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Chlorella nana sp. nov. (Chlorophyceae): a New Marine *Chlorella*

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Abstract

Chlorella nana sp. nov. (Chlorophyceae) from the Northern Adriatic Sea (about 8 km off Venice) is described, using both light and electron-microscopical techniques. Its prominent feature is the very reduced size (1.5-3 μ).

Moreover it has an either thick or thin cell wall, a parietal, cup-shaped chloroplast without pyrenoid. The cells are spherical and propagate by division into two, sometimes four, individuals.

Introduction

In spite of the large number of *Chlorella* species there is very little information on those from marine or brackish environments.

Till now, to the authors' knowledge, only five marine species (Shihira and Krauss 1965) have been observed and studied: *Chlorella spärckii* (Alvik 1934), *Chlorella stigmatophora* (Butcher 1952), *Chlorella salina* (Butcher 1952), *Chlorella ovalis* (Butcher 1952), *Chlorella marina* (Butcher 1952).

We have recently isolated a *Chlorella* from the Northern Adriatic Sea. As its size and structure markedly differ from those of the known marine chlorellae, we think it is a new species.

Diagnosis

Chlorella nana sp. nov.

Cellula sphaerica vel sphaeroidea, membrana glabra, tenui vel conspicua; chromatophoro laete viridi, parietali, ollaeformi cellulam 1/2 implente. Pyrenoide nulla visa. Incrementum est per cellulae matris in 2 vel 4 cellulas filias divisionem. Cellula 1.5-3 µ diametro.

Cells spherical or spheroidal, surrounded by a smooth, thin or thick cell wall. Chromatophore parietal, bright green, cup-shaped, occupying about 1/2 of the cell. No pyrenoid has been observed. Propagation by division of mother cell into two or, more rarely, four daughter cells which are liberated by the bursting of the cell wall.

Isolation: from the Northern Adriatic Sea, 8 km off Venice.

Holotype: Text – Fig. 4–5.

Materials and Methods

Chlorella nana sp. nov. was isolated from a sample of sea-water from the Northern Adriatic Sea, 8 km off Venice.

The light microscopy observations were carried out by means of a Leitz Ortholux microscope. For the TEM (Transmission Electron Microscopy) observation, the material was fixed two hours in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 6.9), washed in the buffer, then postfixed two hours in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 6.9), dehydrated in a graded series of ethyl alcohols and propilen oxide. Staining with uranyl acetate was made while dehydrating with alcohol, 75%. The sample was embedded in a Epon-Durcupan ACM mixture; the thin sections, cut with a LKB Ultratome III, were poststained with lead citrate and examined with a Hitachi HS 9 electron microscope operating at 75 kV.

For the SEM (Scanning Electron Microscopy) observation, the material was fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 6.9), dehydrated using a series of ethyl alcohols in steps of 10% up to absolute alcohol, then in amyl acetate and critical point dried. The Danton Vacuum Critical Point Apparatus of the Fondazione

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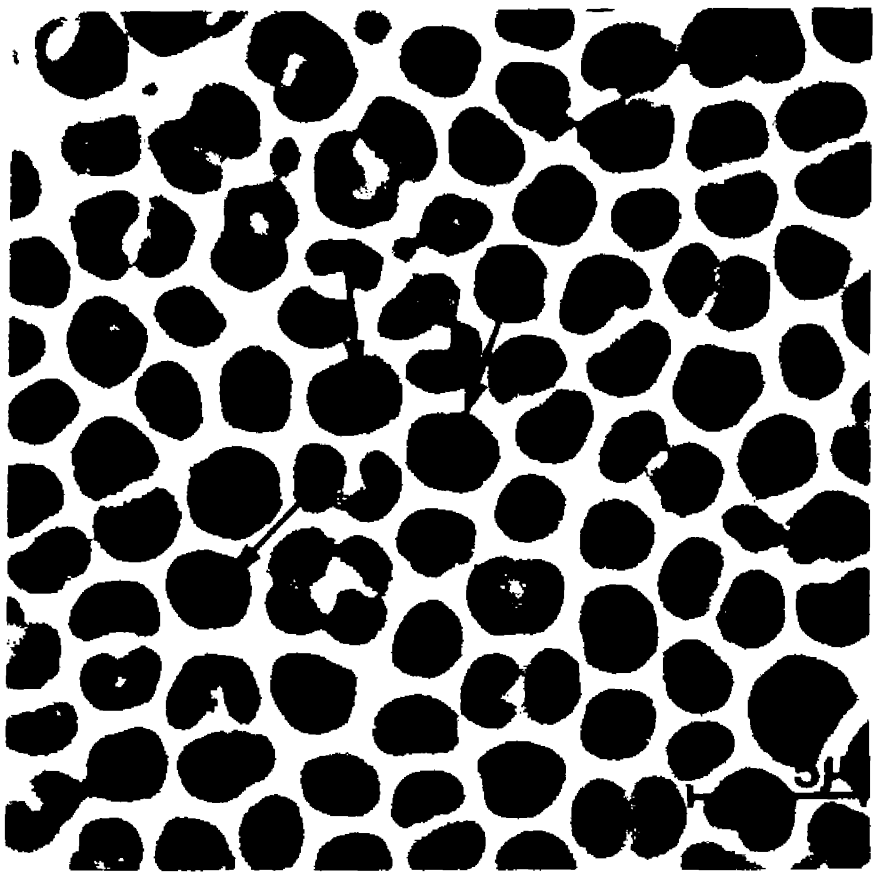

Fig. 1. Photograph of living cells. They appear roundish with a light region (arrow). Cells divided into two or four individuals can also be seen (× 3,000).

Bozza, Istituto di Fisica Tecnica of the Politecnico of Milan, was used. The sample was coated with carbon and gold in a vacuum evaporator type Jeol JEE 4B and observed by a Jeol scanning electron microscope JSM-U3 at the „Centro Universitario Grandi Apparecchiature Scientifiche" (CUGAS) of the University of Padua, at an accelerating voltage of 25 kV.

Results and Discussion

When seen in the light microscope (Fig. 1), the cells appear roundish and sometimes show a light region that can be either central or lateral. Moreover, cells divided in two and sometimes four individuals may be found.

As the cells are very small-sized (1.5-3 μ), internal structures cannot be distinguished in the light microscope. When seen by the SEM, the cells appear spherical and with a smooth surface (Fig. 2). Sometimes it is possible to see large and lenghtened cells that show an equatorial groove; they can be interpreted as dividing organisms (Fig. 3). These cells also show a smooth external surface. The chloroplast could be observed only by means of transmission electron microscopy. It is parietal, cupshaped and occupies almost half of the cell volume (Fig. 4 and 5). No pyrenoid could be observed. An interesting characteristic is that the cell wall can be either thin or thick (Fig. 4 and 5). Comparing the various marine chlorellae we could evidence considerable differences (Tab. I) between *Chlorella nana* and other known species (Shihira and Krauss 1965). In fact *Chlorella nana* sp. nov. partakes only of the roundish shape with *Chlorella salina* (Butcher 1952) and shares only the absence of pyrenoid with *Chlorella ovalis* (Butcher 1952), *Chlorella spärckii* (Alvik 1934) and *Chlorella marina* (Butcher 1952). Finally, *Chlorella nana* sp. nov. participates with *Chlorella stigmatophora* (Butcher 1952) only in the propagation by division of the cell into two or four individuals. So we can conclude that, as the characteristics of *Chlorella nana* are quite different from those of the marine chlorellae described to date, it really represents a new species.

We thought to name it *Chlorella nana* sp. nov.; the specific epithet having been suggested by its very small size.

Acknowledgements

We wish to thank Professor Pierre Bourelly (Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle – Paris) for his helpful suggestions.

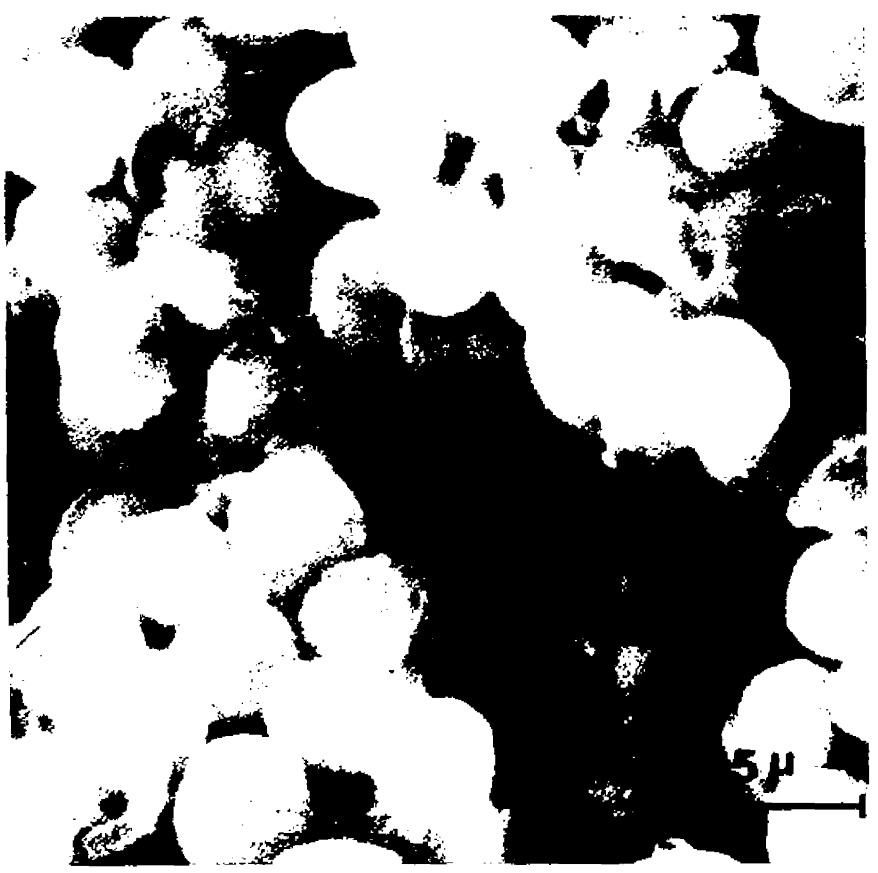

Fig. 2

Fig. 3

Figs. 2–3. Scanning electron microscopy of *Chlorella nana*. Fig. 2 shows several spherical cells with smooth surfaces (× 5,000). Fig. 3: a cell with an equatorial groove (arrow) is dividing into two individuals (× 25,000).

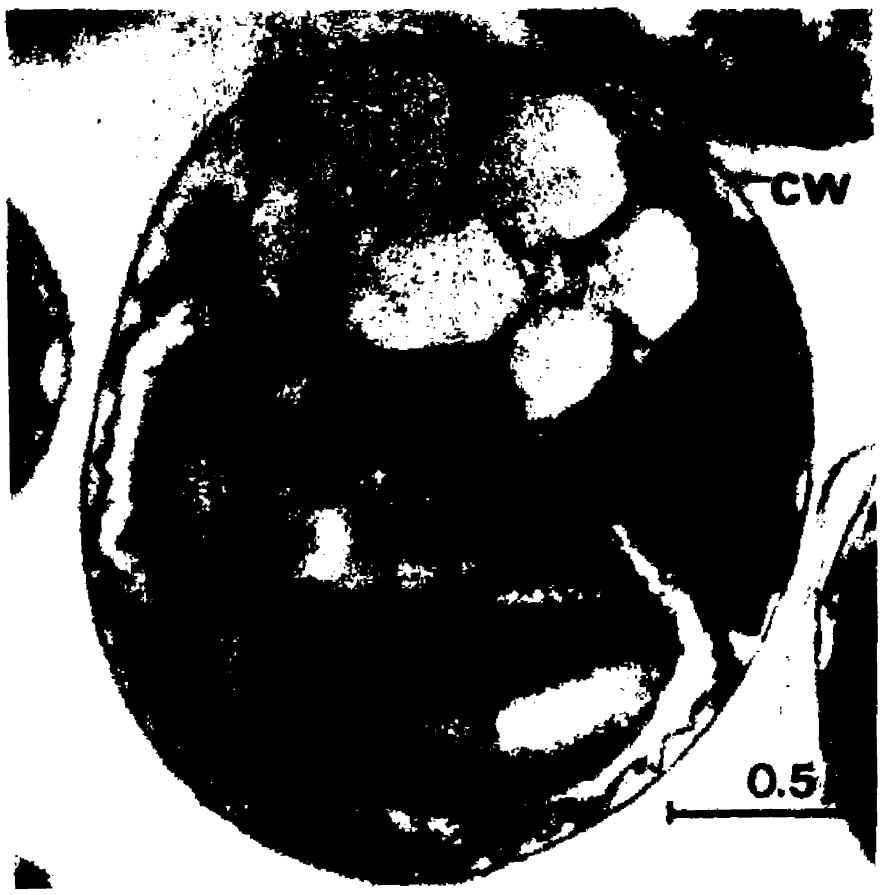

Fig. 4

Fig. 5

Key of labeling

CW: cell wall
M : mitochondrion
N : nucleus
S : starch
Th : thylakoids

Figs. 4–5. Transmission electron microscopy of *Chlorella nana*. The cell in Fig. 4 shows a chloroplast, parietal, cup-shaped, occupying almost half of the whole volume and with large starch granules. The cell wall is very thin, (× 34,000). In Fig. 5 an individual with the same characteristics as the previous one in Fig. 4 is represented; however, it has a thick wall demonstrated by the one to the right. (× 33,000).

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Tab. I. A schematic representation of the structural characteristics of *Chlorella nana* and of the other marine chlorellae

SPECIES	CELL SHAPE	CELL SIZE	CHLOROPLAST	CHLOROPLAST SIZE	PYRENOID	CELL WALL	PROPAGATION	PARTICULAR FEATURES
Chlorella salina (Butcher, 1952)	Spherical	4–7 μ	Saucer-shaped	Almost filling the cell	Large, central and surrounded by starch sheath	Thin, smooth	8 daughter cells	
Chlorella ovalis (Butcher, 1952)	Ovoid or ellipsoidal	3–5 μ × 5–10 μ	Slightly lobed, parietal	About 3/4 of the cell	Absent	Smooth, thin	8 daughter cells	
Chlorella marina (Butcher, 1952)	Ovoid	4–6 μ × 7–10 μ	Parietal, granular, lobed	Almost filling the cell	Not observed	Smooth, thin	8 or 16 daughter cells	
Chlorella stigmatophora (Butcher, 1952)	Spheroidal or elongate	4–6 μ	Saucer-shaped, rather granular	Filling most of the cell	Conspicuous, central or terminal surrounded by starch sheath	Smooth, thin	2 or 4 daughter cells	1 or more stigmata
Chlorella spärckii (Alvik, 1934)	Ellipsoidal or almost globose	2,8–7 μ × 2,5–5 μ	Parietal, slightly lobel and granular	Not described	Absent	Thin	2 daughter cells	
Chlorella nana sp. nov.	Spherical or spheroidal	1,5–3 μ	Cup-shaped parietal	About 1/2 of the cell	Not observed	Smooth, thin or thick	2 or 4 daughter cells	