

## A study of the soil flagellate *Phalansterium solitarium* Sandon 1924 with preliminary data on its ultra-structure

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### Summary

*Phalansterium solitarium* Sandon 1924, a common soil flagellate, was isolated and a clonal culture was examined using light and electron microscopy. The first preliminary observations of its ultrastructure show that the cells of *Ph. solitarium* have the same main characters as an earlier investigated species of the genus, *Ph. digitatum* Stein 1878, including a collar-like structure surrounding the basis of the single emerging flagellum, tubular cristae, a single basal body, surrounded by x, y and z zones with radiating microtubules and a fibrillar rootlet associated with the dictyosome. In contrast to *Ph. digitatum*, no spherules were seen either inside or outside the cell of *Ph. solitarium*.

**Key words:** *Phalansterium solitarium*, soil flagellate, soil protozoa, ultrastructure, protists

### Introduction

The genus *Phalansterium*, which contains three named species, was created by Cienkowski (1870) for *Monas consociatum* Fresenius 1858. *Ph. digitatum* Stein 1878 from freshwater and *Ph. solitarium* Sandon 1924 from soil were later been added to the genus. Hibberd (1983) considered it possible that *Phalansterium consociatum* and *Ph. digitatum* are conspecific.

As defined by light microscopy, members of the genus *Phalansterium* are colourless flagellates with a single flagellum. The proximal part of the flagellum is surrounded by a collar-like structure. The cells excrete mucilage, which results in the formation of well-structured colonies in *Ph. digitatum* and *Ph. consociatum*, and in loose unstructured lumps of cells in *Ph. solitarium*. Because of the single flagellum and the collar-like structure, members of *Phalansterium* were previously associated with the choanoflagellates (e.g. Grandori and Grandori, 1934; Starmach, 1985). *Ph.*

*solitarium* was originally described by Sandon (1924) from soil collected at Spitsbergen (Norway). Sandon (1924) also found the species in soil from Rothamsted and the West Indies. It has also been observed in Danish agricultural soil, rain forest soil from Central Africa, and in Casuarina plantations in Australia (F. Ekelund, unpubl. data).

Hibberd (1983) used electron microscopy to show that the cell structure of *Phalansterium digitatum* essentially differs from that of the choanoflagellates. *Ph. digitatum* has mitochondria with tubular cristae whereas the choanoflagellates have flattened cristae in the mitochondria and while the collar-like structure of the choanoflagellates is formed by microvilli that of *Phalansterium* is solid. Skuja (1964), Hibberd (1983), and Karpov (1990) provide a more detailed discussion on the morphology and taxonomy of *Ph. digitatum*. While Hibberd provided strong evidence that *Ph. digitatum* has a unique identity, the taxonomic position of the other species presently assigned to the genus is still unknown.

The purpose of this work was to use light and electron microscopy to establish the taxonomic position of *Phalansterium solitarium*.

### Material and Methods

Cultures of *Phalansterium solitarium* were obtained from barley rhizosphere soil collected at Research Center Foulum (Denmark). Pure cultures were established by repeatedly growing and diluting the flagellates. The cultures were kept on Tryptic Soy Broth (Difco, 0.1 g/l) in Amoeba Saline (Page, 1988) in 50 ml Nunclon flasks (Nunc), 10 ml of liquid per flask. The cultures were kept at 12°C in darkness. In this condition cultures survived for several months without addition of fresh substrate. Dense cultures for light and electron microscopy were obtained by adding the food bacterium, *Pseudomonas chlororaphis* (ATCC no. 43928), to a concentration of about 10<sup>8</sup> to the cultures. *Ph. solitarium* was examined directly in the culture flasks by an inverted Olympus CK2 microscope equipped with phase contrast, and by differential interference contrast on a Zeiss Axioplan microscope. Photography was performed as in Patterson (1982).

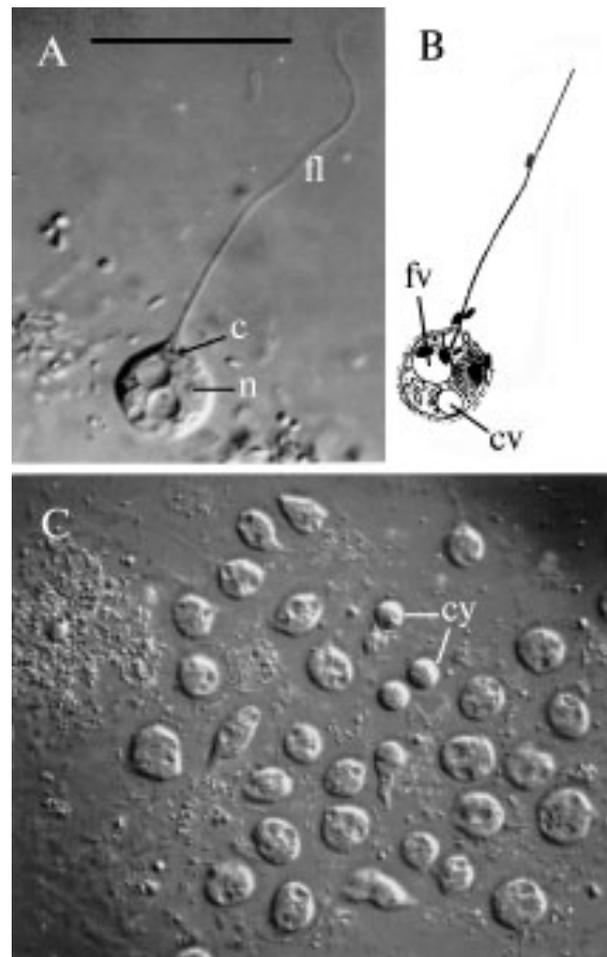
Material for electron microscopy was obtained by adding 5 ml 2% osmium tetroxide in 0.1 M sodium phosphate to 10 ml culture. Cultures were fixed for 2 minutes, then 5 ml 2 % glutaraldehyde was added, and the mixture was fixed for further 30 minutes, fixation was carried out at 5°C. After fixation the sample was centrifuged, rinsed for 30 minutes three times in 0.1 % sodium phosphate, and refixed for 90 minutes in 1% osmium tetroxide, the sample was dehydrated in a cold ethanol series in 20 min. steps and embedded in Spurr's mixture (Vørs, 1992). Sections were cut with a diamond knife using an LKB Ultratome 4, stained with lead citrate and uranyl acetate and examined in a JEOL JEM-100SX transmission electron microscope.

### Results

#### LIGHT MICROSCOPY

Cells are usually more or less globose with an average diameter of 6-8 µm (Figs 1, A-C), a few cells reach a diameter of 11 µm. In old cultures some more elongated to pyriform cells occur (Fig. 1, C). The cells have a single flagellum 4 to 6 times the cell length. Cells tend to resorb their flagellum and become amoeboid when they are placed under a coverslip for microscopical examination. Amoeboid cells, however, were never observed in the culture flasks.

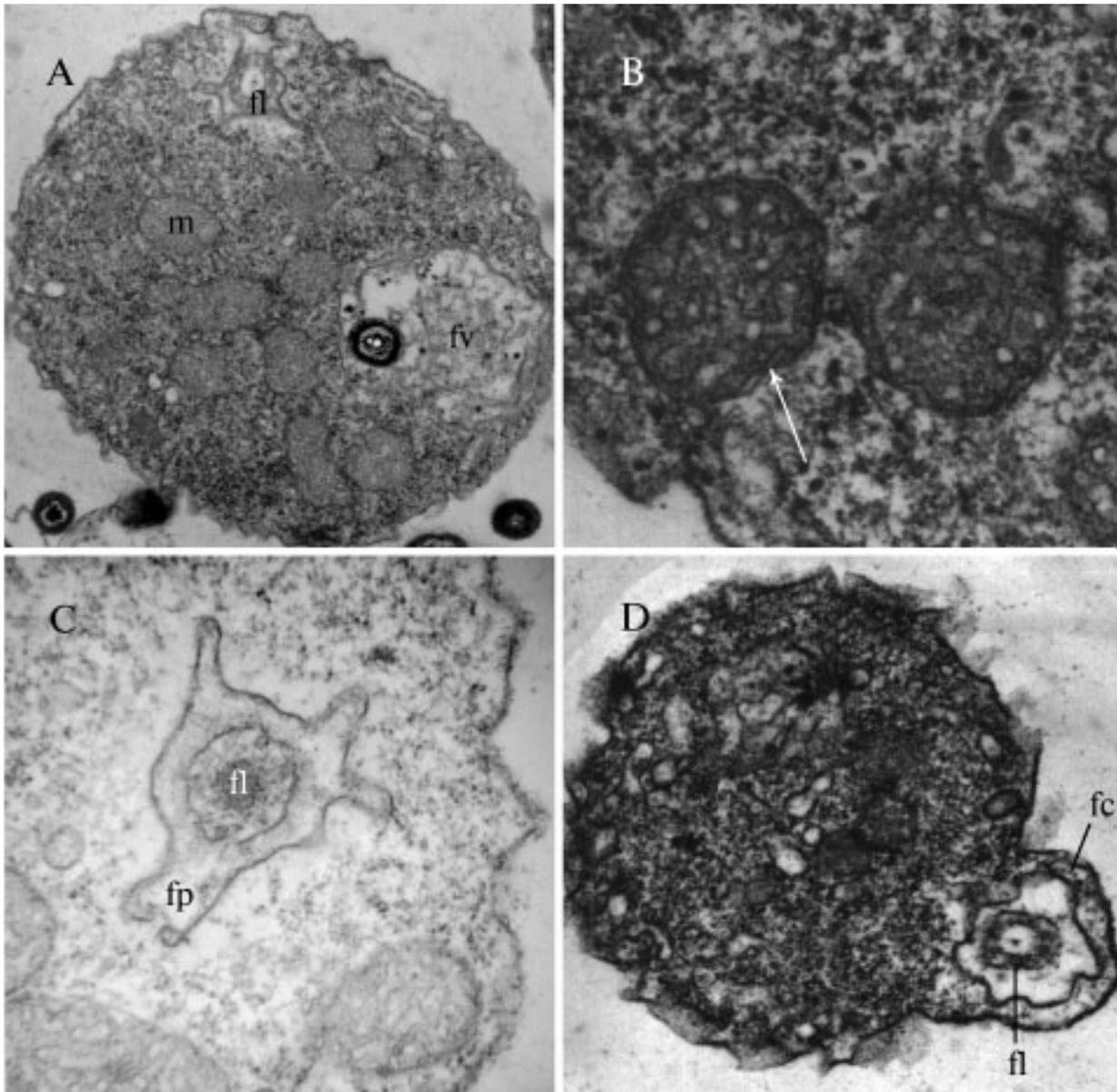
At the base of the flagellum there is a small collar about 1 µm long. The flagellum arises from a small



**Fig. 1.** *Phalansterium solitarium*. Light microscopy. A - trophic cell; B - trophic cell, drawing; C - unstructured colony with both spherical and elongate cells as well as cysts, bacteria and detritus particles are included in the colony. Abbreviations: *c* – collar-like structure, *cv* – contractile vacuole, *cy* - cysts, *fl* - flagellum, *fv* – food vacuole, *n* - nucleus. Scale bar: A - 19 µm, B - 24 µm, C - 36 µm.

pocket. In many individuals the collar and pocket are hardly visible though they are rather conspicuous in some cells (Figs 1, A, C). The nucleus is usually located laterally in the anterior half of the cell. In specimens from cultures to which bacteria have recently been added, one or more conspicuous food vacuoles are present at the anterior end. A single contractile vacuole, and an elaborate vacuole system, which is connected to the contractile vacuole, is located in the posterior part of the cell (Figs 1, A, B). The cytoplasm appears granular. The cells are surrounded by a layer of mucus with a thickness of about 1/2 to 2/3 of the cell diameter.

Most cells in a culture are sessile attached to the surfaces of the culture flask by their mucus layer. Some

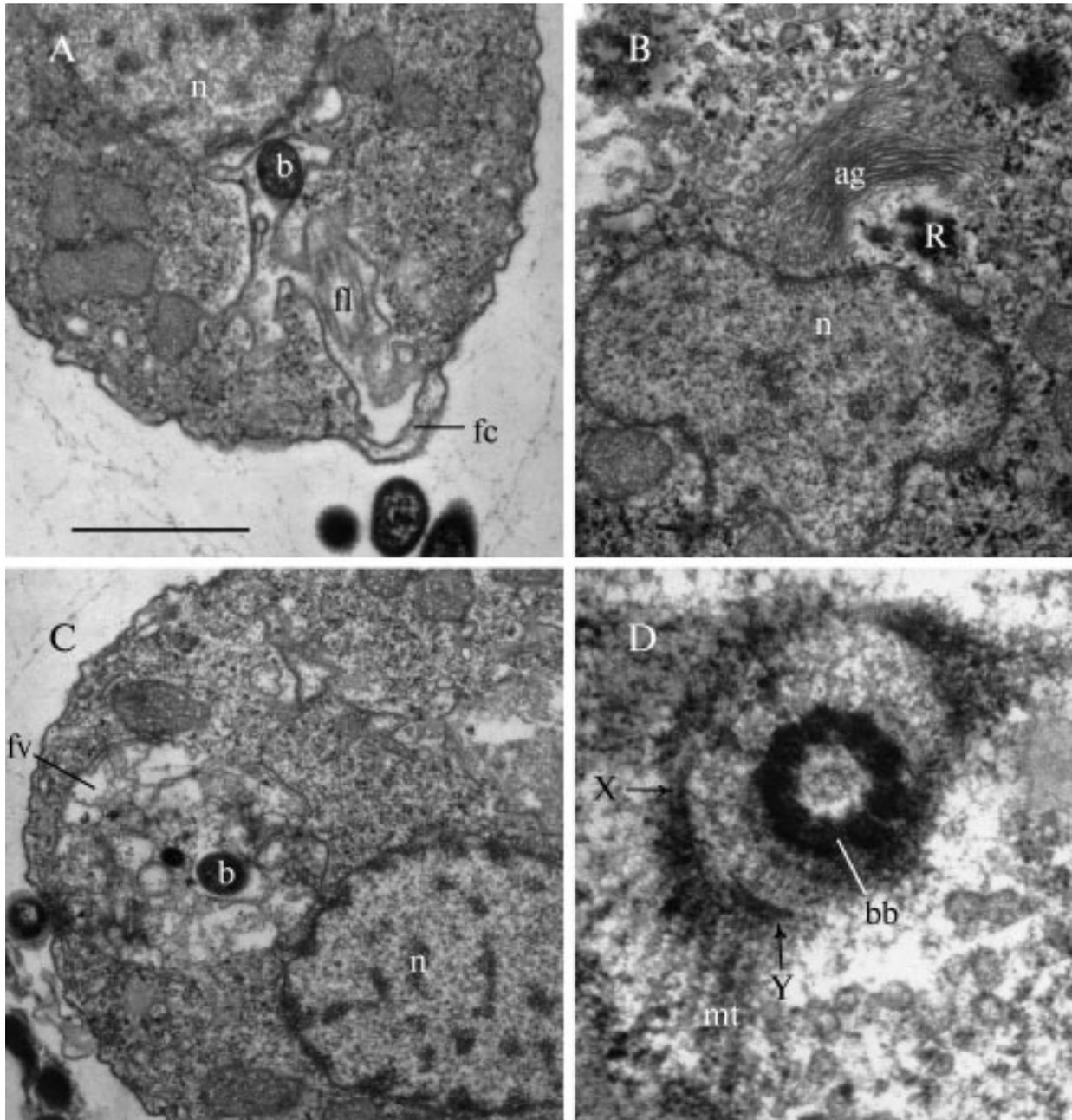


**Fig. 2.** *Phalansterium solitarium*. Electron microscopy. A - whole trophic cell with mitochondrial profiles (*m*); B - mitochondria displaying tubular cristae; *arrow* shows the branching crista; C - flagellum (*fl*) in flagellar pocket (*fp*); D - flagellum (*fl*) surrounded with collar (*fc*). Scale bar: A - 2.7  $\mu\text{m}$ , B - 0.45  $\mu\text{m}$ , C - 0.64  $\mu\text{m}$ .

of the cells, however, swim freely in the liquid phase with the flagellum directed forward. The flagellum beats very stiffly and flagellar beat is hard to observe in healthy specimens, because of the frequency of beating. In sessile specimens the flagellar activity creates a water current towards the base of the flagellum where detritus and bacteria accumulates. Bacteria are often seen moving along the flagellum towards its base. The actual uptake of bacteria through collar and cavity has not been observed. In specimens

with a low beating frequency, it is observed that the flagellum beats with a sine wave.

In cultures with few cells, most cells are single but in dense cultures cells tend to stick together and form unstructured colonies (Fig. 1, C). Bacteria and detritus will also stick to the cells because of the mucilage surrounding them. Cysts are often observed in cultures; they are globular in outline with an average diameter of 4–5  $\mu\text{m}$  (Fig. 1, C). No apparent surface structures are visible. Nucleus, granules and



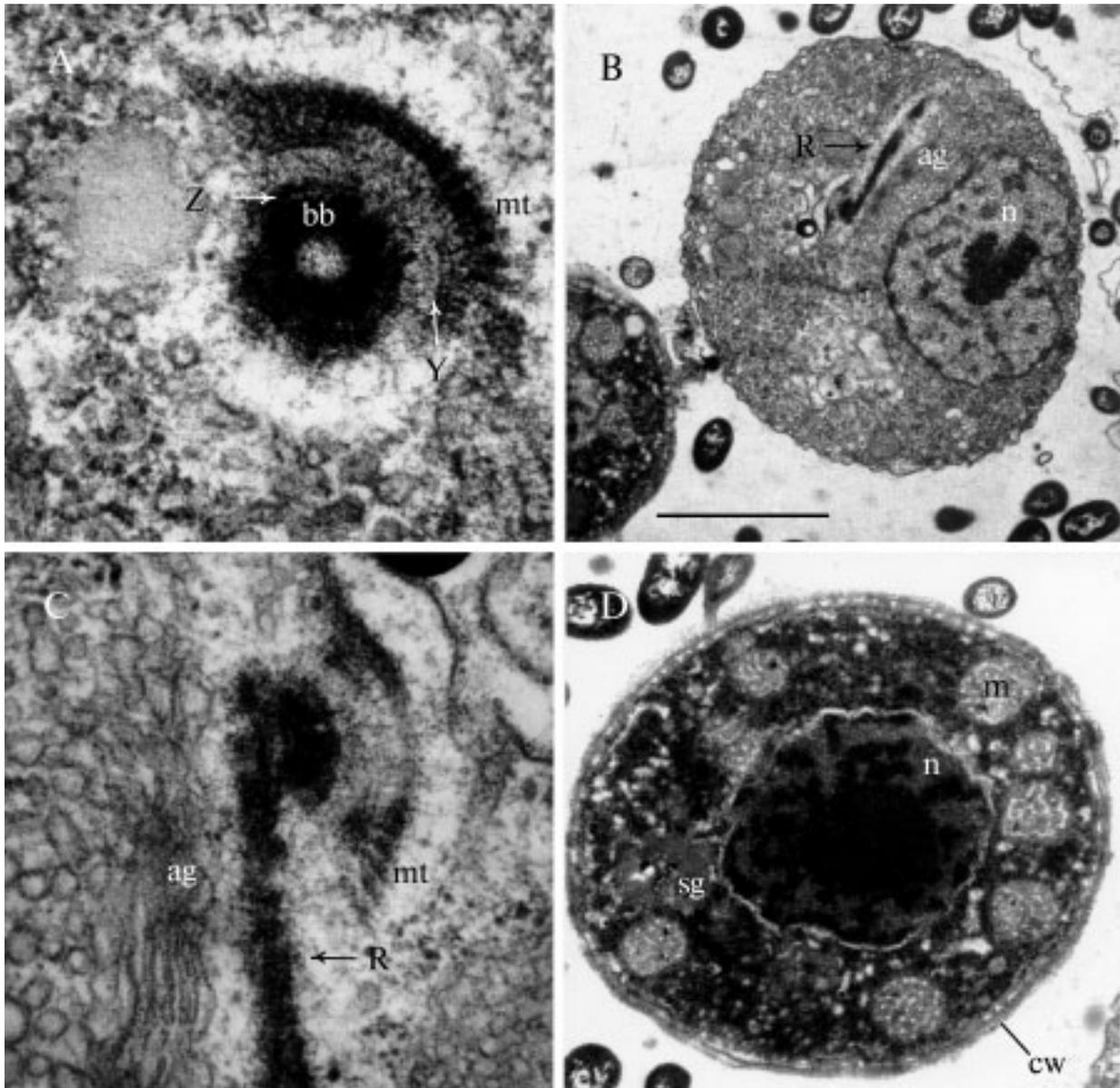
**Fig. 3.** *Phalansterium solitarium*. Electron microscopy. A - trophic cell with bacterium (*b*) in the flagellar pocket; B - dictyosome of the Golgi apparatus (*ag*) in association with fibrillar root (*R*) and nucleus (*n*); C - whole trophic cell displaying food vacuole (*fv*) and nucleus; D - basis of flagellum (*bb*) displaying the *x* and *y* band as well as the radiating microtubules (*mt*). Scale bar: A - 1.8  $\mu$ m, B - 1.8  $\mu$ m, C - 3.2  $\mu$ m, D - 0.3  $\mu$ m.

an active contractile vacuole are often present in the cysts.

#### ELECTRON MICROSCOPY

The only visible structure that surrounds the cell is the smooth plasmalemma. The conspicuous mucus

layer, easily visible in the light microscope, is hardly visible using the present method of fixation. The single flagellum arises approximately in the centre of the cell and ascends through a hollow tube which ends in a flagellar pocket and collar (Figs 2, C, D). Bacteria are often observed in the tube, and this is probably how they get into the food vacuoles (Fig. 3, A), though the passage



**Fig. 4.** *Phalansterium solitarium*. Electron microscopy. A - flagellar basis (*bb*) and the *y* and *z* bands with radiating microtubules (*mt*); B - whole trophic cell with section of the oblique fibrillar root (*R*), associated with dictyosome (*ag*), and nucleus (*n*) with clearly visible nucleolus; C - enlarged fragment of 4B with focus on the flagellar basis with the oblique fibrillar root (*R*) and the dictyosome (*ag*); D - cyst with cyst wall (*cw*), mitochondria (*m*), nucleus (*n*) and storage granules (*sg*). Scale bar: A - 0.4  $\mu$ m, B - 2.7  $\mu$ m, C - 0.5  $\mu$ m, D - 1.8  $\mu$ m.

of a bacterium into the cavity has not been observed on living material.

The flagellar basal body is surrounded by three concentric structures: two outer similar structures (X and Y), each consisting of a pair of parallel fibers and an inner ring (Z) with a more amorphous structure. About 50 microtubules originate close to the two outer rings (Figs 3, D, 4 A). The flagellar root originates at the base of the flagellum and continues obliquely into

the cell. The dictyosome is located very close to the flagellar root (Figs 4, B, C). The nucleus is slightly elongated and usually located laterally in the cell between dictyosome and cell membrane. It contains a nucleolus and several smaller dense staining bodies (Figs 3, B, C). In the posterior part of the cell the elaborate vacuole system is located (Fig. 3, C). In trophic cells several mitochondrial profiles are present, they are randomly distributed in the cytoplasm. The mitochondrial profiles

are about 0.5  $\mu\text{m}$  long and have tubular dichotomously branching cristae (Figs 2, A, B).

The cysts are circular in outline like the trophic cells. The cytoplasm of cysts is denser than that of active cells; the wall is about 0.1  $\mu\text{m}$  thick. Mitochondria are visible in cysts and so is the nucleus, which has a more irregular outline than in active cells. Bodies staining lighter about 1.5  $\mu\text{m}$  in diameter, which are observed in cysts but not in active cells are probably storage granules. Several small vacuoles are seen in the cysts, too (Fig. 4, D).

## Discussion

The flagellate described here, isolated from a Danish agricultural soil, is very similar to *Phalansterium solitarium* as described by Sandon (1924). However, it differs in some characters. Sandon found the flagellum/cell-length ratio to be 3–4 while the ratio in the organism described above was 4–6. Since the flagellate will often retract its flagellum quickly after being put under a coverslip, this difference cannot be considered an important character. Sandon observed that the flagellum tapered towards the anterior end; this was not observed in the present study. While the Danish isolate had a very conspicuous contractile vacuole, this was not the case with Sandon's isolate. Sandon reported his organism to have a length of 10–15  $\mu\text{m}$  while the above described organisms usually had a length of 6–8  $\mu\text{m}$  and only occasionally reached a length of 10–11  $\mu\text{m}$ . Despite the differences, the organism described here overlaps with *Phalansterium solitarium* sensu Sandon in characteristics and they are best regarded as belonging to the same species.

*Phalansterium solitarium* is in some respects strikingly similar to *Ph. digitatum* when observed with the light microscope. Both species have a single flagellum which beats stiffly, the flagellum is surrounded by a narrow collar extending from the cytoplasm; and in both species the flagellum arises from a funnel-shaped pocket. Another shared feature is the mucus layer embedding the cells.

*Phalansterium solitarium* and *Ph. digitatum* also share many ultrastructural characters. In both species the single flagellum emerges from the bottom of a funnel-shaped pocket that forms a cytoplasmic collar at the distal end, the basal body is surrounded by three concentric rings (X, Y and Z) with radiating microtubules and the fibrillar rootlet is associated with a dictyosome. *Ph. solitarium* also seems to lack a second basal body but this was not finally confirmed by the serial sections in this study.

On the other hand, certain differences between *Phalansterium digitatum* and *Ph. solitarium* are very

pronounced, and the ultrastructural similarity is somewhat surprising considering the many differences revealed by light microscopy. *Ph. digitatum* is always colonial, forming colonies with a characteristic regular morphology, while *Ph. solitarium* appears either single or in masses of cells of in distinct shape. The collar of *Ph. digitatum* is larger (4  $\mu\text{m}$  vs. 1  $\mu\text{m}$  long). In *Ph. digitatum*, spherules are embedded in the mucilage, whereas no spherules are present in *Ph. solitarium*. *Ph. digitatum* cells are normally elongate, while *Ph. solitarium* is normally spherical in shape. Flagellum/cell-length ratio is different, 2 in *Ph. digitatum* and 4–6 in *Ph. solitarium*. Two contractile vacuoles could be observed in *Ph. digitatum* and only one in *Ph. solitarium*.

The ultrastructural examination revealed that the spherule-producing vesicles which occupy much of the cell volume in *Ph. digitatum*, are absent in *Ph. solitarium*. This is not surprising since no spherules were observed in the mucilage of *Ph. solitarium*. The branching tubular cristae in *Ph. solitarium* were not observed in *Ph. digitatum* (Hibberd, 1993) and the fibrillar rootlet is more prominent in *Ph. solitarium* than in *Ph. digitatum*; the system of vacuoles connecting to the contractile vacuole is also more elaborate in *Ph. solitarium* than in *Ph. digitatum*.

Cienkowski (1870) and Skuja (1964) both described a very characteristic bipartite cyst in *Phalansterium digitatum*, while Hibberd (1983) observed no cysts. The cysts formed by *Ph. solitarium* displayed no bipartite structure. A common feature, however, between cysts of the two species is the activity of the contractile vacuole, even long time after formation, Skuja (1964) observed this phenomenon for *Ph. digitatum*.

Sandon (1924) did not observe feeding in *Phalansterium solitarium*, and there for supposed (1927) that the organism took up dissolved organic matter. Despite careful and prolonged observation, feeding was not observed in this study; however, since bacteria were observed moving along the flagellum, and bacteria taken up by the flagellate were observed in the flagellar groove, the flagellate obviously feeds on bacteria. It seems likely that solid particles are taken up in the flagellar groove and that the food vacuoles are formed from the bottom of the groove.

Only a few original descriptions of heterotrophic flagellates are based on soil material. Some of these flagellates, e.g. *Apusomonas proboscidea* Aléxéieff 1924 and *Allantion tachyploon* Sandon 1924, were later reported from aquatic environments (Patterson and Zölffel, 1991; Vørs, 1992); while others, e.g. some species of *Cercomonas*, are described so inadequately that they are impossible to identify. Soil flagellates like *Adriamonas peritocrescens* Verhagen, Zölffel, Brugerolle et Patterson 1993, *Apusomonas australiensis* Ekelund et

Patterson 1997 and *Sciviamonas terricola* Ekelund, Patterson and Vørs 1997 (in: Ekelund and Patterson, 1997) have been described so recently that aquatic protozoologists have had no time to search for them. Thus, *Phalansterium solitarium* is one of the very few flagellates with a clear light microscopical identity originally described from soil material (Foissner, 1991) that has been known for a long period. This suggests that the reason it has never been found in aquatic systems is that it is a true soil flagellate. The reasons, however, why this flagellate should be specially adapted to the soil environment are not obvious, except perhaps that it does not form large colonies as its relative *Ph. digitatum*.

### Acknowledgements

Part of this study was carried out at the School of Biological Sciences, University of Sydney. The support of the Australian Biological Resources Study (ABRS), the Australian Research Council (ARC) and the Danish Environmental Research Programme 1998–2001 (BIOPRO) is acknowledged. Lisbeth Haukrogh is thanked for her skillful technical assistance, and David J. Patterson and Øjvind Moestrup are thanked for their valuable comments on the manuscript.

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