Ultrastructure of Flagellated Chrysophytes. IV. *Chrysosphaerella*

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ABSTRACT—Information on the fine structure of naturally occurring cells of *Chrysosphaerella brevispina* and *C. longispina* is given and some new data are presented. The ultrastructure is generally typical for the Chrysophyceae although both perinuclear cisternae and cytoplasmic endobiotic bacteria occur. A paraflagella rod occurs in the photoreceptor area of the short, second flagellum. Siliceous scale and bristle origin are illustrated.

Introduction

The motile, colonial genus *Chrysosphaerella,* of the class Chrysophyceae, was erected by Lauterborn (1) for the type species *C. longispina.* He first illustrated this alga three years later (2). The cells were described as bearing two siliceous rods, a single long flagellum, and containing two plastids, a stigma, and several vacuoles. Two species have been added to the genus since.

Korshikov (3) effectively emended the generic and specific descriptions by noting the presence of a second minute whiplash flagellum and the presence of more than two, usually five, bristles (rods) per cell; and by illustrating the scales and bristles. He also described a second species, *C. brevispina.* Fott and Ludvik (4) published the first electron micrographs of *Chrysosphaerella* scales and bristles. Wujek (5) published the first electron micrograph illustrating the fine structure of the cell. Based on light and electron microscopy, Nicholls (6) revised the generic description in his review of the genus. Later, Nicholls (7) transferred the unicellular taxa to the genus *Spiniferomonas,* retaining only the colonial forms in *Chrysosphaerella.*

Comparative ultrastructural studies have considerably extended our knowledge of chrysophycean structure (8). In this study, cells from natural populations are used to describe the fine structure of *Chrysosphaerella longispina.* The present observations also add to those of Preisig and Hibberd (9) on *C. brevispina,* which are included in their ultrastructural study of *Paraphysomonas* species. The study, while largely substantiating previous chrysophycean cell ultrastructure, gives additional information on flagellar structure and scale and bristle origin.

Materials and Methods

Natural populations of *Chrysosphaerella brevispina* Korsh. and *C. longispina* Laut. were collected in the Lake Itasca region, Minnesota. Fixation was started either directly in the field or upon return to the laboratory. Material was fixed and embedded as previously described (5). Some sections were stained sequentially with uranyl acetate and lead citrate. All sections were examined on a RCA 3F or Philips 300 electron microscope.

Results and Discussion

The fine structure of *Chrysosphaerella* (Figure 1) is similar to that first described by Gibbs (10) for *Ochromonas,* and reviewed in Hibberd's (8) survey of chrysophycean ultrastructure and illustrated by Preisig and Hibberd (9) for *C. brevispina.* The cell's two plastids contain three thylakoids per lamellae, one of which is continuous around the margin of the plastid (Figure 1); surrounding the plastid is an ER cisternae (PER; Figure 3). Promastigonemes are also observed within this compartment (Figure 1). Located adjacent to the PER near the flagellar bases are two bundles each composed of five microtubules (Figure 2). No pyrenoid is observed.

Cells are joined posteriorly, forming colonies. Figures 1, 4, and 5 show that adjacent cell surfaces are free of scales and bristles. No adhesive material is visible in the micrographs. Harris and Bradley (11) have illustrated mucilage by light microscopy in this region; it is presumably derived from the cell's muciferous bodies.

A large chrysolaminarin vesicle fills the posterior part of the cell (Figures 1, 3). The contractile vacuole system occupies a position directly above the anteriorly placed nucleus (Figure 1).

Food vacuoles in both species are distinguishable from other cytoplasmic vesicles by their irregular contents; most of these vesicles contain unrecognizable objects (Figures 1, 4, 5), although one vacuole contains an ingested scale (Figure 4). All food vacuoles are observed in the posterior end of the cells. Bacteria (Figures 6, 7) are observed in the lower two-thirds of the cell's cytoplasm; they are possibly endosymbiotic. Intracellular bacteria have been previously reported as occurring within the perinuclear cisternae of *Paraphysomonas* (9) and *C. brevispina* (12), but the cytoplasmic

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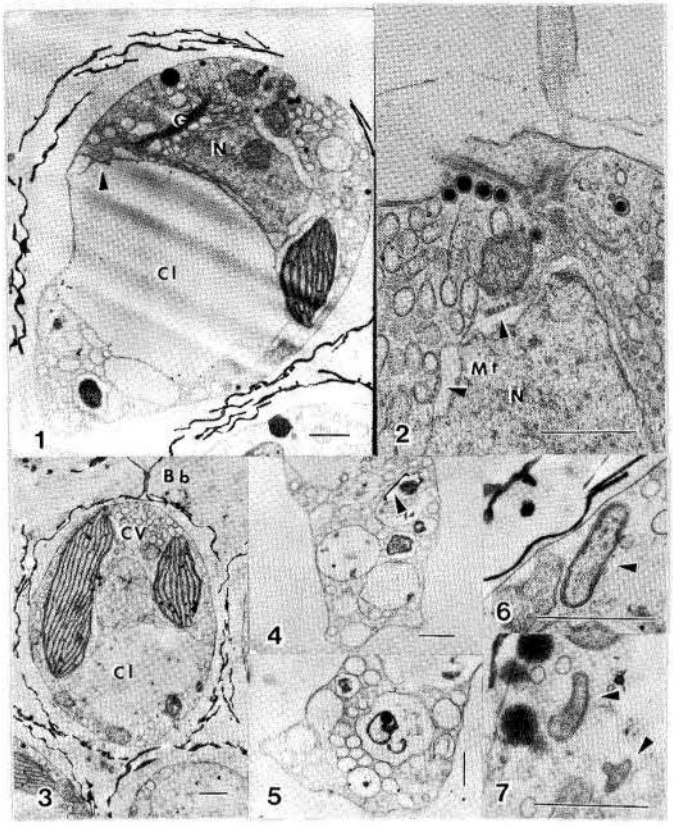


Figure 1. *C. longispina.* Longitudinal section showing large chrysolaminarin vesicle (C1) and vesicle containing promastigonemes (arrow). Numerous food vacuoles are present in the posterior end of the cell. **Figure 2.** *C. brevispina.* Anterior end of a cell showing two flagella. Two groups of five microtubules (Mt) lie near the outer nuclear (N) membrane. **Figure 3.** *C. longispina.* Oblique section showing contractile vacuole (CV), chrysolaminarin vesicle (C1) and the base of a bristle (Bb). One food vacuole is present. **Figures 4, 5.** *C. brevispina.* Posterior portion of two cells with numerous phagocytic vacuoles, with one containing an ingested scale (arrow). **Figures 6, 7.** *C. brevispina.* Endosymbiotic bacteria in the cytoplasm. Scale bar = 10 μm.

bacteria observed here appear to differ in morphology from the nuclear bacteria. Endoplasmic bacteria are not seen within the PER.

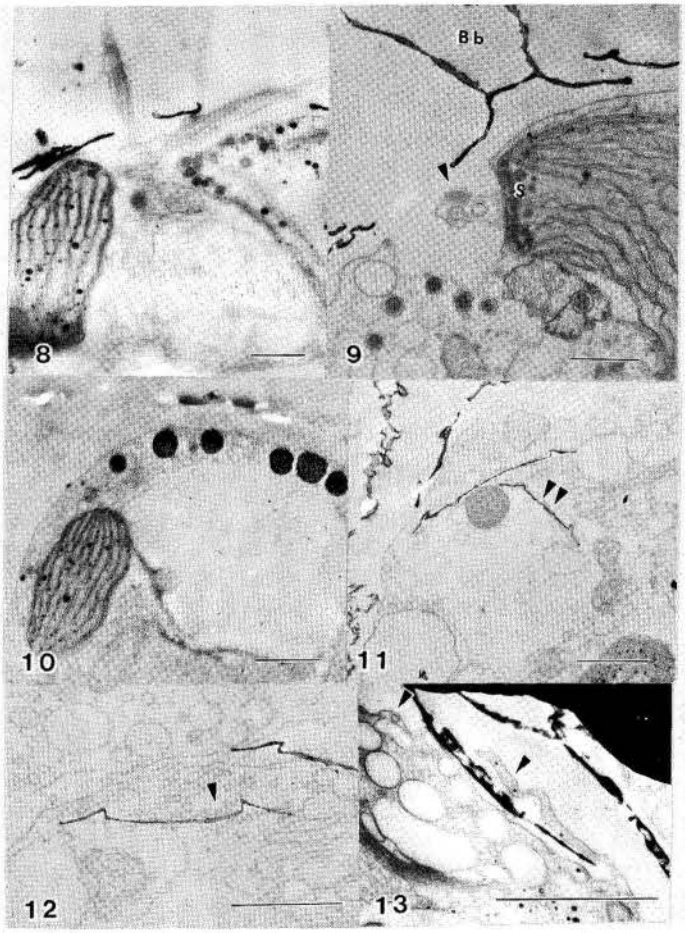
There are two flagella inserted laterally into the cell at an oblique angle to each other (Figures 2, 8); the flimmer flagellum is about the same length as the body of the cell. The whiplash flagellum, not mentioned in Lauterborn's (1) original description, was observed by Korshikov (3) in his emended description of the genus. Electron microscopy confirmed Korshikov's observation (5). This short flagellum bears a swelling (photoreceptor) at its proximal end that lies in a shallow depression in the cell surface, beneath which lies the eyespot (Figure 8).

A dense paraflagellar rod is present at the opposite side of the axoneme from the flagellar swelling (Figures 8, 9). Hibberd (13) observed a flagellar swelling in *Ochromonas* that contained "spotted contents"; the flagellar swelling of *C. brevispina* sometimes contains one to three small vesicles (Figures 8, 9).

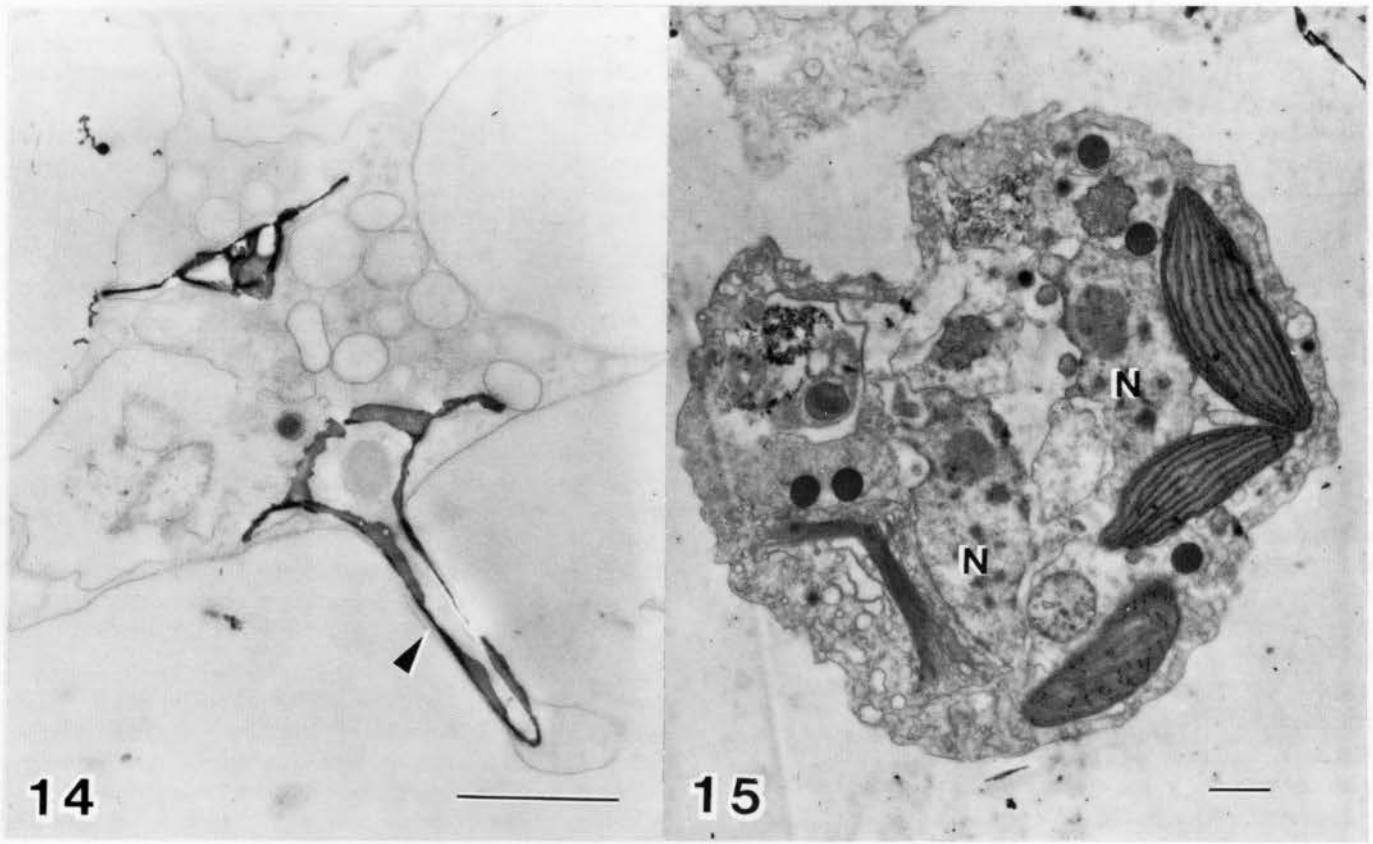
To establish the siliceous nature of the scales and bristles, an entire block of material was immersed in 10% hydrofluoric acid (HF) for 36 hours prior to sectioning (14). Figure 8 shows that normally opaque scales are dissolved by HF treatment. Other acids (HCl, H_2SO_4, HNO_3) have no observable effect on the scales and bristles. This result indicates that both scales and bristles are composed of silica.

Scale and bristle origin have been reported in other chrysophytes, e.g., *Synura, Paraphysomonas, Mallomonas* (15, a review; 9). All the taxa described to date have scales and bristles that are endogenous in origin and acquire their final shape inside vesicles of as yet unknown origin. Wujek and Kristiansen (16) suggest that in some taxa the scales and bristles may originate in the PER. *Chrysosphaerella brevispina* and *C. longispina* scale and bristle seem to have the same origin. Synthesis occurred near the outer surface of a plastid, beginning at the anterior end and proceeding toward the posterior end of the cell. Although the cell possesses two parietal plastids, only one is involved in scale or bristle production.

Scale formation stages are comparable to those observed in *Synura* and *Mallomonas* (15-18). Initial setting up occurred at the PER surface that folds to shape the edges of the scale (Figure 11). Small vesicles of Golgi origin are seen near, and sometimes connected to, the scale vesicle, apparently fusing with it (Figure 12). Although microtubules and microfila-



Figures 8, 9. *C. brevispina.* Longitudinal and cross sections of flagella. Note the paraflagella rod (arrow) in the photoreceptor. The flagellar swelling also contains small vesicles. The short, second flagellum arises from a small depression adjacent to the eyespot (S). **Figure 10.** *C. longispina.* Cell treated with hydrofluoric acid demonstrating the siliceous nature of the scales and bristles. **Figures 11, 12.** *C. brevispina.* Scale vesicles in various degrees of development. Note in figure 12, the large scale vesicle contains an almost completely developed scale (double arrow) and the smaller vesicles (arrow) are fusing with the larger. **Figure 13.** *C. brevispina.* Liberation of scale at surface of plasma membrane (arrow). Scale bar = 10 μm.



Figures 14, 15. *C. brevispina.* Figure 14. Detail of a bristle being extruded from the posterior end of a cell. The bristle vesicle (arrow) is still present and has not fused with the plasma membrane. Figure 15. Late telophase cell. Note the absence of scales. Scale bar = 10 μm.

ments may sometimes be located near scale vesicles, the quality of the fixation does not permit their origin to be determined.

Figure 13 shows a scale in the process of being extruded. The scale vesicle has fused with the plasma membrane, and one edge of the scale is projecting during the exocytosis. Although scales can be released anywhere on the cell surface, more than 95% were observed being released onto the portion of the cell surface that is devoid of scales.

In principle, bristle formation appears to take place in the same way as scale formation. The bristle vesicle, however, appears to be a little more complicated because of the presence of a tubular shaft, which flares abruptly to form the *guard* at its base. From the guard it contracts gradually or rapidly to the union with the *pummel,* which is discoidal and serves to anchor the bristle to the cell (Figures 1, 9). The portion between the guard and pummel may be referred to as the *grip.* The bristle shaft is tubular and not flat. The release of a bristle from the cell is shown in Figure 14. The bristle vesicle has not yet fused with the plasma membrane. Once the bristle has taken its definite position, body scales will overlap the pummel (Figure 3). In Figure 14 cytoplasmic contents are still visible in the bristle's shaft. However, once released, no cytoplasm is present.

Dividing cells normally maintain their armor of scales (12). However, one cell, observed in late telophase, lacked its cell covering (Figure 15).

Ultrastructural features confirm the position of *C. longispina* and *C. brevispina* in the Chrysophyceae *sensu* Hibberd (8). The most striking feature of *Chrysosphaerella* is the morphogenesis of its scales and bristles. Although the endoplasmic reticulum and coalescence of Golgi vesicles are involved in scale/bristle formation, neither the scale/bristle vesicle's shape nor its size corresponds to those of a mature scale/bristle.

The cells forming the colonies are clearly stalked and are all joined posteriorly as in the closely related genus *Synura.* The colonies in the material examined do not appear to be surrounded by mucilage. The insertion of the cell's flagella at approximately right angles to each other differs markedly from such apparently related genera as *Mallomonas* and *Synura.* In these, the flagella are inserted parallel into a shallow pit at the extreme anterior end of the cell and lack an eyespot. Scales have also been demonstrated on the subequal flagella of *Synura* (19), but as yet have not been observed on *Chrysosphaerella.*

All of these observations support the position of *Chrysosphaerella* in the family Paraphysomonadaceae (9).

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