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Morphology and molecular characterization of the epiphytic benthic dinoflagellate *Ostreopsis* cf. *ovata* in the temperate waters off Jeju Island, Korea

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ABSTRACT

Ostreopsis is an epiphytic and potentially toxic dinoflagellate. Here, we have provided the first report about the occurrence of *Ostreopsis* cf. *ovata* in Korean waters. In general, the morphology of the Korean strain of *O.* cf. *ovata* isolated from the waters off Jeju Island, Korea, was similar to the original description of *O. ovata* isolated from Japanese water. However, the cell length of the Korean strain was 20–30% smaller than that of the Japanese strain. In addition, the cingulum of the Korean strain was displaced and undulated in the ventral view, but that of the Japanese strain was not displaced. We have reported, for the first time, the presence of a line of small knobs on the Po plate. The plates of the *O.* cf. *ovata* Korean strain were arranged in a Kofoidian series of Po, 3′, 7″, 6c, 6s, V_p, R_p, 5‴, 1p, and 2⁗. When properly aligned, the small subunit (SSU) rDNA sequence of the *O.* cf. *ovata* Korean strain was 5.8% different to the *O.* cf. *ovata* Malaysian strain, which is the closest strain. In addition, compared to the south-east Asian, Pacific, Atlantic, and Mediterranean strains of *Ostreopsis* sp., *O. ovata*, and *O.* cf. *ovata*, the sequences of the internal transcribed spacer region (ITS1 and ITS2) plus 5.8S, D1–D3, and D8–D10 large subunit (LSU) rDNA of the *O.* cf. *ovata* Korean strain showed a difference of 3.6–16%, 15.8–19.7%, and 0–3%. Furthermore, in the phylogenetic trees based on D1–D3 LSU rDNA regions, the *O.* cf. *ovata* Korean strain was located in the basal position of the other *O. ovata* and *O.* cf. *ovata* clades. Therefore, we suggest that the *O.* cf. *ovata* Korean strain is genetically distinct among the *O. ovata* and *O.* cf. *ovata* strains reported to date.

1. Introduction

Recently, interest in epiphytic and benthic dinoflagellates has rapidly increased, because some of these genera are known to cause harm to other organisms (Mohammad-Noor et al., 2007; Laza-Martinez et al., 2011; Jeong et al., 2012a). For instance, some species belonging to the genera *Ostreopsis* and *Gambierdiscus* are reported to produce toxic substances that could seriously threaten human health and cause enormous economic losses to fisheries and tourism (Sato et al., 2011; Parsons et al., 2012; Hwang et al., 2013). Thus, the presence of such dinoflagellates in certain waters is of critical concern to scientists, in addition to the aquaculture and tourism industries of the country.

Ostreopsis spp. are primarily benthic dinoflagellates that have been observed in a diverse range of habitats, particularly on the surface of sands, corals, macroalgae, and mangroves (Faust et al., 1996; Holmes et al., 1998; Pocsidio and Dimaano, 2004; Aligizaki and Nikolaidis, 2006; Kim et al., 2011; Laza-Martinez et al., 2011). However, some specimens have also been observed in the water column (Faust et al., 1996). In addition, many epiphytic and benthic dinoflagellates have been found in both tropical and subtropical waters (Fukuyo, 1981; Faust, 1999; Parsons et al., 2012), even though some cells have been found in temperate waters (e.g. Selina and Orlova, 2010; Kim et al., 2011; Jeong et al., 2012b; Lim et al., 2013). Therefore, it is worth exploring the distribution of *Ostreopsis* spp. at higher (i.e., more temperate) latitudes.

Ostreopsis siamensis is a type species of the genus *Ostreopsis*, and it was first described by Schmidt (1902). Eight additional *Ostreopsis* species were subsequently established on the basis of various morphological features. Such discriminatory features include

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shape, size (absolute or relative), ratio of dorsoventral (DV) length relative to anteroposterior (AP) length, ratio of DV to width, length of the Po plate, size of the thecal pores, and the shape of the 1p plate of cells (Fukuyo, 1981; Norris et al., 1985; Quod, 1994; Faust and Morton, 1995; Faust et al., 1996; Faust, 1999; Penna et al., 2005). However, there has been a lot of debate on the taxonomy of *Ostreopsis ovata* because: (1) strains have been found in various locations, (2) the intra-specific variation among these strains is considerable, and (3) the range in the size of these strains is very broad. In contrast, there is relatively little variation in the shape and plate pattern of the strains. Moreover, there are many reports on *O. ovata* morphology, without any supporting genetic characterization. In particular, there has been only one report about the sequence of the small subunit (SSU) rDNA of *Ostreopsis* spp. Thus, some studies have named *O. ovata* strains as *O.* cf. *ovata* (e.g. Penna et al., 2010). Therefore, intensive studies on the morphological and genetic characteristics of more strains are needed.

Recently a clonal culture was isolated and established from dinoflagellate cells, from seaweeds collected in the waters off Jeju Island, Korea. This culture was used to conduct morphological and genetic analyses. The morphology of this strain was very similar to that of the originally described cells of *O. ovata* (Fukuyo, 1981). Until now, there have been no formal reports for *O. ovata* or *O.* cf. *ovata* in Korean waters. Therefore, in the present study, we provide the first report about the morphological and genetic (SSU, ITS1, 5.8S, ITS2, D1-D3, and D8-D10 LSU rDNA)

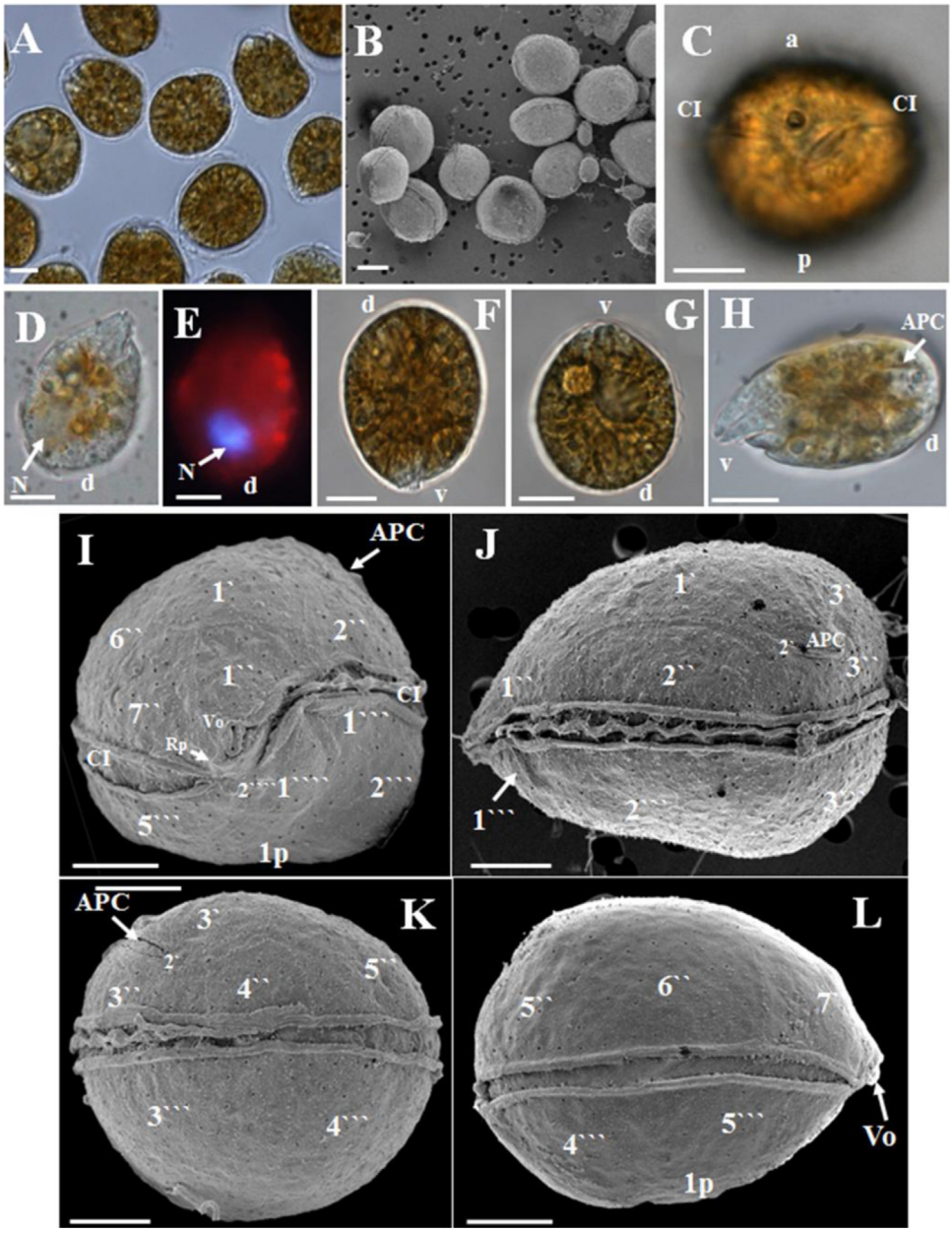

Fig. 1. Micrographs of the *Ostreopsis* cf. *ovata* Korean strain. Light microscopy (A) and scanning electron microscopy (B) micrographs showing cells of various sizes and shapes. Light (C, D, F–H) and epi-fluorescent (E) microscopy micrographs. (C) Ventral view of an almost spherical outline of a cell with a compression between the anterior (a) and posterior ends (p) and a somewhat narrow cingulum (CI). Apical view showing the nucleus (N – visible as a gray body in D and a blue body in E) located dorsally (d). Cells become narrower in the ventor (v), compared to the dorsa (d) in apical (F) and antapical (G) views. (H) Lateral view showing a beak-like feature on the ventral side (v) and a round feature on the dorsal side (d) with the apical pore complex (APC). (I–L) Scanning electron microscopy micrographs. (I) Ventral view of the epithecal and hypothecal plates, CI, ventral opening (Vo), ridged plate (Rp), and APC. (J) Dorsal-left side lateral view of the epithecal and hypothecal plates and APC. (K) Dorsal view of the epithecal and hypothecal plates. (L) Right side lateral view of the epithecal and hypothecal plates. Scale bars = 10 μm for (A)–(H) and 5 μm for (I)–(L).