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Newly identified resting stage cells of diatoms from sediments collected in Ago Bay, central part of Japan

Ken-Ichiro Ishii[1,*], Akira Ishikawa[2] & Ichiro Imai[1,3]

[1] *Laboratory of Marine Environmental Microbiology, Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606–8502, Japan*
[2] *Graduate School of Bioresources, Mie University, Kurima-Machiya, Tsu, Mie 514–8507, Japan*
[3] *Present address: Plankton Laboratory, Division of Marine Bioresource and Environmental Science, Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041–8611, Japan*

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Abstract: Resting stage cells of six diatom species, *Actinoptychus senarius*, *Biddulphia alternans*, *Lithodesmium variabile*, *Odontella longicruris*, *O. mobiliensis*, and *Detonula pumila* were newly identified from bottom sediments of Ago Bay, central Japan. The resting stage cells of *A. senarius*, *B. alternans* and *L. variabile* are very similar in morphology to their vegetative cells; hence, these resting stage cells are "resting cells". The resting stage cells of *O. longicruris* and *O. mobiliensis* closely resemble the vegetative cells in morphology, therefore, they are regarded as resting cells. However, they have some morphological differences compared with the vegetative cells, indicating a possibility of them being resting spores. The resting stage cells of *D. pumila* are quite different in morphology from the vegetative cells; therefore, these are "resting spores". The morphology of the resting spore of *D. pumila* is circular in valve view. In girdle view, each valve shows the same shape, slightly vaulted, and has a distinct mantle. As a common feature of the resting stage cells of the six species, the plastids show a darker color than those of vegetative cells, and are positioned unevenly in the cells.

Key words: plastid, resting cell, resting spore, resting stage cells, vegetative cell

Introduction

In temperate coastal waters, the species composition of phytoplankton communities frequently changes in the water column along with changes of season. Diatoms are the most important members of phytoplankton communities in coastal environments. However, diatoms often disappear during the course of species succession, especially after a period of active vegetative life, and then occasionally reappear again in the water column, especially in temperate coastal waters (Cupp 1943) where environmental conditions fluctuate greatly. Many diatom species show an adaptation to fluctuating environments, and many species survive the periods of unfavorable conditions through the formation of resting stage cells, i.e. "resting spores" and "resting cells" (Hargraves & French 1983, Garrison 1984, Itakura et al. 1997). Resting spores are quite different from vegetative cells in appearance as well as physiology, having thicker frustules, which often have a rounder shape and less elaborate surficial patterns. The term "resting cell" identifies cells that have undergone physiological and cytoplasmic change but remain morphologically very similar to vegetative cells of the species (McQuoid & Hobson 1996). Resting stage cells have an ability to tolerate adverse conditions for vegetative cells, such as darkness, nutrient depletion and temperature changes, allowing the species to survive until more favorable conditions return (Hargraves & French 1975, 1983, Garrison 1984, Itakura 2000). Furthermore, diatom resting stage cells in sediments also play an important role in providing seed populations for subsequent blooms in water columns (Gran 1912, French & Hargraves 1980, Garrison 1981, Itakura 2000). Imai et al. (1984) quantified the densities of diatom resting stage cells in sediments with the extinction dilution method (Most Probable Number method). This method has made it possible to reveal abundances of resting stage cells in the bottom sediments. Investigations of resting stage abundance in the bottom sediments have been carried out in the Seto

* Corresponding author: Ken-Ichiro Ishii; ken1ro@kais.kyoto-u.ac.jp

Inland Sea (Imai et al. 1984, 1990, Itakura et al. 1997, 1999), Ago Bay of central Japan (Ueno & Ishikawa 2009), Funka Bay of northern Japan (Asami & Ban 2000), and the East China Sea (Ishikawa & Furuya 2004). The densities of diatom resting stage cells were reported to be 10^3 to 10^7 cm^{-3} by these studies.

Ishikawa et al. (2007) developed a "plankton emergence trap/chamber (PET Chamber)" to measure the number of germinations/rejuvenations of the resting stage cells in the field. During the course of the study using the PET chamber at a station in Ago Bay (Fig. 1), we often found various vegetative cells of diatom species of which the resting stage cells were previously unknown. We presumed from this finding that many unreported diatom species may have resting stage cells in the bottom sediments of coastal seas. McQuoid & Hobson (1996) compiled a list of the diatom species capable of forming resting stage cells. In their paper, 149 species of 32 genera were listed and the genus *Chaetoceros* had the highest number of species (75 in total). Lewis et al. (1999) found that the diatom species, *Thalassiosira angulata*, *T. pacifica*, *T. punctigera*, *T. eccentrica*, *T. minima* and *T. anguste-lineata*, which were not listed by McQuoid & Hobson (1996), appeared after incubation of sediment collected from the coast of Scotland. In the present study, we discovered unknown diatom resting stage cells and newly identified the resting stage cells of six species from the bottom sediments of Ago Bay.

Materials and Methods

The investigation was conducted monthly from July 2006 to July 2010 at a station in Ago Bay (Fig. 1). The water depth at the sampling point was about 11.5 m. Sediment samples were collected using a sampler designed by Yokoyama & Ueda (1997), in which a plastic coring tube is set inside an Ekman grab to obtain the sediment and the overlying water together without disturbance. The top 3 cm of sediment in the coring tube was collected, put in plastic bottles and tightly sealed, and then stored for over two months in a dark, cold chamber (7°C). Sediment samples were sieved with 100 μm and 20 μm mesh size nets to concentrate resting stage cells (Matsuoka & Fukuyo 2000). Isolation of diatom cells from sediment was conducted by capillary with an inverted microscope (Nikon ECLIPSE TE200, Nikon, Tokyo, Japan). The cells were put in conditions for germination/rejuvenation (temperature of 20°C and continuous light with an irradiance of 50 μmol photons m^{-2} sec^{-1}), and species were identified based on the morphology of germinated or rejuvenated vegetative cells. Germination/rejuvenation was confirmed by the beginning of cell division.

Results and Discussion

Resting stage cells from the sediments were identified as *Actinoptychus senarius* (Eherenberg) Eherenberg, *Biddul-*

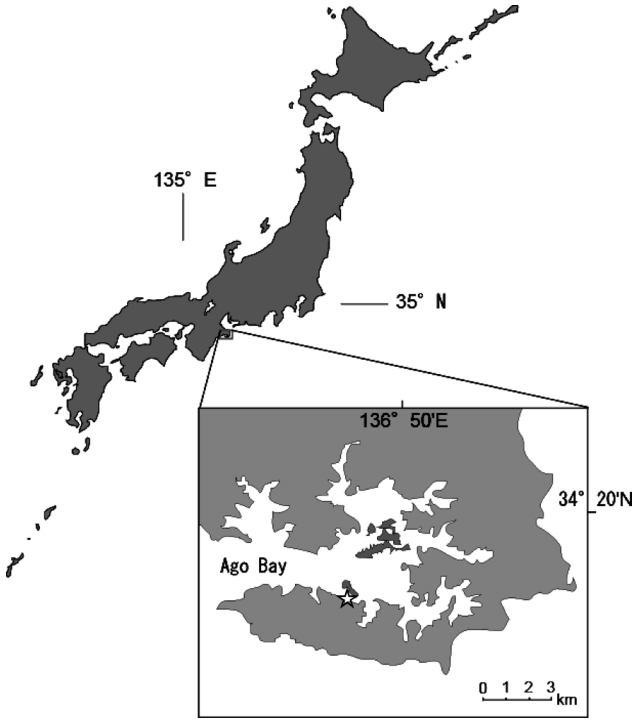

Fig. 1. Location of the sampling station (☆) in Ago Bay, Mie prefecture, central Japan.

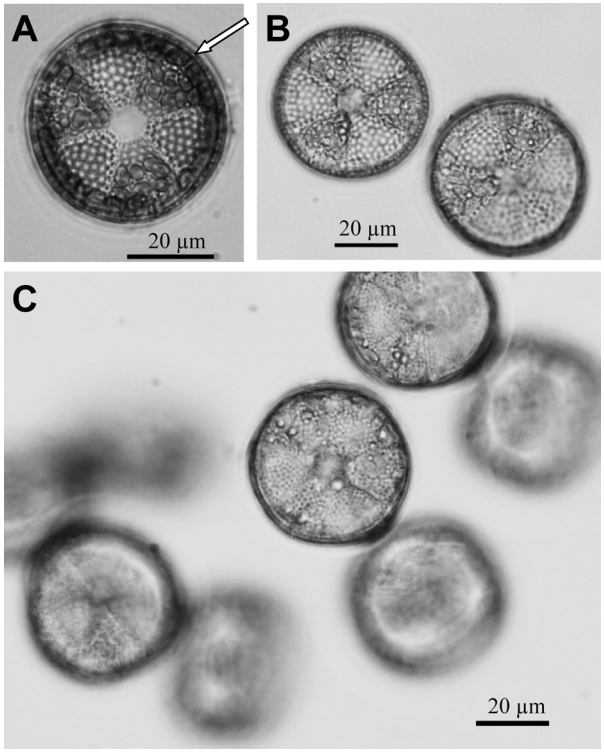

Fig. 2. Photomicrographs of *Actinoptychus senarius*. A: A resting cell. B: Rejuvenated cells 24 hours after the incubation of the resting cell shown in A. C: Cultured cells established from the rejuvenated cells shown in B. A white arrow indicates aggregated plastids, which are darker in color than in the vegetative cells. Scale bars indicate 20 μm.

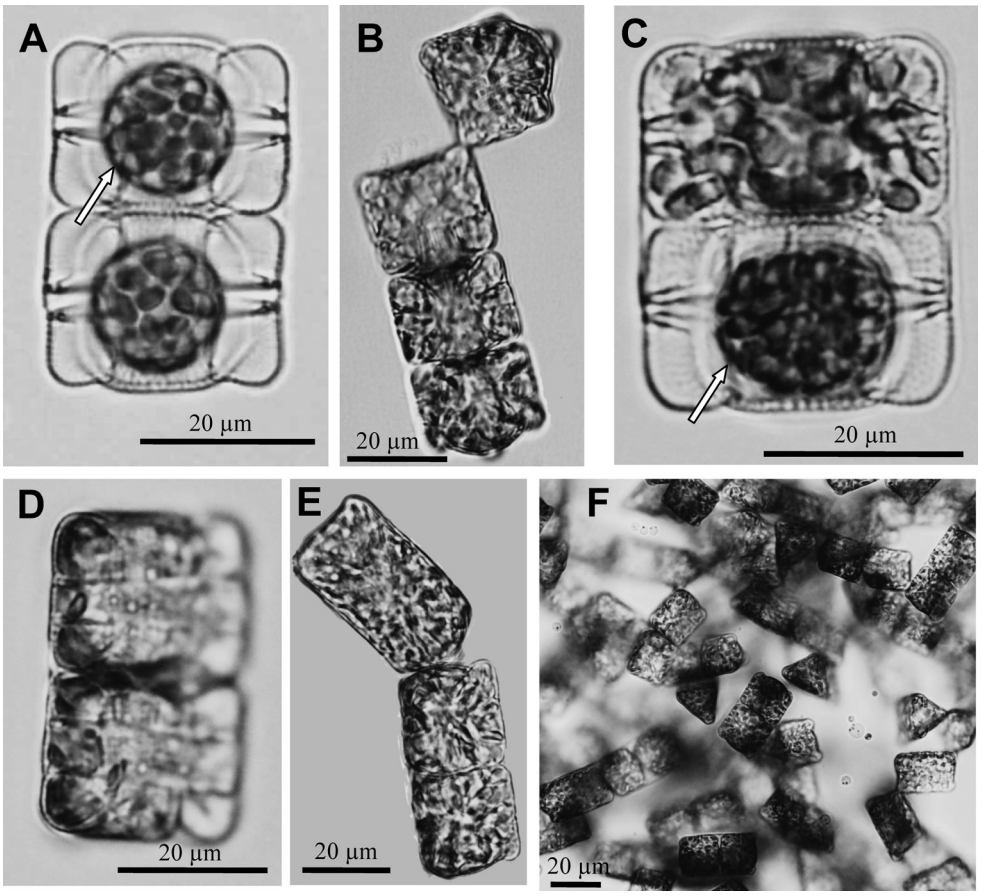

Fig. 3. Photomicrographs of *Biddulphia alternans*. A: Two resting cells. B: Rejuvenated cells 24 hours after the incubation of the resting cell shown in A. C: A resting cell (lower) and a cell just starting rejuvenation (upper). D: Cells before cell division 24 hours after the incubation of the resting cells shown in C. E: Rejuvenated cells 48 hours after the incubation of the resting cells shown in C. F: Cultured cells established from the rejuvenated cells shown in E. White arrows indicate the aggregated plastids darker in color than in the vegetative cells. Scale bars indicate 20 μm.

phia alternans (J. W. Bailey) Van Heurck, *Lithodesmium variabile* Takano, *Odontella longicruris* (Greville) Hoban, *O. mobiliensis* (Bailey) Grunow, and *Detonula pumila* (Castracane) Schütt (Figs. 2–7). Resting stage cells of these six species have not been recorded in previous studies. These resting stage cells were observed two or more times without a seasonal signal.

The resting stage cells of *A. senarius*, *B. alternans* and *L. variabile* are very similar in morphology to vegetative cells (Figs. 2–4); therefore, these resting stage cells are considered resting cells. The resting cell of *A. senarius* has many granulated plastids, which are darker in color than those in vegetative cells, and are unevenly distributed (Fig. 2A, arrowed). The plastids of *B. alternans* resting stage cells are darker in color and gather in spherical form at the center of the cell (Fig. 3A). The plastids of the resting stage cells were observed to expand their distribution in the cytoplasm during less than one hour of microscopic observation, probably due to strong light from the microscope illuminator (Fig. 3C, upper cell). The plastids of *L. variabile* resting cells are darker in color and unevenly distributed (Fig. 4A). The plastids of these three species spread their distribution throughout the cell after cell rejuvenation.

The resting stage cells of *O. longicruris* and *O. mobiliensis* closely resemble the vegetative cells in morphology (Figs. 5, 6), therefore, they are regarded as resting cells. However, they also had a characteristic feature of resting spores in the following respect. Sediment particles always attached to one side of the cell of *O. longicruris* (Fig. 5A), suggesting morphological differences in integumental structure from vegetative cells. In *O. mobiliensis*, resting stage cells sometimes lacked processes on one of two valves (Fig. 6C), which was not observed in vegetative cells. The plastids of these two species are darker in color than those of the vegetative cells, and are unevenly distributed (Figs. 5A, 6C). Plastids of vegetative cells were observed throughout the cell after cell rejuvenation (Figs. 5B, 6B, D).

The resting stage of *D. pumila* shows quite different morphology from vegetative cells (Fig. 7A, F); therefore, these resting stage cells are resting spores. The resting spores are formed in parent cells (Fig. 7C) and are released

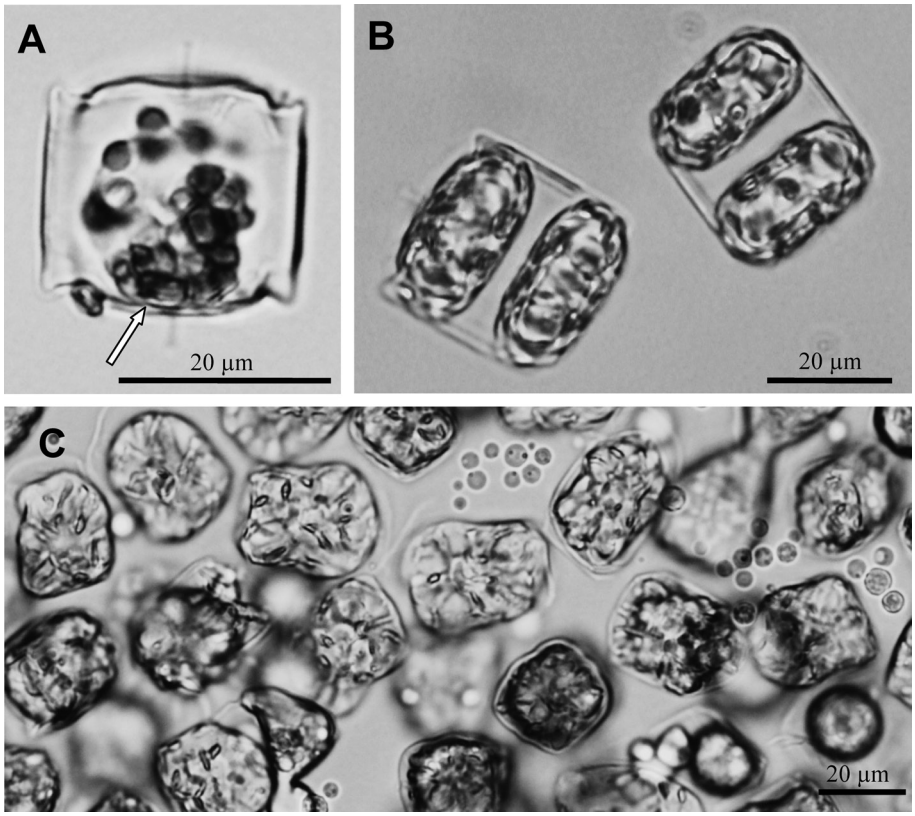

Fig. 4. Photomicrographs of *Lithodesmium variabile*. A: A resting cell. B: Rejuvenated cells 24 hours after the incubation of the resting cell shown in A. C: Cultured cells established from the rejuvenated cells shown in B. A white arrow indicates the aggregated plastids, which are darker in color than in the vegetative cells. Scale bars indicate 20 μm.

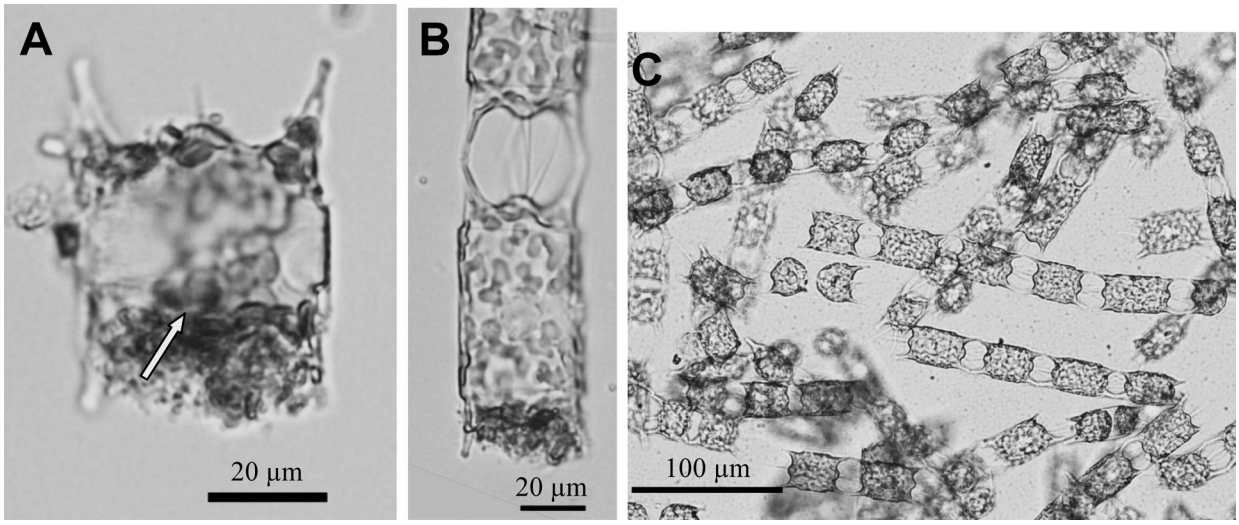

Fig. 5. Photomicrographs of *Odontella longicruris*. A: A resting cell. B: Rejuvenated cells 24 hours after the incubation of the resting cell shown in A. Remaining sediment particles attached to the resting cell are visible. C: Cultured cells established from the rejuvenated cells shown in B. A white arrow indicates the aggregated plastids, which are darker in color than in the vegetative cells. Scale bars indicate 20 μm in A, B and 100 μm in C.

from the parent cells (endogenous spores). The valve shape is circular in valve view (Fig. 7A). In girdle view, each valve shows the same shape, weakly vaulted, and has a distinct mantle (Fig. 7B, C). There are many granulated plastids that are darker in color than those of vegetative cells as in the above species. The plastids were unevenly distrib-

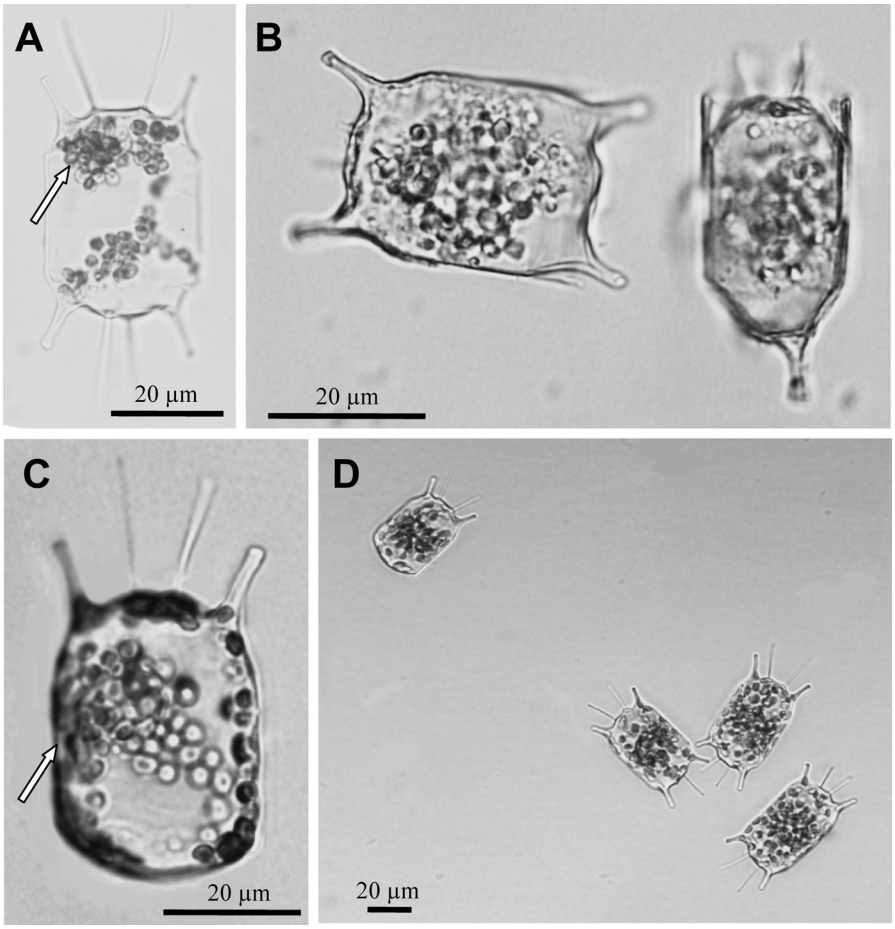

Fig. 6. Photomicrographs of *Odontella mobiliensis*. A: A resting cell. B: The rejuvenated cells 24 hours after the incubation of the resting cell shown in A. C: A resting cell. The structures of one side are deficient. D: Rejuvenated cells 24 hours after the incubation of the resting cell shown in C. White arrows indicate the aggregated plastids, which are darker in color than in the vegetative cells. Scale bars indicate 20 μm.

uted in the cytoplasm. Those of germinated cells were distributed more evenly all over the cell (Fig. 7D).

The present study revealed that the above six species have resting stage cells in their life cycle. These species were occasionally observed in water samples in the study area (unpublished data). However, their resting stage cells have not been reported hitherto. A possible reason for this is that these cells exist in very low densities in sediments. For example, our preliminary study revealed that the density of resting cells of *B. alternans* was equal to or less than 1 cell in sediment of 0.2 g wet mass. Under such low densities, it is very difficult to detect rare resting stage cells using the standard most probable number (MPN) method for the enumeration of diatom resting stage cells in sediments, because the detection limit is 200 cells g^{-1} wet sediment (Imai et al. 1990, Itakura et al. 1999, Itakura 2000).

Most diatom resting stage cells are categorized as resting spores (McQuoid & Hobson 1996). The morphology of resting spores markedly differs from that of vegetative cells, and it is easy to distinguish the resting spores from the vegetative cells. In this study, the resting cells of *A. senarius*, *B. alternans* and *L. variabile* were newly identified. Resting cells have frustules very similar to vegetative cells, in thickness as well as in pattern and shape (McQuoid & Hobson 1996). Therefore, it is very hard to distinguish resting cells from vegetative cells based only on their morphology. However, resting cells are somewhat morphologically distinct from common vegetative cells, i.e. possessing dense, dark cytoplasmic matter (Hargraves 1979, Sicko-Goad et al. 1989, Itakura et al. 1993) and more rounded plastids (Gibson & Fitzsimons 1990). We confirmed that the plastids of the resting stage cells are darker in color than those of the vegetative cells during this study (Figs. 2–7). In addition, plastids are distributed unevenly and locally in the cytoplasm of resting cells. These differences are important features for the identification of resting cells.

It is considered likely that resting stage cells exist in more species than is presently known, especially in the genera from which species having resting stage cells are already known. At present, it is not clear as to what kinds

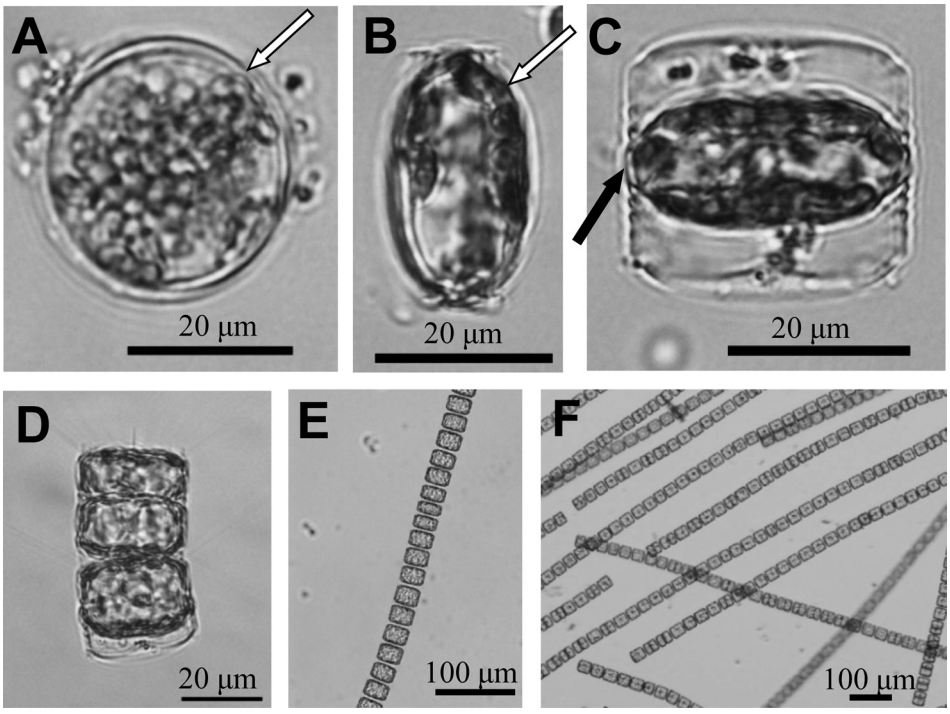

Fig. 7. Photomicrographs of *Detonula pumila*. A: A resting spore in the valve view. B: The same resting spore as A in the girdle view. C: A resting spore in the girdle view. Black arrow indicates the mantle. D: Germinated cells 24 hours after the incubation of the resting cell shown in C. Parent cell remains outside the germinated cells. E, F: Long chained-cells of strain culture established from the germinated cells shown in D. White arrows indicate the aggregated plastids, which are darker in color than in the vegetative cells. Scale bars indicate 20 μm in A-D and 100 μm in E, F.

of species have the ability to form resting stage cells. Kooistra et al. (2010) reported that *Chaetoceros* species forming resting spores can be grouped by certain morphological traits. However, more information is necessary in order to understand what determines the formation of resting stage cells in diatoms.

The present study revealed the morphology of the resting stage cells of six diatom species, which makes it possible to study their physiology and ecology, especially the role of their resting stage cells in population dynamics in coastal waters. However, as for most of the other diatom species it is still unknown as to whether they have resting stage cells in the sediments of coastal seas. More detailed information on the resting stage cells of diatoms in coastal sediments is critically important to fully understand their population dynamics and life cycles.

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