Chlorella neustonica Bourrely (Chlorophyta, Oocystaceae) from Montemar Cove, Chile

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Abstract.- The genus *Chlorella* (Beijerink) is found in a state of taxonomic confusion. Identification of numerous strains lack validity. In this study *Chlorella neustonica* (Bourrely) from Montemar cove, Valparaíso, Chile (32° 57' S – 71° 33'W), is described using both light and electron microscopic techniques. Cells are spherical to lightly ovoid (10 µm), surrounded by a cell envelope and limited externally by a trilaminar pattern, probably sporopollenin. The chloroplast is smoth and cupshaped, with a large pyrenoid crossed by a pair of thylakoids. Asexual reproduction occurs by division of the mother cell into four or more, rarely eight individuals. Both in the field and in laboratory culture, cells are distributed in a green monolayer on the surface of the seawater. After the original description of *C. neustonica* by Bourrely (1957), this paper describes the fine structure of this algae. *C. neustonica* is reported for the first time in the Pacific coast of Chile, extending its distribution to South America.

Keywords: Biodiversity, microalgae, isolation, culture, ultrastructure, new record, Chile.

Resumen.- En la microalga *Chlorella* (Beijerink), existe una gran confusión taxonómica y muchos de los nombres dados a las estirpes de colecciones de cultivo, presentan variaciones nomenclaturales. En este contexto, usando técnicas de microscopía óptica y electrónica de trasmisión, se describe a *Chlorella neustonica* (Bourrely) de caleta Montemar Valparaíso, Chile (32° 57' S – 71° 33'W). Las células se caracterizan por ser esféricas a ligeramente ovoides (10 µm), rodeadas por una pared celular limitada externamente por una capa con un patrón estructural trilaminar, probablemente esporopolenina. El cloroplasto es liso y con forma de copa, provisto de un pirenoide atravesado por un par de tilacoides. La reproducción asexual ocurre por divisiones de la célula madre en cuatro y a veces ocho células. En el laboratorio y en terreno las células se presentan formando una capa verde espumosa sobre la superficie del agua. En este trabajo se caracteriza por primera vez la estructura fina de *C. neustonica*, complementando la descripción original de Bourrely (1957), efectuada bajo microscopía óptica. *C. neustonica*, es también registrada por primera vez en las costas del Pacífico de Chile, extendiendo su rango de distribución a Sud América.

Palabras clave: Biodiversidad, microalga, aislación, cultivo, ultraestructura, nuevo registro, Chile.

Introduction

Chlorella was one of the first algae to be isolated as a pure culture (Beijerink 1890). The small size of cells and the difficulty in discerning the inner structure led to the conclusion that morphological and structural characteristics are not sufficient for taxonomical evaluation of the species (Fott & Nováková 1969). The first investigators of *Chlorella* were microbiologists; they studied the isolated species through physiological methods. Previously no monograph that includes morphological, ultrastructural and molecular data of the genus have been produced, some identifications keys are incomplete and are thus misleading. The taxonomy of *Chlorella* has been in such confusion for years that many of the names given to various strains in culture collections are of little value. For instance, *C. pyrenoidosa* Chick 251 (T) is the same as *C. vulgaris* C-211-8b (G), *C. emersonii* C-211-8b (C) and C. *fusca* var. *vacuolata* S. & K. 211-8b (G). Similarly, *C. pyrenoidosa* Chick 1230 (T) is the same as *Chlorella* strain Tx-7-11-05, *C. vulgaris* f. *tertia* 211-8k (C), *C. sorokiniana* Shihira and Krauss 211-8k (G), *C. pyrenoidosa* 211-8k (C) and *C. sorokiniana* Shihira and Krauss 22521 (M) (Oh-Hama & Miyachi 1988).

Although some literature reviews indicate about one dozen marine species (Chrétiennot – Dinet 1990), at present time, only six have been studied formally through electron microscopy: *C. nana* Andreoli, Rascio et Casadoro (Andreoli *et al.* 1978, Rascio *et al.* 1979), *C. ovalis* Butcher (Rascio *et al.* 1980a), *C. stigmatophora* Butcher (Rascio *et al.* 1980b), *C. spaerckii* Alvik (Rascio *et al.* 1980c), *C. salina* Butcher (Rascio *et al.* 1980d) and *C. marina* Butcher (Rascio & Casadoro 1981).

The original description of *Chlorella neustonica* by Bourrely (1957), presents a brief diagnosis of the species and specific characteristics by photonic

microscopy . The specific epithet of *C. neustonica* was suggested by Bourrely (1957) due to the peculiar distribution of cells in a monolayer on the surface of the water.

The marine chlorellae have not been studied in Chile. The present paper deals with the fine structure of *C. neustonica,* which is not yet characterized by electron microscopy.

Material and Methods

C. neustonica was isolated from a sample of seawater from the intertidal zone of Montemar Cove, Chile, (32° 57' S – 71° 33'W). Cultures were grown at Provasoli medium, 20 ± 2 °C and 60 µmol m^{-2} s^{-1} of continuous light. Isolation and culture of clone cells were obtained by capillary pipette. Light microscopical observations were made with a Nikon Labophot microscope. For transmission electron microscopy (TEM), cells were fixed two hours in 2% glutaraldehyde with 2 % formaldehyde in a 0.1 M cacodylate buffer (pH 7.2). Samples were then washed in the buffer, postfixed two hours in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) and dehydrated in a graded series of ethyl alcohol. The sample was embedded in a Medcast resin, the thin sections cut with a Reitcher OMU2 Ultrotome, were poststained with uranyl acetate and lead citrate and examined with a Zeiss EM 900 electron microscope operating at 50 kV.

Results and Discussion

Under the light microscope the cells of *C. neustonica* from Montemar cove appear roundish (10µm) (Figs. 1a, 2). The vegetative cells of *C. neustonica* like *C. salina* are larger than other chlorellae. The size of *C. salina* range from 9 to 11 µm while the other species vary from a minimum of 1.5 µm in *C. nana* (Andreoli *et al.* 1978) to a maximun of 6.0 µm in *C. ovalis* and *C. stigmatophora* (Rascio *et al.* 1980 a, b). Internal structures can be distinguished, revealing a cupshaped chloroplast, which occupies about half of the cell volume. Inside the chloroplast, a large and roundish pyrenoid is prominent (Figs. 1a, 2), whereas in *C. ovalis, C. spaerckii* and *C. marina* the pyrenoids have not been observed by light microscopy (Rascio *et al.* 1980a, c, Rascio & Casadoro 1981). As in *C. stigmatophora* a small spot like stigma is observed in the plastid (Rascio *et al.* 1980b). Asexual reproduction occurs by division of mother cells into four, or more rarely eight daughter cells that are liberated by the bursting of the cell wall (Figs 1b, 3).

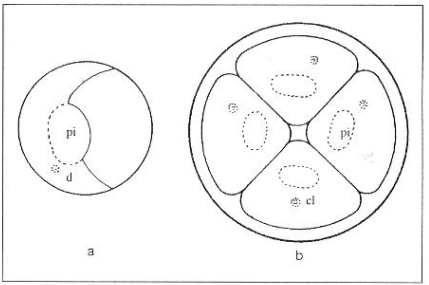

Figure 1

***Chlorella neustonica* . a) Scheme of vegetative cell; b) asexual reproduction. pi, pyrenoid; cl, chloroplast.**

Chlorella neustonica. a) Esquema de la célula vegetativa; b) reproducción asexual. pi, pirenoide; cl, cloroplasto.

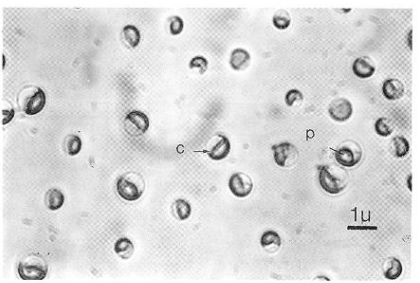

Figure 2

***Chlorella neustonica.* External morphology of vegetative cell and asexual reproduction. Chloroplast (c), pyrenoid (p); x 400.**

Chlorella neustonica. Morfología externa de la célula vegetativa y reproducción asexual. Cloroplasto (c), pirenoide (p); 400 x.

When seen by transmission electron microscopy, the chloroplast of *C. neustonica* is surrounded by a double membrane. Inside the chloroplast the lamella are composed of two to four thylakoids interconnected by single thylakoids. Between these llamella, some osmiophilic granules are observed, which probably are lipid reserves (Fig. 4). The pyrenoid appears to be surrounded by a conspicuous starch sheath usually formed by three to four segments. A pair of thylakoids penetrate the pyrenoid matrix (Figs. 4, 5, 6). The presence of one orange body which was interpreted

under light microscopy as a stigma is not confirmed in the fine structure of *C. neustonica*. A single group of small osmiophilic granules observed between the thylakoids was not clear enought to confirm the presence of a stigma (Fig. 4). Ultrastructural studies by Rascio *et al.* (1980b) in *C. stigmatophora* also did not confirm the presence of the stigma described by Butcher (1952) under light microscopy. Dodge (1973) has indicated that the eyespot is characteristic of mobile cells and gamets or zoospores of inmobile cells, assigning it a photoreceptor function.

Figure 3

***Chlorella neustonica*. Asexual reproduction. The arrow indicates the formation of four vegetative cells; x 400.**

Chlorella neustonica. Reproducción asexual. La flecha indica la formación de cuatro células vegetativas; 400 x.

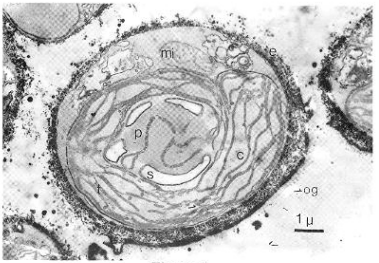

Figure 4

***Chlorella neustonica*. Ultrastructure of vegetative cell. Chloroplast (c), pyrenoid (p), starch (s), thylakoids (t), mitochondrion (mi), cell envelope (e), vacuolar system (v), osmiophilic granules (og); x 4000.**

Chlorella neustonica. Ultraestructura de la célula vegetativa. Cloroplasto (c), pirenoide (p), almidón (s), tilacoide (t), mitocondria (mi), envoltura celular (e), sistema vacuolar (v), gránulos osmiofílicos; 4000 x.

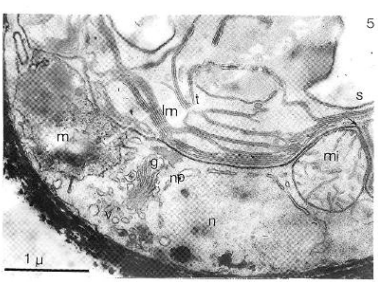

Figure 5

***Chlorella neustonica*. Ultrastructure of vegetative cell. Nucleus (n), nuclear pores (np), mitochondrion (mi), Golgi apparatus (g), vesicles (v), microbody (m), lamelle (lm), thylakoids (t), starch (s); x 24000.**

Chlorella neustonica. Ultraestructura de la célula vegetativa. Núcleo (n), poros nucleares (np), mitocondria (mi), aparato de Golgi (g), vesículas (v), microcuerpo (m), lamela (lm), tilacoide (t), almidón (s); 24000 x.

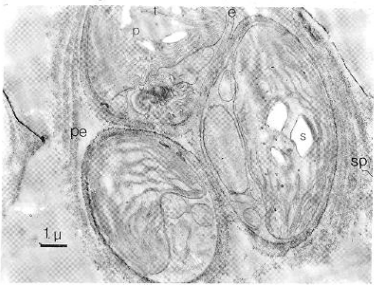

Figure 6

***Chlorella neustonica*. Ultrastructure of dividing cells. Parental envelope (pe), cell envelope (e), sporopollenin (sp), pyrenoid (p), starch (s), thylakoids (t); x 11900.**

Chlorella neustonica. Ultraestructura de la envoltura celular y células en estado reproductivo. Envoltura parental (pe), envoltura celular (e), esporopolenina (sp), pirenoide (p), almidón (s), tilacoides (t); 11900 x.

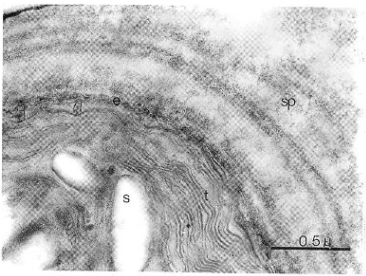

Figure 7

***Chlorella neustonica*. Ultrastructure of cell envelope of dividing cells. Cell envelope (e), sporopollenin (sp), starch (s), thylakoids (t); x 36000.**

Chlorella neustonica. Ultraestructura de la envoltura celular y células en división. Envoltura celular (e), esporopolenina (sp), almidón (s), tilacoides (t); 36000 x.

In *C. neustonica* the nucleus is observed near the inner cell wall surface (Fig. 5), and normally one or seldom, two mitochondrial profiles are visible in a cell section (Figs. 5, 6). In *C. neustonica* like *C. stigmatophora*, *C. spaerckii*, and *C. nana*, the mitochondrial reticulum, if present, is not very irregular, and eventual ramifications are always near the plastid surface (Rascio *et al* 1980b, c, Rascio *et al.* 1979). A Golgi apparatus is visible between the nucleus and a dense microbody (Fig. 5). Two vacuolar systems appear developed neighboring the cell wall (Fig. 4). In *C. salina* a huge central vacuole occupies most of the cell volume and appears to be completely surrounded by a plastidial profile (Rascio *et al.* 1980d).

In the outer surface, over the cell wall, *C. neustonica* is surrounded by a cell envelope of variable thickness (*ca.* 0.6 µm). This structure presents two external dense electron regions surrounding an internal hyaline zone (Figs. 4, 6 and 7). This characteristic has been observed in the marine species *C. nana* in which the trilaminar pattern presents asymetric external bands not evident in *C. neustonica*. Although in this study acetolysis techniques were not employed, analysis made in *C. nana* and other freshwater species have shown that this structure is composed of sporopollenin. This kind of cell envelope is a significant taxonomic characteristic for *C. nana* and *C. neustonica*, distinguishing them from other marine species. This complex material is typically found in the walls of spores and pollen of higher plants, contributing greatly to their viability and preservation over long periods of time (Pickett–Heaps 1975).

After the original description by Bourrely (1957), this paper describes for the first time the fine structure of *C. neustonica*. The morphological characteristics which clearly separate this microalga from previosly described marine chlorellae are, large cellular size, regular shape of its chloroplast, large size of the pyrenoid and reduced vacuolar system. *C. neustonica* is reported for the first time in the Pacific coast of Chile, extending the distribution to South America.

Acknowledgements

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