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Morphological Characteristics of *Peridinium bipes* f. *occcultatum* (Dinophyceae) Isolated from Three Geographically Segregated Aquatic Systems of Korea

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To resolve some of the most pressing uncertainties of *Peridinium* identity, morphological characteristics of Korean *Peridinium* were examined using light and scanning electron microscopy. The *Peridinium* samples were collected from three different regions of Juam, Sang-sa and Togyo Reservoirs, when seasonal blooms occurred. Formula of the epithecal plate was recorded with 7 precingular, 3 intercalary, and 4 apical plates (4′, 3a, 7″). An apical pore, 3~5 μm in size, was apparently present. The cingulum was easily observed under light microscope, and was considerably offset by about 15 μm (2~3 times per cingulum width). The sulcus was straight longitudinally and widened apparently towards the antapex. None of spine was found on the surface of the thecal plates on scanning electron micrographs. The average body length was 50.4 μm with a range of 29~63 μm. The geometric dimension, as designated to the body length:width ratio, was found from calculation to be 1.12 with a range of 1.00~1.35, therefore, the cell was shown slightly elongated. Based on their morphology, the causative organisms of red tides in three different Korean waters were identified as *P. bipes* f. *occultatum*, which was reported for the first time in Korea.

Key words : morphological characteristics, *Peridinium bipes* f. *occultatum*, red tide

INTRODUCTION

The armoured dinoflagellate *Peridinium* Ehrenberg is one of the most important genera in freshwater phytoplankton, as it is widely distributed around the world and contains a large number of species (Pollingher, 1987). The species is the unicellular, eukaryotic algae that is a very diverse group containing autotrophic, heterotrophic or symbiotic forms. A few photosynthetic species at least may form significant blooms. So far, the freshwater red tide, which mainly consists of dinoflagellate *Peridinium*, has been observed in many reservoirs or lakes (Nakamoto, 1975; Pollingher and Serruya, 1976; Wynne *et al*., 1982; Ikeda *et al*., 1993; Kawabata and Hirano, 1995; Fukuju *et al*., 1998; Yamada *et al*., 1998). In Korea, similar blooms were observed at Lake Soyang (Lee and Cho, 1994), Togyo Reservoir (Han *et al*., 1995; Ki 1998), and Juam Reservoir (Lee, 1994). Main problems by large populations of these organisms are to clog filtration systems and give unpleasant taste and smell to drinking water (Nakamoto, 1975; Kawabata and Hirano, 1995). Recent studies have, therefore, been focused on their ecological features and population controls of *Peridinium* for water quality management (Kawabata and Hirano, 1995).

Recently, these organisms in Korean waters were morphologically identified as *Peridinium bipes* Stein (Ki, 1998) or its relatives as *P. willei*

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Huitfeld−Kaas (Han *et al.*, 1995) and *P. cinctum* Muller (Lee *et al.*, 1996). However, because of their morphological similarity, many uncertainties on their proper identity have remained. The identification problems might also be caused mainly by the microscopic size of cells and numerously resembled species comprising *Peridinium* in freshwater. For the proper identification of *Peridinium* species, it is essential to be able to observe both number and arrangement of the armoured plates: in particular, apical pore complex and the number of cingular plates are important for *Peridinium* (Huber−Pestalozii, 1968).

In this study, we described the morphological characteristics of the Korean *Peridinium* in order to collect decisively morphological evidences for bipes−group, which has two uniquely morphological characteristics−e.g. the presence of apical pore and 4′, 3a, 7″ of thecal formation (Elster and Ohle, 1968).

MATERIALS AND METHODS

1. Water Sampling

Water samples were collected monthly for one year (April 1997 to March 1998), including winter seasons, in Togyo Reservoir (Fig. 1), in which its water quality and population dynamics of *Peridinium* were described in our previous studies (Han *et al.*, 1995; Ki, 1998). Further water samples were collected from Juam and Sang−sa Reservoirs (Fig. 1), when seasonal *Peridinium* blooms occurred (April, 2003). These field samples were immediately fixed with Lugol's solution or formalin at 1% final concentration.

2. Morphological observations

The samples collected from wild populations were used to examine the morphological features of the genus *Peridinium*, using a light microscope (Axioplan, Carl Zeiss, Germany). For scanning electron microscopy, *Peridinium* samples collected from field were fixed with glutaraldehyde to a final concentration of 1%. The samples were filtered onto a Nucleopore filter (2−, 5−μm pore size), washed several times with distilled water (D.W.), dehydrated by using a series of alcohol and dried via liquid CO_2 in a critical point drying apparatus (Baltec CPD 010, Liechtenstein). The filters were subsequently glued to the stubs with double−adhesive carbon disks, sputter−coated with gold, and observed under a scanning electron microscope (XL 20, Philips, Netherlands).

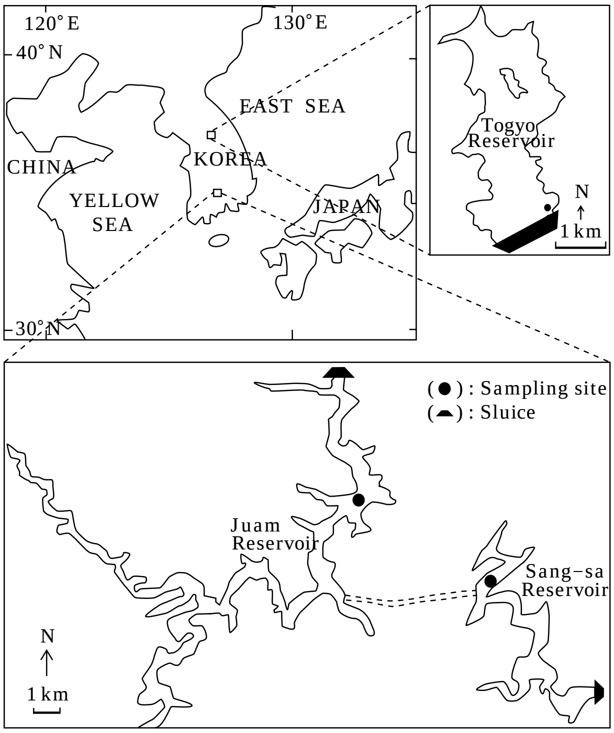

Fig. 1. Locations of sampling station (●) at Juam, Sang−sa and Togyo Reservoirs, Korea. Togyo Reservoir was constructed in 1976 for agricultural irrigation in the demilitarized zone (DMZ) in Korea. Its water qualities were characterized by low content of nutrients and mesotrophic status.

3. Cell size determination

Cell size was calculated by measuring more than 10 cells collected from field samples with the above light microscope.

RESULTS

1. Body shape and thecal plate formula

The Korean *Peridinium bipes* f. *occultatum* cells from Togyo Reservoir were pyriform in outline, and were more or less flattened dorsoventrally (Fig. 2). Thecal plates were thick, and aleolae, like net−ornament, was apparent under scanning electron micrographs. Formula of the epithecal plate was recorded with 7 precingular, 3 intercalary, and 4 apical plates (4′, 3a, 7″) (Figs. 2b, c, 3c). First apical plate was rhomboidal, and one side of the plate grooved due to connection of anterior sulcal plate. The fourth precingular plate (4″) was rectangular and larger than the other precingular plates, and the third intercalary plate (3a) was trapezoid and also larger than the other intercalary plates. An apical pore, 3 ~ 5 μm in size, was apparently present in Korean *Peridinium*, evidenced by the apicoventral and ventral views in light micrographs (Fig. 2a, c). The thick plate suture was easily seen in a scan-

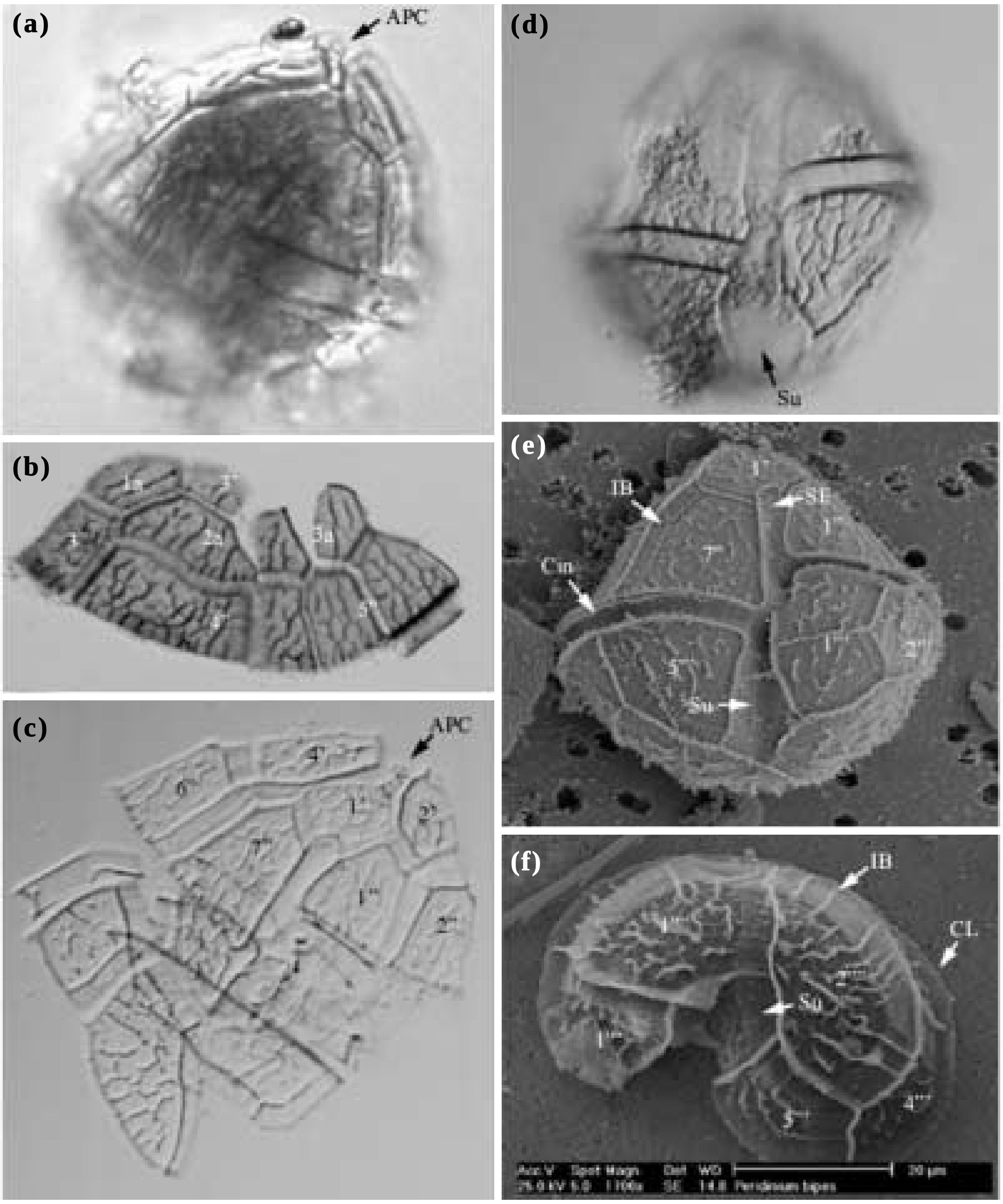

Fig. 2. Light and SEM micrographs of the Korea *P. bipes* f. *occultatum* collected from Togyo Reservoir. Apicoventral view of vegetative cell (a), apical views of thecal plate (b, c), ventral views of vegetative cell (d, e) and antapical view of the cell (f). The numberings of thecal plate indicate as 4′, 3a, 7″ on the corresponding plate. Arrows indicate a typical apical pore complex (APC), cingulum (Cin), cingular list (CL), intercalary band (IB), sulcal extension (SE), and sulcus (Su). The formula of thecal plate recorded is 4′, 3a, 7″, 5‴, 2⁗. Scale bar = 10 μm.

ning electron micrograph (Fig. 2f).

The cingulum was easily observed under light microscope, and the girdle was considerably offset by approximately 15 μm (2 ~ 3 times per cingulum width) (Figs. 2d, e, 3a). The left-side position of cingulum was nearly in center, but its right side was more posterior. Thus, the displacement was left handed, and the upper part was slightly larger than the lower part of the cells. The cingular list was apparent from the lateral edges of the cingulum (Fig. 2f), whereas none of spine in *Peridinium bipes* f. *occultatum* was found on the surface of the thecal plates on scanning electron micrographs (Fig. 2e, f). The sulcus was straight longitudinally and widened apparently towards the antapex (Figs. 2d, e, 3a). The sulcal extension lengthened in a measurable length toward the apex (Figs. 2c, d, e, 3a). An anterior sulcal plate adjoined with the first apical plate to groove one side of the plate.

All *Peridinium* cells collected from Juam and Sang-sa Reservoirs were observed as the same morphology of *Peridinium bipes* f. *occultatum*: ovoid in shape, 4′, 3a, 7″ of thecal formation, presence of apical pore complex, absence of spine.

2. Body size and geometry

The average body length of the Korean isolate of *Peridinium bipes* f. *occultatum* was 50.4 μm with a range of 29 ~ 63 μm (Fig. 4a), which was calculated by measuring 99 cells from the samples collected monthly from Togyo Reservoir, and the body width of the cells was 45.3 μm with a range of 22 ~ 62 μm (Fig. 4a). As shown in Fig. 4, the body length of the Korean *P. bipes* f. *occultatum* was grouped into two ranges (29 ~ 40 μm and 45 ~ 63 μm).

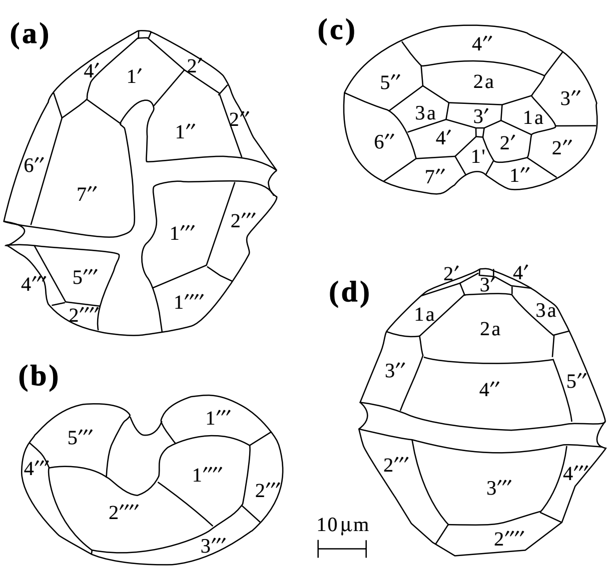

Fig. 3. Line drawings of the Korean *P. bipes* f. *occultatum*, based on the present work (e.g. Fig. 2): ventral view (a), antapical view (b), apical view (c), dorsal view (d). The numberings of thecal plate indicate as 4′, 3a, 7″, 5‴, 2⁗ on the corresponding plate. Arrows on the drawing indicate the prominently morphological characteristics (e.g. sulcal extension, girdle-offset, apical pore, the shape of 1′ plate, non-presence of spine) of *P. bipes* f. *occultatum*. Scale bar = 10 μm.

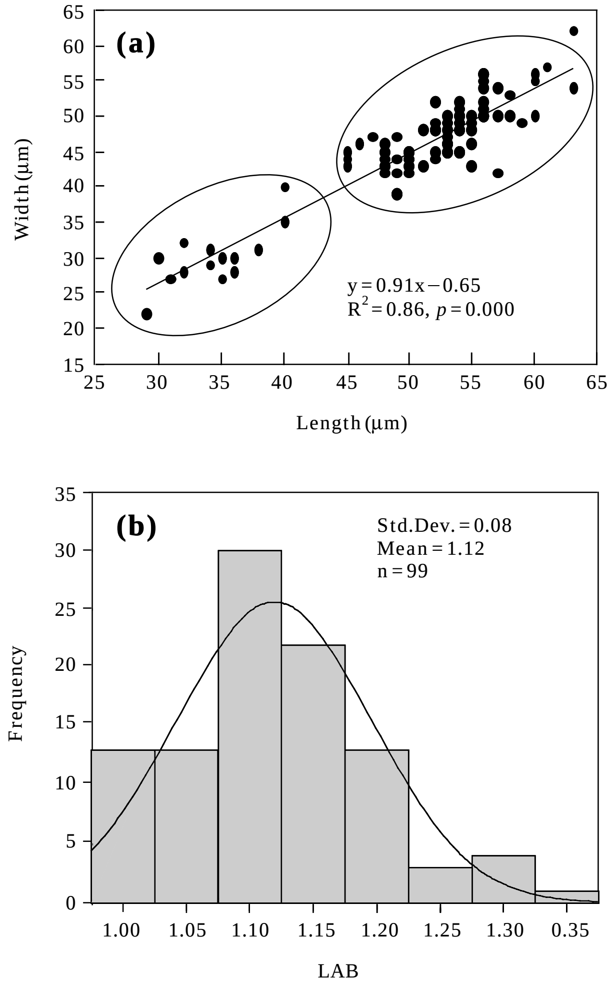

Fig. 4. Relationship between body length and width (a), and the LAB frequency distribution (b) of the Korean *P. bipes* f. *occultatum*. The least squares linear regression equation of (a) is y = 0.91x − 0.65 (r^2 = 0.86, p = 0.00, n = 99). The line displays the normal curve on the histogram (n = 99).

The geometric dimension was described as the body length:width ratio, called as LAB (long as broad). The LAB of the Korean *Peridinium bipes* f. *occultatum* isolate was found from calculation to be 1.12 with a range of 1.00∼1.35 (Fig. 4b), and the cell was therefore shown slightly elongated.

DISCUSSION

Dense blooms of the dinoflagellate *Peridinium* have occurred regularly in three Korean waters (Juam, Sang-sa and Togyo Reservoirs) every year. We described finely the morphological characteristics of Korean *Peridinium* cells, and then identified them as species level.

All of the Korean *Peridinium* cells collected from three different Korean waters showed the identical morphology: cells ovoid to pyriform in shape, the presence of apical pore, 4′, 3a, 7″ of thecal formation, and prominent sulcal extension. Mainly on the basis of the plate formation, the Korean *Peridinium* was included into bipes-, cinctum-, or willei-group, by following the Elster and Ohle's groupings (1968). The three groups are subdivided into two subgenera, *Poroperidinium* (e.g. *P. bipes*) and *Cleistoperidinium* (e.g. *P. cinctum*, *P. pseudolaeve*, and *P. willei*), based on the presence or absence of an apical pore complex (e.g. Popovský and Pfiester, 1990). Therefore, the Korean *Peridinium* cells were morphologically included into bipes-group (or *P. bipes*).

According to Stein (1883), *Peridinium bipes* possesses fine reticulation on the thecal plates and prominent antapical spines. Abé (1981) also reported that a minute spine is often found on the left of the flagellar pore springing from the left sulcal edge, and two short antapical spines on ventral area of the antapical plates. None of spine in the Korean *P. bipes*, however, was found on the surface of the thecal plates. It suggested that the Korean *P. bipes* was a variant of the species resulted from the ecological adaptation or evolution for the geographical segregation for a long time. Indeed, the species of *P. bipes* is divided into several intraspecific taxa such as *P. bipes* var. *excisum* (Lemm.) Lef., *P. bipes* f. *globosum* (Lindem.) Lef., *P. bipes* f. *occultatum* (Elster and Ohle, 1968). Of these subspecies, *P. bipes* f. *occultatum* does not have spine on ventral area of the antapical plates. The morphological feature allowed the Korean *P. bipes* to be identified as *P. bipes* f. *occultatum* within subspecies level. This decision was strongly supported by the previous study, in which *P. bipes* f. *occultatum* was characterized by its tabulation, a distinctive apical pore complex, well-developed girdle and sulcus, and absence of the spine (e.g. Elster and Ohle, 1968; Huber-Pestalozzi, 1968).

The girdle-offset was easily seen in light micrographs, and might be one of the important features of *P. bipes* (or *P. bipes* f. *occultatum*) separated from its relatives like *P. cinctum*, *P. volzii*, and *P. willei*. The torsion of the body, similar to the girdle-offset, is frequently observed in the unarmoured dinoflagellate Gymnodiniidae, and the prominent feature has expertly been reported in the unarmoured marine dinoflagellate *Gymnodinium aureolum*, *G. mikimotoi* (Hansen *et al*., 2000) and the genus *Cochlodinium* (Kofoid and Swezy, 1974). Although the girdle-offset is generally slight in the armoured dinoflagellates, it is of interest to observe that the displacement was significantly wide at 2∼3 times per cingulum width in the Korean *P. bipes* f. *occultatum*. In earlier study, Huber-Pestalozzi (1968) reported that the girdle displaces 2∼3 times its width in *P. bipes* f. *occultatum*, which is in good agreement with the present result.

Interestingly, the body length in the Korean *P. bipes* f. *occultatum* was divided into two ranges. The size in the Korean cells was more broad-ranged than in the Huber-Pestalozzi's work (36∼58 μm in length; 1968), but the range of the large cells alone was generally consistent with the Huber-Pestalozzi's work. The small cells could be observed limitedly in field samples collected during February and May, and the large cells were commonly observed in the others. In earlier studies, Pollingher (1988) described in detail that the size variation was a natural feature of the *Peridinium* populations, depending on both cell nutrition and stages in cell cycle, and that the broad spectrum of body size should be correlated with relatively low division rate and long span of life. In particular, the two separations of the size-range in Korean *P. bipes* f. *occultatum* may be explained by sexual reproduction of the dinoflagellate: the vegetative cells are haploid, and gametes are formed by a longitudinal division of the peridinoid dinoflagellate (Pollinger, 1988). The differentiation gives rise to cells lower in mass and poorer in plastids and pigments than the vegetative cells. Apart from their smaller size and lighter color, the gametes are not obviously different from vegetative cells (Lee, 1989). However, the variations of size in natural samples of *P. bipes* f. *occultatum* remain to be understood in more detail through future studies.

The LAB ratio could be used to the geometric description of the body shape, and the Korean *P. bipes* f. *occultatum* cells were therefore shown slightly elongated (1.12 LAB). Although the ratio will suffice to numerically describe the body shape of *Peridinium*, few data have been available on the dinoflagellates, except 1∼1.5 LAB of *P. willei* (Bellinger 1992) and some *Gymnodinium* species (Hansen *et al*., 2000). The LAB ratio was first used to numerically describe the body shape of *P. bipes* f. *occultatum*, and could also be partly used as a taxonomic key in the dinoflagellate. As a taxonomic key, the LAB ratios have been used for the description of the body shape, particularly in diatom classification (Bellinger, 1992).

This study is the first report that *P. bipes* f. *occultatum* is responsible for the freshwater red tides in three different Korean waters.

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