

*Florenciella ultra* gen. and sp. nov. (Dictyochytriales, Heterokontophyta) a small flagellate isolated from the English Channel (If anyone has a suggestion for a different title, please let me know, the present one is very dull).

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**Abstract** (we will write in the end?)

### **Material and methods**

The culture was isolated from a 3 µm pre-filtered surface sample by Florence LeGall in the English Channel (48,45N and 3,57 W) on December 4, 2000. It is maintained in the Roscoff Culture Collection (as RCC 446) and grown in K-medium (Keller et al. 1987) at ??°C under white fluorescent light with a PFR of about ???µmol photons m<sup>-2</sup> s<sup>-1</sup> and a ??:??h L:D cycle (Florence or Daniel fill in).

#### Light microscopy

The cells were studied and photographed live under a Nikon Microphot FX fitted with phase contrast and differential interference contrast optics.

#### Electron microscopy

Positively stained whole-mounts were prepared by placing a drop of the culture on a carbon and formvar coated grid. The drop was allowed to dry on the grid that was subsequently rinsed in distilled water. Thereafter the cells were contrasted in 2% uranyl acetate for 20 min. Finally the grid was rinsed in distilled water and dried. Negative stains were produced by not rinsing the grids properly, just dipping the grids very quickly in distilled water and before they were left to dry. Some of the whole-mounts were shadowed with gold-palladium using an Edward's speedivac 12 E6 coating unit, angle c. 30.

Thin-sections were prepared according to the following protocol: Fixation in glutaraldehyde for 2 h. Rinsing 3x 30 min in medium and 2x10 min in 0.1 M Na cacodylate buffer (pH 8). Post fixing in 1% osmium tetroxide in 0.1M Na cacodylate buffer for 3 h. Rinsing 3x 15 min in Na cacodylate buffer and 2x10 min in distilled water. Dehydration was accomplished in an ethanol series starting at 30% and gradually rising to 96%. The dehydration was concluded with 4x10 min in 100% ethanol and 2x10 min in propyleneoxide. The pellets were left over night in a 1:1 mixture of propyleneoxide and Epon (Burke & Wayne Gieselmann 1971) embedding resin. Finally the cells were given 3x1h in Epon before they were polymerized at 50 C for 12 h. The thin-sections were contrasted with lead citrate. Thin sections and whole-mounts were viewed in a Jeol 1200ex at the laboratories for Biosciences, Department of Biology, University of Oslo.

## Results

### Morphology and ultrastructure.

The cells measure 3-4 microns and have two flagella (Fig 1). The long anteriorly directed flagellum (8-10  $\mu\text{m}$ ) has long tubular mastigonemes (easily lost during fixation. Fig. 2. Whole mount arrow) that are produced in the perinuclear compartment (Figs 3,4,6). The short flagellum (ca 1  $\mu\text{m}$ ) is smooth and the central pair of microtubules extends beyond the flagellum proper, creating a thin terminal extension, which can be one to two times the length of the short flagellum (Fig. 1.). Usually the cells contain two chloroplasts each with an immersed pyrenoid that may be traversed by tubules and a girdle lamella (Fig. 3). The nucleus is placed centrally, at the anterior end of the cell just beneath the basal bodies. The nucleus has a depression where the basal bodies fit in (Fig. 3). The nuclear membrane is continuous with the outer chloroplast membrane (Fig. 4). The mitochondrion has tubular cristae (Fig.3). The dictyosome is situated in proximity of the flagellar bases (Fig. 7, need a better picture). No microtubular roots have been observed. The basal bodies seem to be interconnected by fibrous roots (Fig. Y). The transition region of the flagella contains a transitional plate and 2 rings (Fig. 6). Several small vesicles are common close to the cell membrane (Fig. X). Sometimes the cells get infected by viruses (Fig 7.).

### Diagnosis

Cells 3-4 microns. Two flagella inserted apically. Long flagellum measuring 10 microns with long mastigonemes produced in perinuclear compartment. Short flagellum, ca 2 microns, smooth with terminal hair twice the length of the flagellum proper. Cells contain two chloroplasts with girdle lamellae and immersed pyrenoids. Fibrous roots connect the basal bodies. No microtubular roots present. Nucleus with depression where basal bodies fit. A single dictyosome is placed in proximity of the flagellar bases. Several vesicles situated underneath the cell membrane. Will be translated into Latin

## Discussion

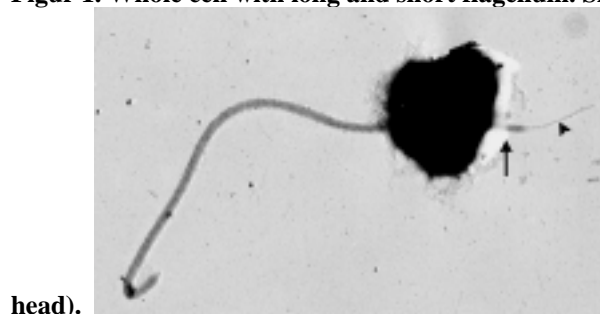
### Morphology and ultrastructure;

The silicoflagellates have earlier been classified with the Chrysophyceae (Christensen 1980), but have been moved (Hibberd 1986, Kristiansen 1986, 1990). Moestrup (1995) proposes to retain the class Dictyochophyceae Silva 1992 and the class is composed of the orders; O. Pedinellales Zimmermann *et al.* 1984, O. Rhizochromulinales O'Kelly & Wujek 1995 and O. Dictyochales Haeckel 1894. The silicoflagellates are widespread and may occasionally reach bloom concentrations. (e.g. Tangen 1974). Currently one genus which contains three extant species of silicoflagellates; *Dictyocha fibula*, *D. speculum* and *D. octonaria* (Deflandre 1952, Moestrup & Thomsen 1990) are recognized. Their most prominent feature is their silicified skeleton which is species specific and the morphology of the skeleton is used as a diagnostic feature. The long flagellum bears tripartite hairs and the membrane

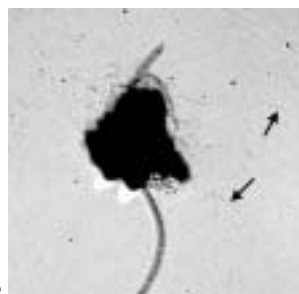
of the flagellum extends into a wing-like structure supported by a paraxonemal rod. The second flagellum is reduced to a basal body. The cells have numerous chloroplasts. The *D. speculum* has a life cycle with at least three distinct stages that have been observed in marine plankton. One of the stages is flagellated and bears a short stubby flagellum in addition to the long hairy one. The pedinellids comprise nine genera and ca 26 species (Moestrup 1995). Genetic studies have shown that within the heterokontophyta, the silicoflagellates are most closely related to the diatoms and the pelagophytes (Saunders et al. 1995, this study).

The ultrastructure of *Florenziella ultra* shows several similarities to the silicoflagellates. The chloroplasts have the typical girdle lamellae and a single pyrenoid. Microtubular roots seem to be lacking which is normal for the class, but two fibrous roots appears to be connecting the basal bodies. The basal bodies fit into a depression of the nucleus, but no distinct rhizoplast can be identified. The transition region of the flagella has two rings that are not found in the other members of the Dictyochales, but is a common structure among the pedinellids and pelagophytes. The flagella of *Florenziella ultra* lack the wing-like structure and the paraxonemal rod and in addition the long tubular hairs that cover the long flagellum do not appear to be tripartite. The ultrastructural evidence for the classification of *F. ultra* as a dictyochophyte is not overwhelming, but the genetic analysis clearly identify it as belonging in the Dictyochophyceae (see genetic part of discussion).

**Figur 1. Whole cell with long and short flagellum. Short flagellum proper (arrow) and terminal hair (arrow**

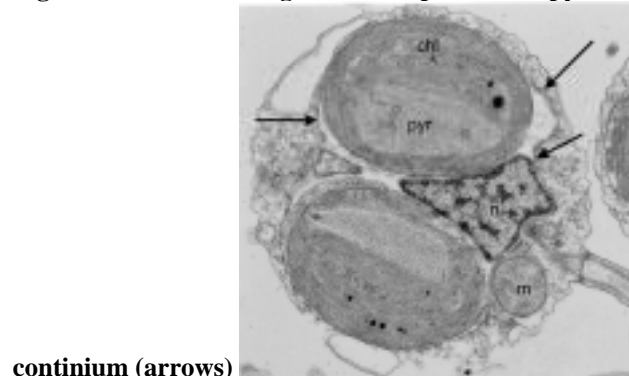


head).

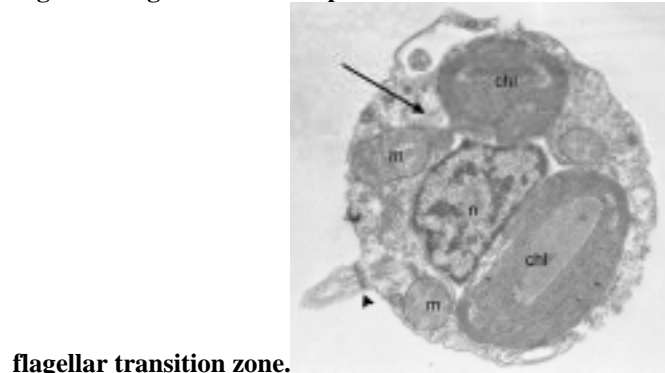


**Figur 2. Detached flagellar hairs**

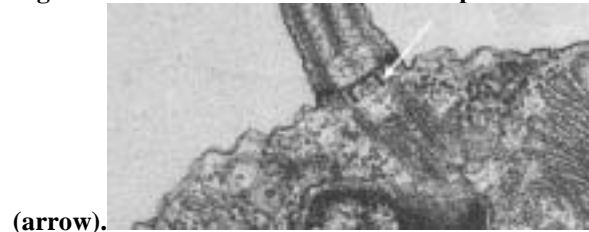
**Figur 3. Section showing two chloroplasts with pyrenoid, mitochondrion, nucleus and the nucleus-ER**



**Figur 4. Flagellar hairs are produced in the nucleus-ER continium (arrow). Arrowhead showing the**



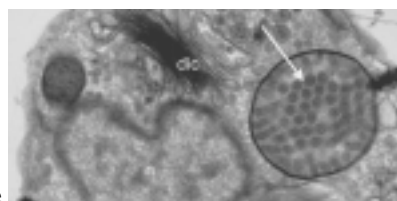
**Figur 5. The transition zone contains a plate and underneath it two rings are found**



**Figur 6. The flagellar hairs are tubular (arrow). The nucleus-ER continium also contain some vesicular**



**Figur 7. Cell infected with virus, n-nucleus, dic-dictyosome**



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