

Morphological Variation of *Lingulodinium polyedrum* (Dinophyceae) in Culture Specimens and Reinterpretation of the Thecal Formula

Keun-Yong Kim* Makoto Yoshida¹ and Chang-Hoon Kim

Department of Aquaculture, Pukyong National University, Busan 608-737, South Korea

¹Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto 862-8502, Japan

Morphological observation of a unialgal culture of *Lingulodinium polyedrum* was conducted under light- and scanning electron microscopes. *Lingulodinium polyedrum* was redefined as having the thecal formula of APC, Q, 5', 6'', 6c, 6s, 5''', 2'''' using the reinterpreted tabulation system. In our tabulation system, the Q, which is equivalent to the 3' in the strict Kofoidian system, was treated as an auxiliary platelet because of its considerable morphological variations in culture specimens. Subsequently, apical plates were redefined as plates directly connected to the APC and/or the Q. The 1''' and the 1p in the conventional Kofoidian system were treated as a component of the sulcus (the Ssa) and as an antapical plate (the 1'''), respectively. Our reinterpreted tabulation system clearly differentiated extant gonyaulacalean taxa.

Key Words: *Lingulodinium polyedrum*, morphological variation, reinterpretation, thecal formula

INTRODUCTION

Photosynthetic, bioluminescent *Lingulodinium polyedrum* (Stein) Dodge is distributed worldwide and one of well-known harmful algal bloom (HAB) species that have sometimes caused mass mortality of marine animals (Torrey 1902; Schradie and Bliss 1962; Blasco 1977; Bruno *et al.* 1990; Turabo *et al.* 1998). Recently, its production of newly recognized yessotoxins (YTXs) was demonstrated in mussels collected during a HAB episode in the Adriatic Sea and in algal culture (Turabo *et al.* 1998; Paz *et al.* 2004). This dinoflagellate has also been widely used as an experimental microorganism in various phases of physiological and molecular researches (e.g., Mittag *et al.* 1994; Le *et al.* 1997; Okamoto *et al.* 2001; Chudnovsky *et al.* 2002; Suzuki *et al.* 2005).

Dodge (1989) redefined the genus *Gonyaulax* Diesing as Po, 3', 2a, 6'', 6c, 4-8s, 5''', 1p, 1'''' and suggested moving other gonyaulacoids that did not fit this thecal formula to other genera. According to his redefinition, *Gonyaulax polyedra* Stein was transferred to the fossil genus *Lingulodinium* (Wall) Dodge because of the different thecal formula of Po, 3', 3a, 6'', 6c, s, 6''', 1p, 1''''.

Its cyst morphology is also different from that of *Gonyaulax spinifera* (Claparède *et* Lachmann) Diesing, the type species of *Gonyaulax* (Wall 1967; Kobayashi *et al.* 1981), further justifying its generic transfer.

Various nomenclatures have been proposed to describe the thecal formula of thecate dinoflagellates since Kofoid (1909, 1911), and taxonomists have interpreted several plates differently (e.g., apical plates as anterior intercalary plates, the Q as the 3', the Ssa as the 1''' and the 1'''' as the 1p). For example, *L. polyedrum* has been described as cl. pl., 4', 2a, 6'', 6, 6''', 1p, 1''''; 2 ap. cl., 5', 6'', 6''', 2''''; and APC, Q, 4', 1a, 6'', 6c, s, 6''', 1p, 1'''' by Kofoid (1911), Eaton (1980) and Fensome *et al.* (1993), respectively. As a result, apparently different taxa were classified under the same genus, and species belonging to the same genus were assigned to different genera (Balech 1980; Eaton 1980; Evitt 1985; Helenes 1986). Thus, Balech (1980) stressed the necessity of normalizing the nomenclatures based on the actual affinities among thecal plates.

In this study, we established a unialgal culture of *L. polyedrum*. Based on morphological observation under a light microscope and a scanning electron microscope (SEM), the thecal formula of *L. polyedrum* was redefined and compared to those of some extant gonyaulacalean taxa using the reinterpreted tabulation system for

*Corresponding author (koby0323@yahoo.com)

consistency.

MATERIALS AND METHODS

Lingulodinium polyedrum DRW0108 was established from a plankton sample collected from Deukryang Bay, Korea in 2001. A motile cell was picked out using a micropipette, washed successively with sterile seawater and finally transferred to a test tube containing f/2-Si medium (Guillard and Ryther 1962). The unialgal culture was maintained at 18-20°C and at *ca* 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ of a cool white fluorescent tube under a 14L:10D photoperiod.

Vegetative cells were photographed under a light microscope (Olympus BX50; Olympus Corp., Nagano, Japan) equipped with the differential interference contrast (DIC) optic. For SEM observation, vegetative cells in the exponential growth phase were concentrated by brief centrifugation and fixed with 1% OsO₄ for 15 min. The cells were then rinsed twice with filtered seawater, placed onto a 0.2 μm pore-size polycarbonate membrane filter (Nucleopore®; Corning, New York City,

USA), dehydrated in a graded ethanol series, critical-point dried using liquid CO₂ and finally coated with Au-Pd. The specimen was examined with a HITACHI S-2400 SEM (Hitachi, Ltd., Tokyo, Japan). Terminology used for the description of the thecal formula was modified from Kofoid (1911) and Balech (1995; Fig. 1).

RESULTS

Morphological observation

Cells of *Lingulodinium polyedrum* were pentagonal in ventral view (Fig. 2A, H), 32.7-46.2 μm long and 30.4-42.9 μm wide ($n = 15$). The thecal formula was APC, Q, 5', 6'', 6c, 6s, 5''', 2'''' using the reinterpreted tabulation system for consistency (see below). The triangular epitheca was usually composed of the apical pore complex (APC), the Q, five apical plates (5') and six precingular plates (6''; Fig. 2A, C). The top of the epitheca was slightly truncated. The ellipsoidal APC was flush with the edges of 2-3 apical plates and the Q, and was laid dorsoventrally at the center of the epitheca. Its center was ornamented with numerous cross-ridges. When present, the small Q, which is equivalent to the 3' in the strict Kofoidian system (Kofoid 1911), was four- or six-sided and situated along the right side of the APC. The irregular 1' directly connected to the APC was long and narrow, and dented inwardly. A ventral pore (vp) was sometimes present on the suture between the 1' and the 2'. The 4', which corresponds to the 2a in the strict Kofoidian system, was situated between the 3' and the 5' at the right side of the epitheca, and usually did not directly contact either the APC or the 1' and 2'. The 6'' was five-sided, with a convex left margin.

The trapezoidal hypotheca was composed of five precingular plates (5''') and two antapical plates (2''''; Fig. 2A, B, D). The 1''', which is homologous to the posterior intercalary plate (1p) in the conventional Kofoidian system, was vertically elongated with a pointed anterior margin. The anterior margin was sometimes truncated and contacted the left posterior margin of the anterior sinistral sulcal platelet (Ssa). The 2'''' was six-sided with a concave ventral margin and was situated at the center of the hypotheca. Evitt (1985) and Fensome *et al.* (1993) categorized this hypothetical organization as "sexiform." There were neither prominent spines nor horns in the antapex.

The deeply incised median cingulum was delineated by ribbed lists and displaced by one girdle width without offset of the two ends (Fig. 2A, I). It was

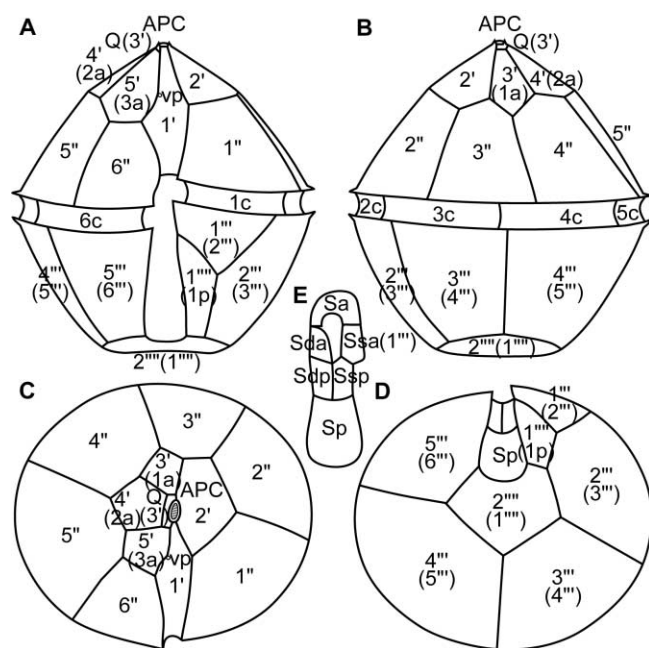


Fig. 1. *Lingulodinium polyedrum*. Schematic diagram of ventral (A), dorsal (B), apical (C) and antapical (D) views, and of sulcal platelets (E). The equivalent Kofoidian tabulation is indicated in parentheses. APC, apical pore complex; 1'-5', apical plate series; 1''-6'', precingular plate; 1'''-5''', postcingular plate; 1''''-2'''', antapical plate; Sa, anterior sulcal platelet; Sda, anterior dextral sulcal; Ssa, anterior sinistral sulcal; Sdp, posterior dextral sulcal; Ssp, posterior sinistral sulcal; Sp, posterior sulcal.

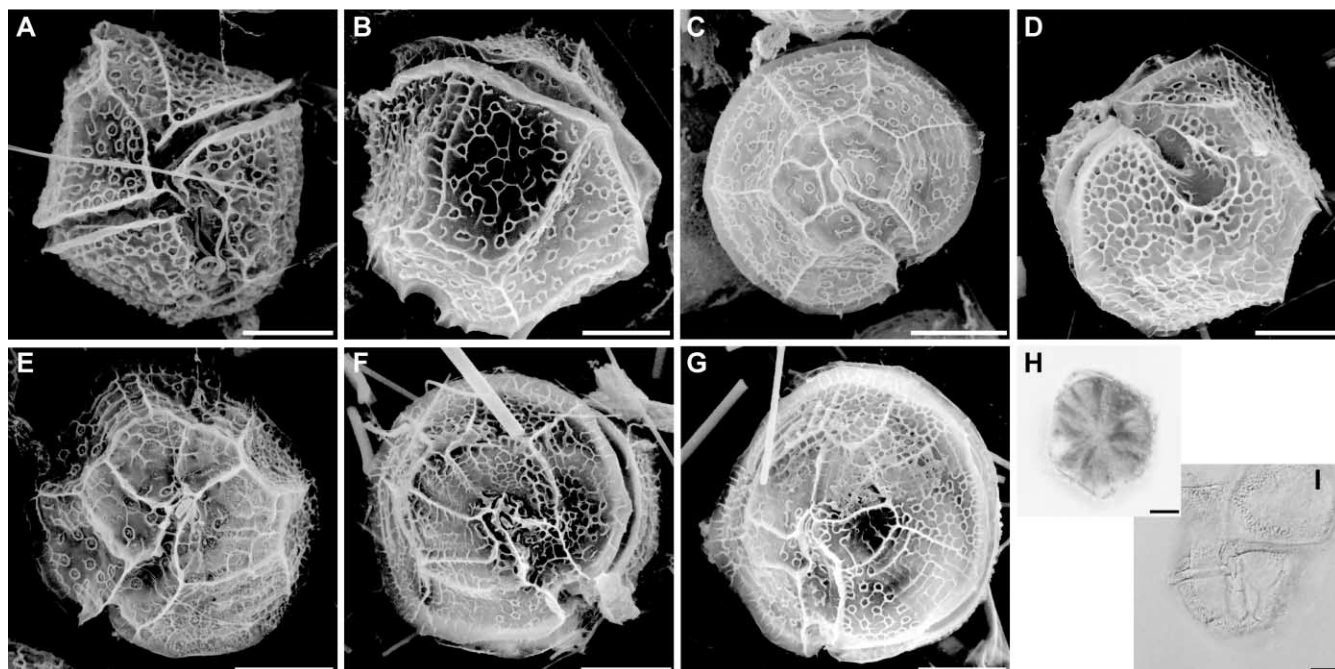


Fig. 2. *Lingulodinium polyedrum*. SEM micrographs of ventral (A), dorsal (B), apical (C) and antapical (D) views, and aberrant epithelial plate patterns (E-G). Light micrographs of a vegetative cell (H) and sulcal platelets (I). Scale bars = 10 μ m.

previously reported to comprise six cingular platelets (Kofoid 1911; Dürr and Netzel 1974; Kobayashi *et al.* 1981; Dodge 1989). The inwardly dented sulcus stretched to the antapex with slight widening and was contiguous to the 1' (Fig. 2A, D). The detailed arrangement of sulcal plates observed in this study was identical to the illustration by Kofoid (1911). It consisted of six platelets: the anterior sulcal (Sa), the anterior dextral sulcal (Sda), the Ssa, the posterior dextral sulcal (Sdp), the posterior sinistral sulcal (Ssp) and the posterior sulcal (Sp; Fig. 2I). The Ssa was inversely pentagonal-shaped, with the right posterior margin bordering the Ssp and the left posterior margin bordering the anterior margin of the 1'''. The Sp with the concave ventral and convex dorsal margins was dorsoventrally elongated with antapical widening. Sulcal lists were slightly developed.

Thecal plates were delineated by ridges along sutures, and hypothecal plates were sometimes interrupted by strong suture bands (Fig. 2B). All thecal plates, including cingular and sulcal platelets, were fairly thick, and ornamented with reticulations and numerous round trichocyst pores (Fig. 2A-G). Cells were yellow-brown, and the major accessory pigment was peridinin (unpublished data). A nucleus was located in the middle of the cell (Fig. 2H).

Morphological variation of the epitheca

In addition to the normal plate tabulation described

above, aberrant epithelial tabulations were often observed in culture specimens of *L. polyedrum* (Fig. 2E-G). Sometimes the Q was considerably diminished, leading to the direct connection of the 3' to the APC (Fig. 2E, F). In other cases, the Q was shrunken and totally absent, resulting in the direct contact of the 4' to the APC, and there were subsequently five Kofoidian apical plates instead of four (Fig. 2G).

DISCUSSION

Morphological observation and variation

The thecal formula of *Lingulodinium polyedrum* was APC, Q, 5', 6'', 6c, 6s, 5''', 2'''' using the reinterpreted tabulation system, which was basically identical to those previously reported (i.e., Kofoid 1911; Dürr and Netzel 1974; Kobayashi *et al.* 1981; Dodge 1989). The thecal formula of Po, 3', 3a, 6'', 6''', 1p, 1'''' of Dodge (1989) was slightly different from those of other taxonomists (i.e., Po, 4', 2a, 6'', 6''', 1p, 1''''; Kofoid 1911; Dürr and Netzel 1974; Kobayashi *et al.* 1981), and this difference in the epithelial tabulation appeared to be caused by the morphological variation of the Q observed in this study. Likewise, Kofoid (1911) also noted the significant reduction of the Q and the direct contact of the 4' to the APC, resulting in the thecal formula of cl, pl, 5', 1a, 6'', 6, 6''', 1p, 1'''' according to his nomenclature. This morphological variation indicates that the Q is not a

good taxonomic criterion and should be treated as an auxiliary platelet. Similar thecal variation has often been documented in diverse gonyaulacalean species in response to varying environmental or culture conditions (Taylor 1975; Wall and Evitt 1975; Hansen *et al.* 1996/97; Delgado *et al.* 1997).

Reinterpretation of the thecal formula

Kofoid (1909, 1911) designated apical plates as those plates that reached the apical pore (Po) and intercalary plates as those between these plates and the precingular plates. Thus Kofoid (1911) did not treat the Q as a separate platelet and included it in the apical plate series. However more recently, it has been treated as an independent platelet separated from apical plates (Eaton 1980; Helenes 1984; Evitt 1985). We also treated the Q as an auxiliary platelet belonging to the APC, primary based on its significant morphological variation found in this study. Subsequently, apical plates were redefined as plates contiguous to the APC and/or the Q. It is consistent with the case of the Peridinales where the "canal platelet (X)" is conventionally treated as a member of the Po. In several species, a certain apical plate is indirectly connected to the APC or the Q and may be considered as an anterior intercalary plate in the strict Kofoidian system, but its 'real affinity' is more important and thus included in the apical plate series [e.g., the 1' in *Alexandrium* spp. in the subgenus *Gessnerium sunsu* Balech (Balech 1995), the 4' in *Ceratium hirundinella* (Müller) Schrank, the 1' in *Ceratium carolinianum* Jörgensen (Wall and Evitt 1975) and the 1' in *Glochidinium penardiforme* (Lindemann) Boltovskoy (Boltovskoy 1999)].

There could be five or six postcingular plates depending on the interpretation of a small platelet, the Ssa (Balech 1980, 1995; Evitt 1985; Dodge 1989), which corresponds to the 1''' in the conventional Kofoidian system (Kofoid 1911). Balech (1995), however, treated it as a component of the sulcus because of its narrow, obliquely depressed nature towards the sulcus, and it was apparently so in *Alexandrium* Halim (Balech 1995), *Amylax triacantha* (Jörgensen) Sournia (Kofoid 1906, 1911), *L. polyedrum* (Kobayashi *et al.* 1981; Dodge 1989; this study), *Pyrocystis* Murray *ex* Haeckel (Swift and Wall 1972; Taylor 1972; Pincemin *et al.* 1982) and *Pyrodinium bahamense* var. *compressum* (Böhm) Steidinger, Tester *et al.* Taylor (Steidinger *et al.* 1980). The developed sulcal list also seemed to be frequently misinterpreted for the 1'''. *Gonyaulax* species (Kofoid 1911; Matsuoka *et al.* 1988;

Dodge 1989; Lewis *et al.* 2001; Ellegaard *et al.* 2003) and *Protoceratium reticulatum* (Claparède *et* Lachmann) Bütschli (Reinecke 1967; von Stosch 1969; Dodge 1989) were such cases. In contrast, Balech (1980) defined antapical plates as those bordering the sulcus without being connected to the cingulum and included the 1p in the antapical plate series. Eaton's (1980) redefinition of antapical plates as the plates posterior to and contiguous to postcingular plates was different from that of Balech (1980), but also interpreted the 1p as an antapical plate. Thus, there are two antapical plates, the 1'''' and the 2''''', according to Balech (1980), which are homologues of the 1p and the 1''', respectively, in the conventional Kofoidian system (Kofoid 1911).

Comparison with other extant gonyaulacalean taxa

In this study, we subsequently adopted the new nomenclatural interpretation described above to modern gonyaulacalean taxa. When our tabulation system was consistently applied, the genus *Gonyaulax* Diesing was recognized as having the thecal formula of APC, Q, 4', 6'', 5''', 2''''', and *L. polyedrum* was clearly different from *Gonyaulax* species by possessing an additional apical plate. *Amylax triacantha* had the identical tabulation to *L. polyedrum*, but was easily distinguishable by the position of the Q at the left side of the APC and by prominent antapical spine(s) (Kofoid 1906, 1911; Dodge 1989). The general thecal formula of goniodomacean taxa including *Alexandrium* and *Pyrodinium* Plate was APC, 4', 6'', 5''', 2''''', and was easily discriminated from other gonyaulacalean members by having four apical plates and lacking the Q (Steidinger *et al.* 1980; Balech 1995). The phylogenetic relationship of these gonyaulacalean members based on rDNA sequence data will be presented in the ongoing study.

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