomonas* Kent) and phototrophic (e.g. *Pedinella* Vysotskii, *Pseudopedinella* N. Carter and *Apedinella* Throndsen) species (Moestrup 1995; Sekiguchi *et al.* 2003). The photosynthetic species contain three, six or many chloroplasts. The more or less spherical cells are pulled by a single flagellum bearing tripartite hairs and beating in a planar sine wave. A wing supported by a paraxonemal rod is usually present within the membrane of the flagellum. Tentacles supported by triads of microtubules may surround the flagellar pole (*Pedinella*), be reduced (*Apedinella*), or lack completely (*Pseudopedinella*). The dictyosome is located in the posterior end of the cell (Moestrup 1995).

The order Rhizochromulinales comprises a single species, *Rhizochromulina marina,* which is amoeboid in its vegetative stage and produces a flagellated zoospore containing one chlo-



Figs 6–12. Electron micrographs of *Florenciella parvula.* Scale bars = 0.5 μm (Figs 8–10, 12), 1 μm (Figs 7, 11) or 2 μm (Fig. 6). b, basal body; chl, chloroplast; dic, dictyosome; m, mitochondrion; n, nucleus; p, pyrenoid.

Fig. 6. Whole mount of an entire cell showing acronematic flagellum (arrow).

Fig. 7. Thin section showing flagellum with swelling (arrow).

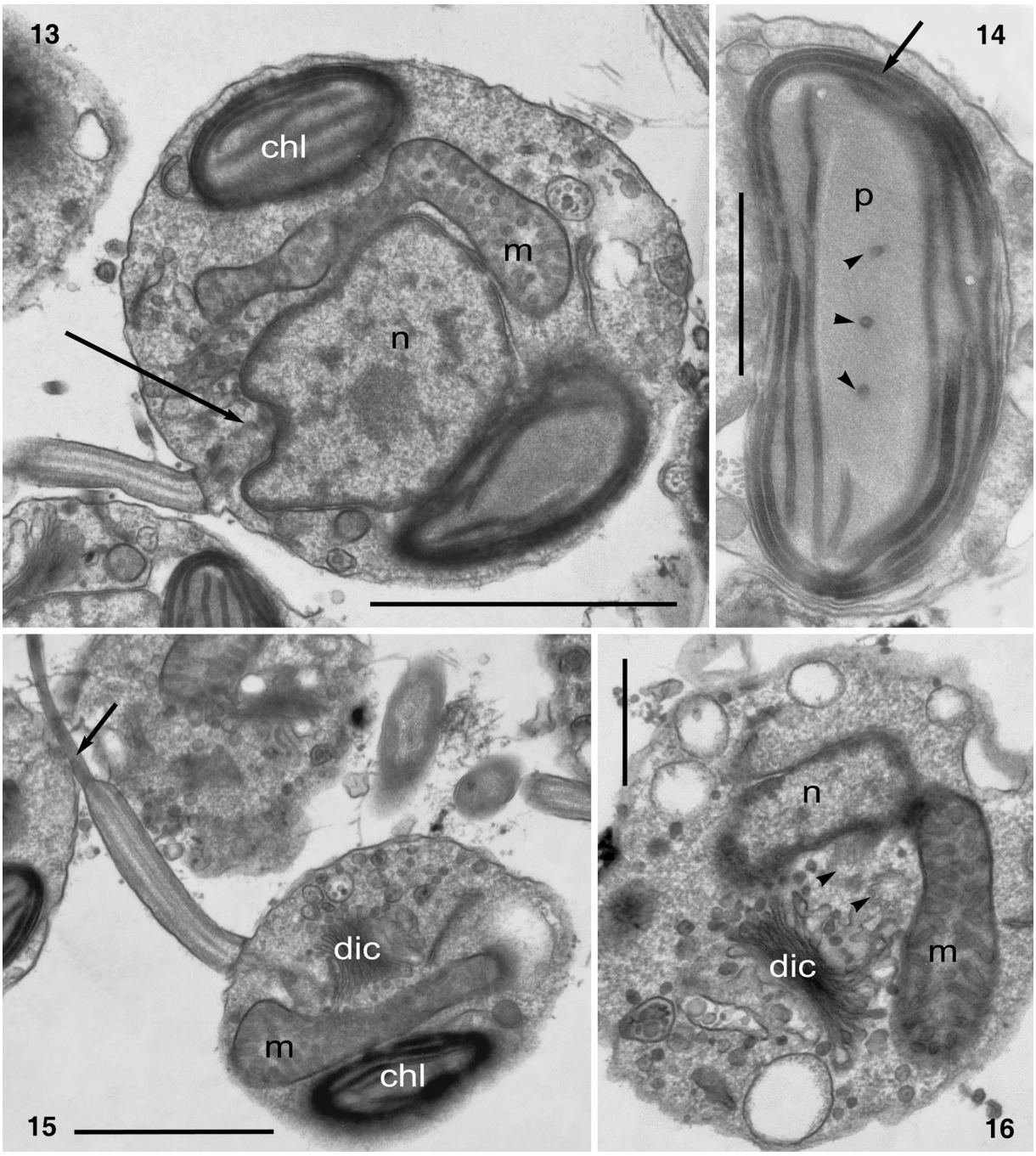
Fig. 8. Thin section of tubular flagellar hairs produced in the perinuclear compartment (arrow).

Fig. 9. Whole mount showing flagellum and hairs (arrows).

Fig. 10. Thin section with view of flagellar hair (arrow).

Fig. 11. Thin section through whole cell containing chloroplasts with pyrenoids, basal bodies (arrowheads) and mitochondrion. Vesicles often occur (arrows) close to the cell membrane.

Fig. 12. Thin section showing the transition zone (arrow) with two proximal rings. The dictyosome is placed at the anterior end of the cell and the basal bodies fit into a depression of the nucleus.



Figs 13–16. Thin sections of *Florenciella parvula.* Scale bars 0.5 μm (Figs 14, 16) or 2 μm (Figs 13, 15). chl, chloroplast; dic, dictyosome; m, mitochondrion; n, nucleus; p, pyrenoid.

Fig. 13. Cross section of cell with single elongated mitochondrion and depression in the nucleus (arrow).

Fig. 14. Detail showing the chloroplast with the girdle lamella (arrow) and embedded pyrenoid penetrated by thylakoid tubes (arrowheads).

Fig. 15. Anterior part of cell with chloroplast, mitochondrion, dictyosome and the short acronematic flagellum (arrow).

Fig. 16. Section through the flagellar base region showing location of the nucleus, mitochondrion, basal bodies (arrow heads) and dictyosome in relation to each other.

roplast. This flagellate bears one emergent flagellum with tripartite hairs. The rhizopodia contain microtubules anchored on the nucleus, which is a common feature in pedinellids. The flagellar transition zone of *R. marina* has a proximal two-gyred helix. As in the pedinellids, the dictyosome is located posterior to the nucleus. The chloroplast lacks a girdle lamella and the chloroplast endoplasmic reticulum does not appear confluent with the membrane of the nucleus. There are no signs of a flagellar rod or other inclusions in the flagellum (Hibberd & Chrétiennot-Dinet 1979; O'Kelly & Wujek 1995).

The order Dictyochales corresponds to the silicoflagellates *sensu stricto.* Currently, one genus is recognized, *Dictyocha* and, commonly, three extant species, *D. fibula* Ehrenberg, *D. speculum* and *D. octonaria* Ehrenberg, are included in the

genus (Deflandre 1950; Moestrup & Thomsen 1990; Moestrup 2000). Their most prominent feature is their skeleton made of silica, the morphology of which is widely used as a diagnostic feature. The morphology of the skeleton varies, however, within the same species (see Henriksen *et al.* 1993) and is therefore not always a reliable character in species identification and delineation. The number of species and varieties remains uncertain and a recent study (Hernández-Becerril & Bravo-Sierra 2001) recognized five morphological species and one variety (*D. calida* Poelschau, *D. californica* Schrader & Murray, *D. fibula* var. *fibula, D. fibula* var. *robusta* Schrader & Murray, *D. octonaria* and *D. speculum*).

The long flagellum of *D. speculum* bears tubular hairs, but their exact morphology has not been firmly established. The membrane of the flagellum extends into a wing-like structure supported by a paraxonemal rod. The second flagellum is commonly reduced to a basal body and the cells have numerous chloroplasts. *Dictyocha speculum* has a life cycle with at least three distinct stages that have been observed in marine plankton. The naked flagellated stage bears a short stubby flagellum in addition to the long hairy one (Moestrup & Thomsen 1990).

In the Dictyochophyceae and some members of the Pelagophyceae, microtubular roots are absent and this seems to be the case in *F. parvula* as well, although fibrous roots appear to be connecting the basal bodies. The basal bodies fit into a depression of the nucleus, but no distinct rhizoplast has been identified. The transition region of the flagella has two proximal rings that are not found in the other members of the Dictyochales, but are a common structure among the pedinellids (Moestrup 1995), the rhizochromulinids (Hibberd & Chrétiennot-Dinet 1979) and the class Pelagophyceae (Andersen *et al.* 1993). *Phaeomonas parva* of the newly described Pinguiophyceae (Kawachi *et al.* 2002) also has a transition region with a proximal 2-gyred helix, as does *Sulcochrysis biplastida* Honda, Kawachi & Inouye (a heterokont of uncertain affiliation). But, unlike the dictyochophyceans, *P. parva* and *S. biplastida* have microtubular roots (Honda *et al.* 1995). In addition, the latter possesses the anterior depression in the nucleus that is a characteristic of many dictyochophyceans.

The flagella of *F. parvula* lack the wing-like structure and the paraxonemal rod that is typical of the longer flagellum of pedinellids and silicoflagellates. The long tubular hairs that cover the long flagellum of *F. parvula* do not appear to have partitions. In general the flagellar hairs of the Heterokontophyta are tripartite, although exceptions occur (e.g. Bolidophyceae).

In the naked flagellated stage of *Dictyocha,* the dictyosomes are located at the anterior end of the cell. The single dictyosome of *F. parvula* is similarly located in the anterior part of the cell. The combination of a lack of microtubular roots and the presence of proximal tubular rings in the flagellar transition region are features that *F. parvula* shares with the pedinellids, the rhizochromulinids and the pelagophyceans. The depression in the nucleus, where the basal bodies are situated, indicates a relationship with the pedinellids, silicoflagellates and pelagophyceans.

Only occasionally does *F. parvula* contain more than two chloroplasts. This distinguishes it from the pedinellids (which either lack chloroplasts or have three, six or many), from *Rhizochromulina* (which has only one) and from *Dictyocha* (which has many). The chloroplasts of *F. parvula* have lamellae composed of three thylakoids, a girdle lamella and a single pyrenoid, features found in many heterokont algal classes (Hibberd 1986). The pyrenoid of *F. parvula* is embedded in the chloroplast matrix and is traversed by single tubular thylakoids. The pyrenoids of *F. parvula* invariably appear inside the girdle lamella. The tubular intrusions, which are seen in *Apedinella* and *Sulcochrysis* Honda, Kawachi & Inouye, have not been observed.

The ultrastructural evidence for the classification of *F. parvula* as a silicoflagellate may not seem overwhelming, but the depression in the nucleus, the anterior location of the dictyosome, the girdle lamella and the chloroplast-immersed pyrenoids are features that *Dictyocha* and *Florenciella* share, not only with each other, but also with other groups to a variable extent. In LM, the cells lacking the short flagellum may resemble the naked flagellated stage of *D. speculum,* although the latter often possesses a marked depression in the periplast at the flagellar pole that has not been observed in *F. parvula.*

Early phylogenetic studies based on molecular evidence showed that, within the Heterokontophyta, the Dictyochophyceae was most closely related to the Bacillariophyceae and the Pelagophyceae (Saunders *et al.* 1995), whereas most recent studies based on larger sets of sequences point to a close relationship between the Dictyochophyceae and the Pelagophyceae (Kawachi *et al.* 2002). The SSU rDNA phylogenetic analysis presented in this paper confirms this close relationship because all three analyses performed (NJ, ML and parsimony; Fig. 1 and data not shown) show that the Dictyochophyceae and Pelagophyceae are sister groups. Within the Dictyochophyceae, the closest relative of *F. parvula* is *D. speculum,* the only *Dictyocha* species for which a sequence is available. The absence of any silicified structure in *F. parvula* suggests that many phylogenetic groups possessing such structures may have close relatives in the picoplankton lacking them, as is the case for the diatoms and their naked relatives, the Bolidophyceae (Guillou *et al.* 1999). The pigment composition of the Dictyochophyceae has not been extensively analysed with high-resolution techniques, but the available results indicate the predominance of the carotenoids fucoxanthin and diadinoxanthin in Rhizocromulinales (Hibberd & Chrétiennot-Dinet 1979) and Pedinellales (Schlüter *et al.* 2000; Daugbjerg & Henriksen 2001). The latter authors, however, found fucoxanthin, 19′ hexanoyloxyfucoxanthin, 19′ butanoyloxyfucoxanthin and chlorophyll c_3 in *D. speculum.* This pigment composition corresponds to the haptophyte type 4 of Jeffrey & Wright (1994) and therefore departs from those of the other two orders. We found no trace of 19′ hexanoyloxyfucoxanthin in *F. parvula,* and therefore its pigment composition establishes a new pigment pattern within the Dictyochophyceae, which corresponds to that of the Pelagophyceae and is in agreement with the phylogenetic data. The occurrence of environmental rDNA sequences very closely related to that of *F. parvula* observed in three different oceanic regions (Antarctic, North Atlantic and Mediterranean Sea; Table 3) suggests that members of *Florenciella* or closely related genera could be important components of the picoplankton in polar and temperate ecosystems. In fact, in the North Atlantic and Antarctic samples (Díez *et al.* 2001), these dictyochophycean sequences were the only ones together with prasinophycean sequences that could be related to photosyn-

Table 3. Comparison between the SSU rDNA sequence of *Florenciella parvula* and partial sequences from environmental samples.

Sequence name	GenBank number	Geographic origin	Length (bp)	% identity
ANT37.15	AF363180	Antarctica	517	99.0
NA11.6	AF363181	North Atlantic	654	97.1
NA37.4	AF363182	North Atlantic	645	99.1
BL001221.35	R. Massana, unpublished	Mediterranean Sea	787	99.4

thetic groups, the other sequences belonging to heterotrophic groups, such as the novel uncultivated stramenopiles described by Massana *et al.* (2002). Therefore, the Dictyochophyceae may contribute significantly to the oceanic 19′ butanoyloxyfucoxanthin pigment pool, which presents a very important component in the picoplankton and is usually attributed to the Pelagophyceae (e.g. Latasa & Bidigare 1997; Barlow *et al.* 2002).

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