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Note

Redescription of *Cryptomonas lima*, collected from Sorrento, Italy, the basionym of *Prorocentrum lima*

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Dinoflagellates are classified based on their morphological features. In taxonomy, characteristics described in the diagnosis of a species in the original publication are thought to be the most fundamental definition of the species. For species that were transferred to another genus from the one allocated originally, its basionym must be clearly cited in the proposal of the revision. However, in descriptions made before the establishment and adoption of the nomenclature codes, the citation of a basionym was sometimes unclear, and its uncertainty caused taxonomic confusion. In this context, *Prorocentrum lima* (Ehrenberg) Stein, one of the species described in the early stages of dinoflagellate taxonomy, has been a source of confusion for later studies, since the morphological characteristics of the original description and the drawing is a little different to *P. lima* as currently understood.

P. lima was described by Ehrenberg (1860) under the name of *Cryptomonas lima*, without a figure. The description of *C. lima* was as follows:

Testula fragili silicea ovata punctis asperis raris obsita, flagello simplici, forma ovata, postica parte turgente rotundata, antica breviter sensim attenuata, leviter emarginata. Intestina flavofusca.

Ehrenberg described that, the cell is ovate, roundly dilated in the anterior margin, tapering apically and slightly emarginate. The cell has a single flagellum. Rare punctations are scattered over the valve. He had collected the organism in Sorrento, Italy. In a following paper, Ehrenberg (1873) attached some drawings of *C. lima* drawn by himself in 1859. Stein (1878) transferred *C. lima* to the genus *Prorocentrum* because the cell shape of *C. lima* was similar to the genus *Prorocentrum*. But Stein's paper has been overlooked and only referred by a small number of researchers. Independently from the revision by Stein, a morphologically similar species resembing *P. lima* was described and named *Exuviaella marina* by Cienkowski (1881). It was collected from the White Sea, and described with the status of a new genus and species.

The two genera, *Prorocentrum* and *Exuviaella* were reclassified on the basis of a series of studies using light microscopy (LM) and electron microscopy (SEM and TEM) by Dodge and one of his colleagues (Dodge 1965, Dodge & Bibby 1973, Dodge 1975). Dodge concluded that there was no clear distinction between the two genera. Dodge (1975) listed *C. lima* as the basionym of *P. lima* and *E. marina* as a synonym of *P. lima*. *P. lima* has been reported from all over the world under the name of *P. lima* (Ehrenberg) Dodge (Fukuyo 1981, Faust 1991, Yoo 2004). However, the disagreement about the basionym of *P. lima* (Ehrenberg) Dodge whether it is *C. lima* or *E. marina*, still exists. In 1997, McLachlan et al. stated that it remained uncertain whether the alga presently referred to as *P. lima* is the same as the organism Ehrenberg described and illustrated as *C. lima*. According to them, Cienkowski's *E. marina* represents the organism presently regarded as *P. lima* (McLachlan et al. 1997). In the present study, the original description of Ehrenberg, the starting point of all studies of *P. lima*, was examined carefully, and compared with the organism, collected in the present day, from the type locality of *P. lima*.

The original drawing: The original drawing of *C. lima* by Ehrenberg in 1859 was opened to the public on the web-site of the Museum für Naturkunde, Berlin, Germany (www.museum.hu-berlin.de:55080/Ehrenberg/). The number of the *C. lima* drawing is 366. Wild sample collection: Seaweeds, on which *P. lima* was expected to attach, were collected on July 11, 2004 in Sorrento, Bay of Naples, Italy, the type locality of the species. A small amount of the seaweed growing on rocks was collected by snorkeling and put in a plastic bottle under water. Three specimens were obtained from that area. The specimens were brought back to the laboratory for observation.

Light Microscopy (LM): Micrographs were obtained using an Axioplan 2 microscope with an Axiophot 2 (Carl Zeiss, Munchen-Hallbergmoos, Germany) using Kodak Ektachrome 320T color reversal film (Kodak, Rochester, NY, USA). Images were digitalized using a Nikon Cool Scan II (Nikon,

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Tokyo, Japan). Cell dimensions were determined by measuring the length and width using an eyepiece micrometer at 1000× magnification.

Scanning electron microscopy (SEM): The cells were pipetted individually from the sample, and placed on poly-L-lysine-coated coverslips. These were fixed in 4% osmium tetroxide (Nisshin EM, Tokyo, Japan) in seawater for 30 min, rinsed three times in distilled water, dehydrated in a series of increasing ethanol concentrations (60%, 70%, 80%, 90%, 95% and 99.5%), and finally placed in 99.0% 2-methyl-2-propanol (Wako, Tokyo, Japan). The cells were freeze-dried, and coated with platinum-palladium, before being observed using a Hitachi S-4000 (Hitachi, Tokyo, Japan) scanning electron microscope at 7 kV.

Original drawing of *C. lima*: The cell is ovoid, broad in the middle region, and tapering toward the anterior margin (Fig. 1A). The posterior margin is rounded. The anterior margin of the right valve is concave. The anterior margin of the left valve is barely convex. One flagellum is described from the anterior concavity of the cell. Many punctuations are scattered over the valve. Some punctuations on the valve margin look like spines. Two large vacuoles are clearly drawn in the anterior region.

Observation of wild specimens by LM: More than thirty cells from all specimens belonging to the genus *Prorocentrum* were collected from Sorrento, Italy, the type locality of *C. lima*. All the cells had the same morphological characters and were thought to be assignable to a single species. Cells were ovoid, broad in the middle region, and tapering toward the anterior margin (Fig. 1B). The cells were 36 μm long, and 27 μm wide. The posterior margin was rounded. Two large pyrenoids were located in the center beneath each valve. Two large vacuoles were observed in the anterior half of the protoplasm. A large nucleus was located in the posterior region. Many valve pores were scattered over the valve, except in the central area (Fig. 1C), and along the margins of both valves. The valve pores looked like the punctuations (as Fig. 1D). The valve margin looked like the teeth of a saw when the focus of the microscopic image was adjusted to the valve pores. However spine-like punctuations on the valve margins were unable to be observed.

Observation of wild specimens by SEM: The surface of both valves was smooth. Valve pores of 0.37 μm diameter were scattered over valves except for in the center areas and larger marginal pores of 0.61 μm diameter ran parallel along the periphery of the valves (Fig. 2A). The anterior margin of the left valve was flat or barely concave, and horizontal or slightly slanted to the left of the left valve (Fig. 2B). The intercalary band was smooth (Fig. 2C). The periflagellar area was composed of eight platelets (Fig. 2D). These platelets surrounded a flagellar pore and an auxiliary pore. The flagellar and auxiliary pores were separated by plate-c. Each platelet could have several pore-like structures, but the number of those structures differed among cells. Plate-b is difficult to see even using high magnification, because it is located at the base of the left side of the auxiliary pore. Plate-b was in contact with plate-e. The surface of the auxiliary pore was composed of plates-a, -c, -d and -e. Four platelets were located adjacent to the left valve.

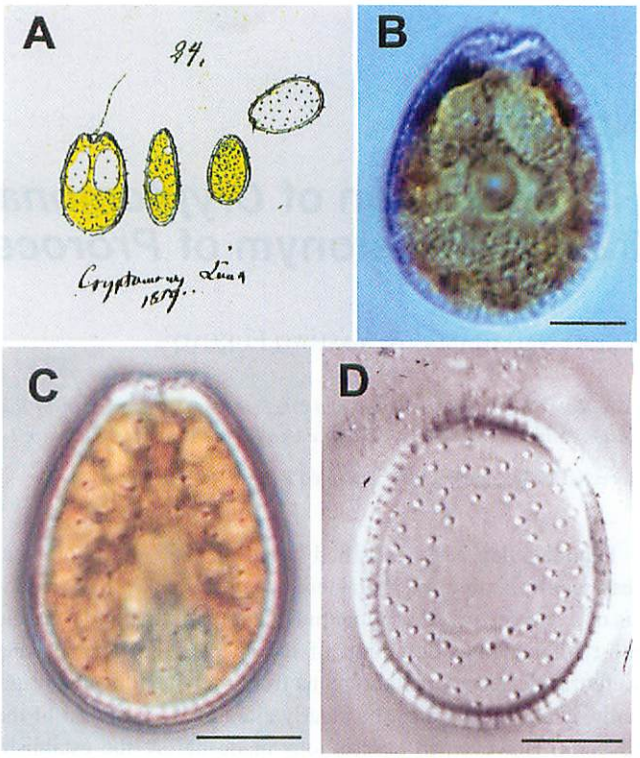

Fig. 1. *Cryptomonas lima*. A. Ehrenberg: Inserted from Ehrenberg's drawing sheet no. 366 (Museum für Naturkunde, Berlin) which was published as figs 24, 25 in 1873. The cell is of ovoid shape, broad in the middle region, and tapering toward the anterior margin. The anterior margin of the left valve is barely convex. Some punctuations on the valve margin appear to be spines. There are two large vacuoles in the anterior region. B. *Prorocentrum lima*: Cell from wild sample from type locality of *C. lima*. The cell is ovoid, broad in the middle region, and tapering toward the anterior margin. They are two large vacuoles in the anterior region. A large pyrenoid is located in the center of the valve. A large nucleus is located in the posterior region. Scale bar=10 μm. C. *Prorocentrum lima*: Culture cell collected from Sorrento, Italy. Many valve pores are scattered over the valve except in the central area and along the margins of both valves. Scale bar=10 μm. D. *Prorocentrum lima*: Culture cell collected from Palawan Island, Philippines. Many valve pores are scattered over the valve except in the central area. The valve pores looked like the punctuations. The valve margin looked like the teeth of a saw when the focus of the microscopic image was adjusted to the valve pores. Scale bar=10 μm.

There were only two characters that differed between the original drawing of *C. lima* and the organism we collected. One is the shape of the anterior margin of the left valve. In the original drawing of *C. lima*, the anterior margin was barely convex. The shape of the anterior margin of cells collected in this study was flat or barely concave according to LM and SEM observations. Depending on the orientation and tilt of organisms under LM observation, however, the anterior margin sometimes looked barely convex, like in the original drawing

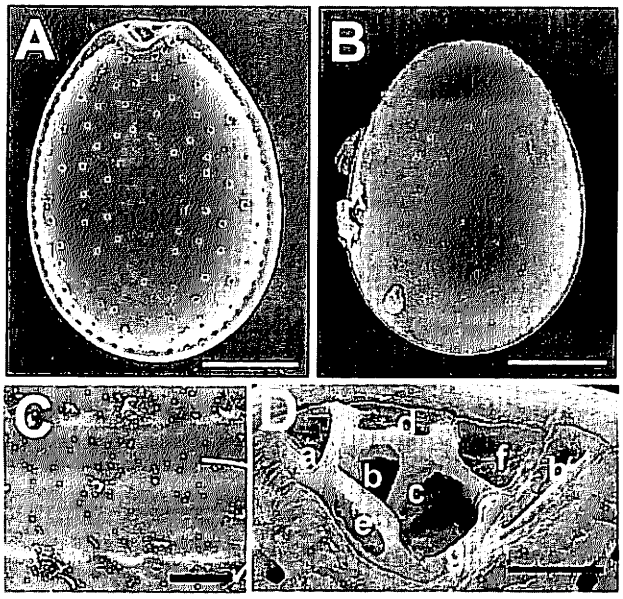

Fig. 2. *Prorocentrum lima*: Cell from wild sample from type locality of *C. lima*. A. Right valve view of the cell, with smooth surface, scattered valve pores except in the central area and marginal pores around the periphery of the valve. Scale bar=10 μm B. Left valve view of the cell. The anterior margin is flat or barely concave, and horizontal or slightly slanted to the left of the left valve. Scale bar=10 μm C. Lateral view of the cell. Intercalary band is smooth. Scale bar=2 μm D. Periflagellar area is composed of eight platelets. These platelets surround a flagellar pore and an auxiliary pore. The flagellar pore and auxiliary pore are separated by plate-c. Scale bar=1 μm

of *C. lima*, because the periflagellar area was shadowed by the V-shaped depression of the right valve. The other differing character concerns the valve pore. The original drawing of *C. lima* exhibited many punctuations over both valves and some punctuations, like spines, along the margins of both valves. However, these are thought to be artifacts due to observation of the penetrating through the transparent valves when observed with a low resolution microscope. The valve pores and marginal pores observed in this study under LM had an appearance similar to those described by Ehrenberg when the observation was made under low magnification, even using a high resolution microscope. The valve margin looked like the teeth of a saw when the focus of the microscopic image was adjusted to the valve pores. Also the valve margin of *P. lima* looked like the teeth of a saw (Fig. 1D). Therefore, the punctuations on the valves must be pores and the marginal spine-like punctuations also a row of marginal pores. The organism collected in this study is considered to be *C. lima*, since the morphological characteristics of the organism were identical to the description, and also very similar to the drawing of *C. lima* by Ehrenberg (1860, 1873).

The organism collected in this study was identical to *P. lima* (Ehrenberg) Dodge, considering the morphological characteristics: that the valve surface is smooth and both valves have pores except in the central area, that there is a row of marginal pores present at the cell periphery, that the intercalary band is smooth, that the periflagellar area is composed of eight platelets, and that these platelets are composed of a flagellar pore and an auxiliary pore. The arrangement of the periflagellar area is very similar to that of *P. lima* drawn by Taylor (1980, Fig. 5C). Taylor suggested that plate-b may be in contact with plate-e, although he could not observe it. His suspicion was confirmed in this study. As described above, the organism collected in this study was identified as *P. lima* (Ehrenberg) Dodge. In the present study, the basionym of *P. lima* was reconfirmed as *C. lima*.

Prorocentrum lima (Ehrenberg) Stein 1878
Basionym: *Cryptomonas lima* Ehrenberg 1860
Lectotype: Ehrenberg 1873, fig. 25
Type locality: Sorrento, Bay of Naples, Italy

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