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Molecular and ultrastructural taxonomy of *Telonema subtilis* Griessmann

Abstract (we will write in the end)

### Introduction

The heterotrophic flagellate *Telonema subtilis* has been reisolated from the Roscoff area, one of the original sites sampled in 1913 by its author Griessman. This characteristic species has been reported from many parts of the world and judged by its distribution it appears to be both eurytherm and euryhaline (Buchanan 1966, Thronsen 1969, 1983, Thomsen 1992, Vørs 1992, Patterson et al. 1993, Kuylenstierna & Karlson 1994, Vørs et al 1995, Tong 1997, Tong et al. 1998, Brandt & Sleight 2000). It was studied in the light microscope by Hollande and Cachon (1950) and the first electron micrographs appeared in the early 1990 's (Thomsen 1992, Vørs 1992, Nagasaki et al. 1993).

The present investigation is the first to combine fine-structural characterization of the species with molecular biology, both performed on a culture isolated from Roscoff on the Atlantic coast of France. Judged only by its anatomical features *Telonema subtilis* is difficult to assign to a specific class of protozoa and at present it is classified as an *insertae sedis*.

### Material and methods

**Isolation and culture conditions:** *Telonema subtilis* was isolated into culture together with *Imantonia rotunda* which also serves as its main foodsource. The isolation took place on 2 April 2000 from a water-sample collected at ASTAN (48° 45' N-3° 57' W) and was carried out by Florence Le Gall. The culture is grown in K-medium (Keller 19xx) at 15° C and under white fluorescent light at a quantum flux of approximately xx  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and a x:x L:D cycle.

**Light microscopy:** Live cells were photographed using a Nikon microphot.

**Electron microscopy:** The cells were fixed in 1% glutaraldehyde in growth medium for 1 h and centrifuged to form a pellet. The centrifugation was followed by three rinses à 15 min in growth medium and three additional rinses à 15 min in 0.1M cacodylat buffer (pH 7.8). The pellet was post fixed over-night in 1% osmium tetroxide and 1.5 % potassium ferricyanide in 0.1M cacodylat buffer. Subsequently, the cells were rinsed three times in cacodylat buffer and twice in distilled water before they were left over night in saturated aqueous uranyl acetate over night. The next morning the cells were dehydrated in an ethanol series starting at 30% and concluded by 4 rinses à 10 min in 100 % followed by two rinses à 5 min in propylene oxide. The cells were left overnight in a 1:1 mixture of propylene oxide and Epon embedding resin. Finally, the pellet was given three fresh changes à 1 h before it was polymerised at 60 degrees Celsius for 12 h.

## Results

Fine-structure; *Telonema* is heterotrophic and the culture RCC 404 grows on the scaly haptophyte *Imantonia rotunda* (Fig 1). The cell dimensions of *Telonema* are 3-4 x 6-8  $\mu\text{m}$ . The 2 smooth flagella are a little shorter than the length of the cell and directed backwards during swimming. The flagella are slightly unequal in length. They emerge from one side of a protruding, pointed posterior end and the uptake of food takes place at the opposite end termed the anterior end of the cell. *Telonema subtile* also contains a mitochondrion with tubular cristae, a central nucleus (Fig. ), a conspicuous food vacuole placed in the posterior part of the cell and some inclusions resembling trichocysts (Fig. ). Structures resembling cortical alveoli may be visible underneath the cell membrane (Fig. ). The most prominent feature of the cell is the sub-cortical lamina (Fig.) which encloses the cell. It is composed of layers of microtubules and fibers and do not seem to be connected to the fairly simple flagellar apparatus (Figs).

Emended diagnosis in English and Latin

## Discussion

The sub-cortical lamina is a quite unique character so far only found in another *Telonema* species, *Telonema antarctica* *sp. nov.* not yet formally described (Klaveness, Kamran & Thomsen in prep.), but a somewhat similar structure exists in the enigmatic dinoflagellate *Oxhyrris marina* (Roberts et al. 1993). This new *Telonema* possess many of the same features as *Telonema subtile*, but its flagella are many times longer than the cell. Also, the longest flagellum bears hairs that appear to be tripartite (Klaveness pers. com.). Phylogenetic studies show that *Telonema subtilis* and the new *Telonema* are closely related to each other and placed at the base of the Stramenopiles (~Heterokontophyta) and the Alveolates, the latter including the dinoflagellates and *Oxhyrris marina* (Shalchian-Tabrizi pers. com.). The occurrence of ultrastructural features like tubular cristae, cortical alveoli and tripartite hairs in the genus *Telonema* supports its phylogenetic position as a possible ancestor to the major eukaryotic lineage with tubular mitochondrion cristae. We believe that *Telonema* should be placed among the heterokonts.



Figur 1. *Telonema subtilis* feeding on *Imantonia rotunda*

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