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Ultrastructure of Endosymbiotic *Chlorella* in a *Vorticella*

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SYNOPSIS. Observations were made on the ultrastructure of a species of *Vorticella* containing endosymbiotic *Chlorella*. The *Vorticella*, which were collected from nature, bore conspicuous tubercles of irregular size and distribution on the pellicle. Each endosymbiotic algal cell was located in a separate vacuole and possessed a cell wall and cup-shaped chloroplast with a large pyrenoid. The pyrenoid was bisected by thylakoids and surrounded by starch plates. No dividing or degenerating algal cells were observed.

Index Key Words: *Vorticella*; *Chlorella*; symbiosis; ultrastructure.

SYMBIOTIC relationships between algae and aquatic invertebrates have long been of interest, and a number of excellent reviews are available (2, 5). Ochsman (8) pointed out that the ultrastructural study of these symbiotic associations is useful in determining the host-symbiote interface, the influence of the invertebrate host on symbiote structure and reproduction, morphologic evidence for movement of nutrients between organisms, and evidence of the ability of the host to digest the symbiotes. In fresh-water hosts, zoochlorellae of the genus *Chlorella* are the most common symbiotic algae, and they have been observed in a number of protozoan genera (2, 5). Ultrastructural studies of endosymbiotic *Chlorella* have been reported for *Hydra viridis* (8) and *Paramecium bursaria* (4), and research on these 2 symbiotic associations has recently been summarized (3, 6). These studies have contributed substantially toward an understanding of the relationships mentioned by Ochsman (8) for fresh water endosymbiotic associations, but more such studies are needed (4).

Zoochlorellae have been infrequently reported in the fresh water ciliate *Vorticella*. In their taxonomic review of this genus Noland & Finley (7) reported that zoochlorellae were observed in the putative species *Vorticella chlorostigma* Ehrenberg, and *Vorticella fasciculata* O. F. Müller. Noland & Finley (7) concluded that the specific identity of these 2 putative species must be established on grounds other than intracellular zoochlorellae, because the stability of the symbiotic relationship was in doubt. With the exception of a report of some unpublished observations by Ochsman (8), the ultrastructure of vorticellids containing algal endosymbiotes has not been previously studied. In the present report the fine structure of a green *Vorticella* is compared with that of *H. viridis* (8) and *P. bursaria* (4) with particular attention to the relations between host and symbiote discussed by Ochsman (8). Although the focus of this paper is not taxonomic, a brief description of the dimensions and features of the green *Vorticella* studied by light microscopy will also be given.

MATERIALS AND METHODS

Repeated collections of a green *Vorticella* were made in a pond on the Loesell Field Laboratory of Eastern Michigan University in Ypsilanti, Michigan. This *Vorticella* typically occurred in large groups which were easily seen as bright green, irregularly shaped macroscopic patches on the undersides of old floating leaves of *Nuphar*, on submerged leaves of *Sparganium*, and on roots of *Spirodela*. Pieces of leaves bearing *Vorticella* patches were excised, fixed overnight in 2% (v/v) glutaralde-

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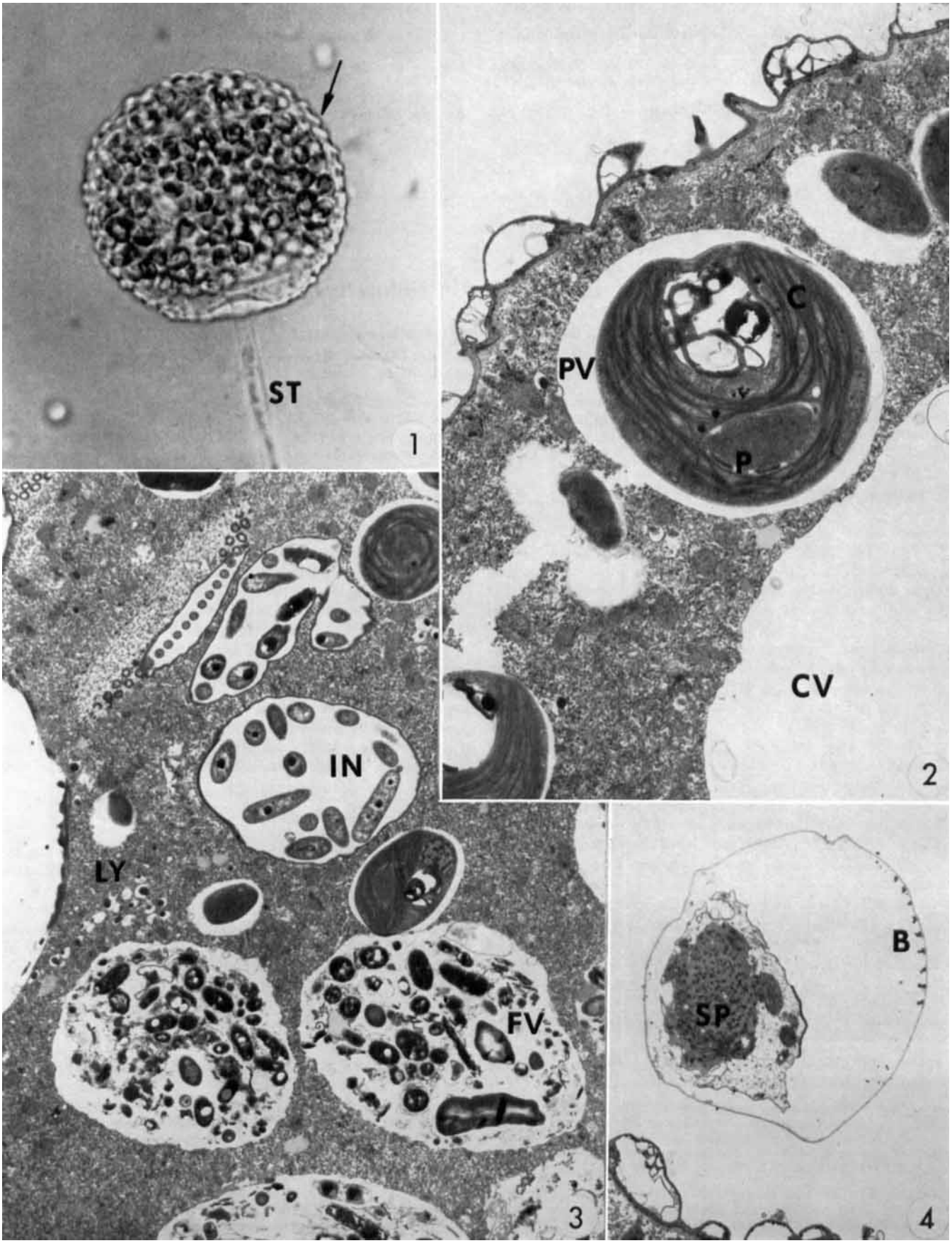
Fig. **1**. Photomicrograph of a single green *Vorticella*. Note the stalk (ST) as well as the distribution of algae and pellicular tubercles (arrow). Bright field, axial illumination. ×800.

Figs. **2-4**. [Electron micrographs of ultrathin sections through various parts of green *Vorticella*.] **2**. An endosymbiotic *Chlorella* is seen in a perialgal vacuole situated near the contractile vacuole (CV). The cup-shaped chloroplast (C) of the alga contains a pyrenoid (P) bisected by thylakoids and surrounded by small starch plates ×15,800. **3**. Sections of the infundibulum (IN) contain intact bacteria, while bacteria in various stages of digestion are evident in food vacuoles (FV). Lysosome-like bodies (LY) are near the food vacuoles. ×6,300. **4**. Spasmoneme (SP) and bâtonnets (B) are seen in a cross-section of a ciliate's stalk. ×8,300.

Figs. **5**, **6**. [Electron micrographs of cross-sections of green *Vorticella*.] **5**. Near the adoral end of the ciliate, note sections of vestibulum (VE) and pellicular tubercles (T), which contain electron-dense material. ×5,700. **6**. Near the aboral end basal bodies (BB) mark a part of the trochal band. Four algae are lodged in perialgal vacuoles with apposed membranes (arrows); these cells may be the products of a recent division. Note also a part of a food vacuole (FV) and the lysosome-like bodies (LY). ×8,600.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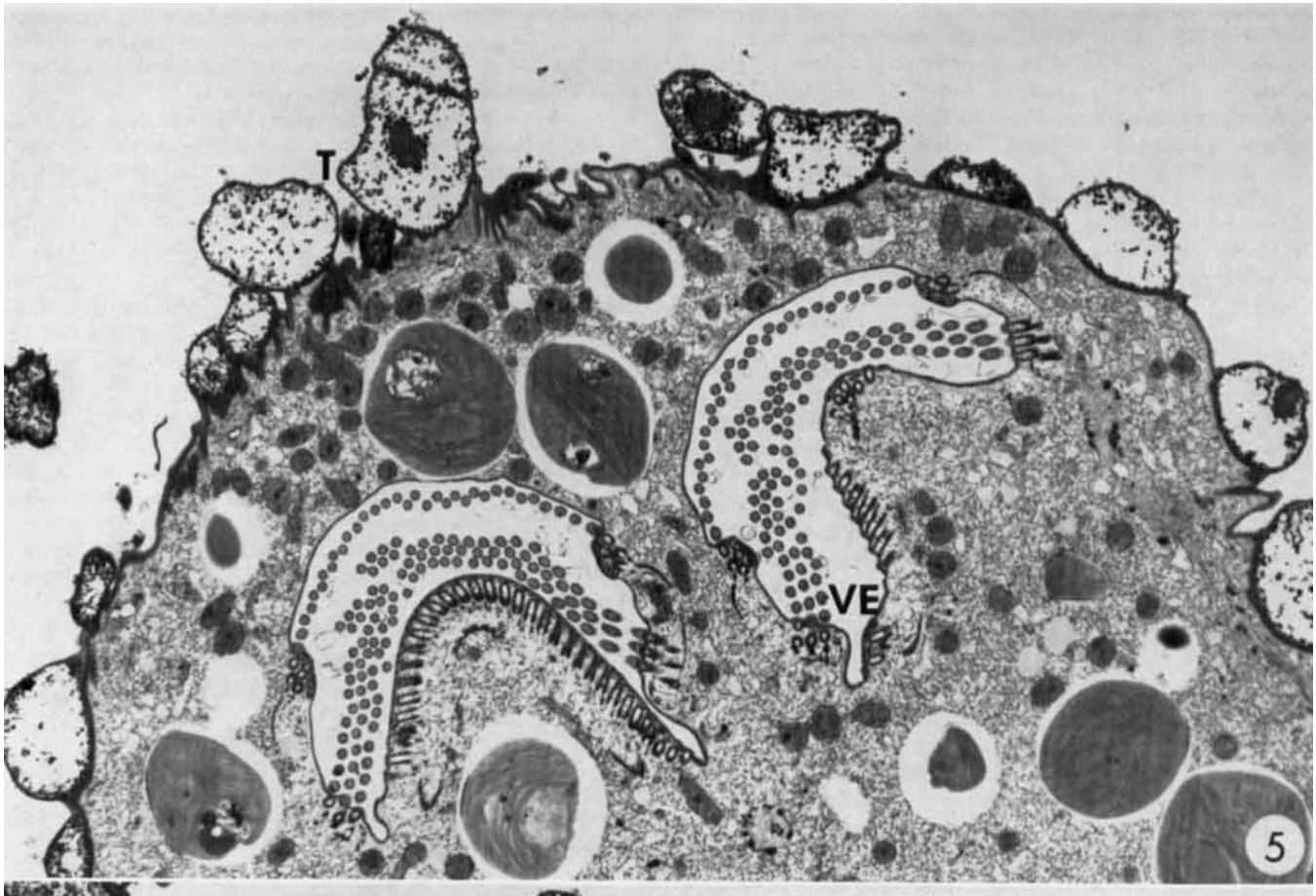
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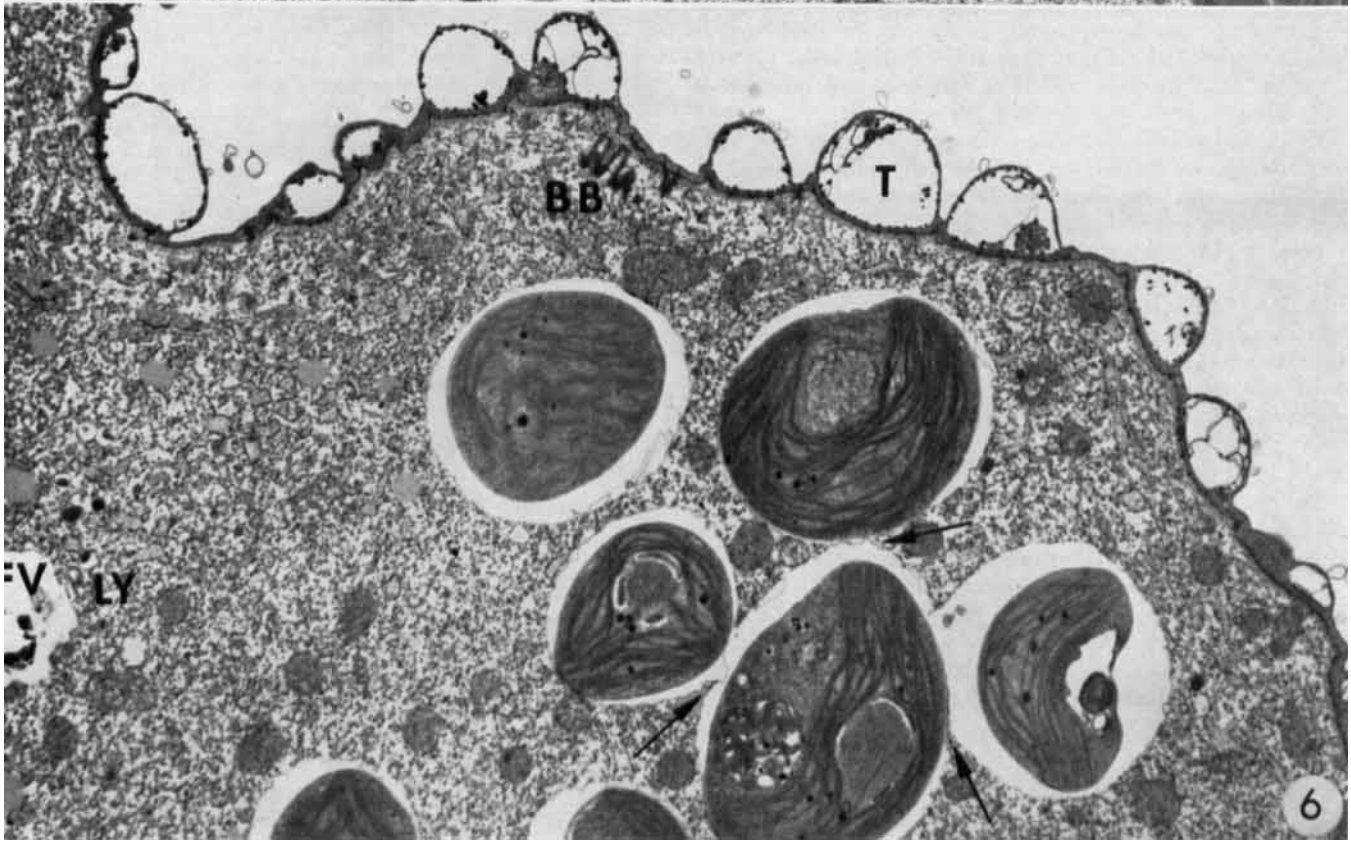
BB
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LY
6

hyde in 0.05 M cacodylate buffer at pH 7.3, washed with buffer, fixed with 2% (w/v) OsO_4 in the same buffer, and washed again with buffer. The specimens were then stained *en bloc* for 15 min in 0.5% (w/v) aqueous uranyl acetate, dehydrated in an ethanol series, and embedded in Epon (11), using propylene oxide as a transitional solvent. Sections were cut on an LBK Ultratome equipped with a diamond knife, stained briefly with lead citrate (10), and examined in an RCA EMU 3G electron microscope operated at 50 kV. The protozoa were oriented in the blocks so that cells were approximately cross-sectioned. Sections were made of at least 10 individuals.

RESULTS

Each green *Vorticella* was attached to the leaf substrate by an unbranched, spirally contractile stalk. Typical shape, size, and distribution of algal cells is shown in a photomicrograph of a single green *Vorticella* (Fig. 1). The mean length of 10 vorticellids, excluding stalk, was 53.4 μm, and the mean width at the widest point of 19 vorticellids was 50.4 μm. The extended stalk was 4.4 μm wide and at least 175 μm long. The length of one complete turn of the spasmoneme spiral was ~ 29 μm. The pellicle was ornamented with tubercles of irregular size and distribution (Figs. 1, 5, 6).

The general ultrastructure of the cell body and stalk of the green *Vorticella* was similar to that of *Vorticella convallaria* (Linnaeus) (1), except for the presence of pellicular tubercles (Figs. 4-6). These tubercles were found to contain varying amounts of an electron-dense granular material. Intact bacterial cells could be observed in the infundibulum, and degraded bacterial cells were present in the food vacuoles surrounded by a single membrane (Fig. 3).

The endosymbiotic *Chlorella* were distributed throughout the cytoplasm of the green *Vorticella*. Each algal cell was contained in a separate vacuole structurally similar to the food vacuoles (Fig. 3). No algal cells, or remnants of algal cells, were observed in food vacuoles containing bacteria, and no bacterial cells were seen in the vacuoles with the algal cells.

The endosymbiotic *Chlorella* were oval in shape. The mean length of 6 cells was 4.1 μm, and the cell walls were 0.02 μm thick. Each alga contained a single nucleus and a single cup-shaped chloroplast with pyrenoid (Fig. 2). The pyrenoid was bisected by thylakoids and surrounded by concavo-convex starch plates. Only small amounts of starch were observed. Mitochondria, Golgi bodies, and ribosomes were also present. Some algal cells contained vacuoles partially filled with an electron-dense material which was not well preserved during processing for electron microscopy. No dividing algae were noted; however, 4 cells with appressed perialgal vacuolar membranes might represent products of a recent division (Fig. 6).

DISCUSSION

The ultrastructure of the endosymbiotic *Chlorella* in the green *Vorticella* described in this paper is very similar to that of the symbiotic *Chlorella* of *P. bursaria* (4) and of *H. viridis* (8). In these hosts the symbiotic algae possess a cell wall. The cell wall of the endosymbiotic *Chlorella* in *Vorticella* appears comparable in thickness to those of the free-living algal species illustrated by Pickett-Heaps (9). Retention of a cell wall in symbiotic *Chlorella* may be related to its capability for independent existence or, as Karakashian et al. (4) suggested, the incompleteness of adaptive changes of *Chlorella* to symbiosis. As in *P. bursaria* and *H. viridis* the symbiotic *Chlorella* in *Vorticella* have a single cup-shaped chloroplast. The pyrenoid of the alga in *Vorticella* is very similar to that in *P. bursaria*. Pyrenoids were absent from the CAL strain of *H. viridis* (8), although they were present in the other 2 strains examined.

The algal symbiotes of *Vorticella*, like those of *P. bursaria* (4) and *H. viridis* (8), lie singly in perialgal vacuoles (4). In *P. bursaria* Karakashian et al. (4) commonly found algae undergoing digestion in food vacuoles along with bacteria, but in culture *P. bursaria* continuously releases *Chlorella* into the medium where they can be ingested by the ciliates. Since the *Vorticella* in this report were obtained in nature, there was probably less chance of ingesting algae than if these ciliates had been in culture. No dividing algal cells were seen in *Vorticella*. As in the case of digestion of algae, however, their division would more likely be observed in rapidly multiplying laboratory cultures than in cells isolated from nature.

There have been relatively few ultrastructural studies of symbiotic *Chlorella* (4, 8). The present report of the ultrastructure of endosymbiotic *Chlorella* in a species of *Vorticella* should facilitate comparison with other algal symbioses. The limited observations suggest that endosymbiotic *Chlorella* in different invertebrate hosts are ultrastructurally similar.

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