

A new record of the dinoflagellate *Lingulodinium polyedra* (Dinophyceae, Gonyaulacales) from Tanjung Api-Api, South Sumatra, Indonesia

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Abstract. Accurate identification of potentially harmful marine microalgae is required for biological risk monitoring, particularly in coastal areas with prominent aquaculture and tourism activities. Although molecular-based identification is becoming the standard method for species delimitation, morphological identification using light microscopy is often preferred as the tools required are easily available and accessible to both the general public and scientific communities. Unfortunately, many reports on microalgal biodiversity in Indonesia still lack reliable photomicrographs and detailed descriptions of the identified species, thus making it challenging to confirm the correct identity and occurrence of the taxa in subsequent inventory studies. In this study, a new record of Lingulodinium polyedra was reported from Tanjung Api-Api, South Sumatra, Indonesia. This planktonic dinoflagellate is listed as potentially harmful by the Intergovernmental Oceanic Commission (IOC) UNESCO. Based on the cell outline, the species was angular and roughly pentagonal in ventral view or polyhedral-shaped, 44.34-48.78 µm wide by 44.94-50.12 µm long. The cell was also armored with thick thecal plates and its surface was coarsely sculptured, with numerous trichocyst pores, while strong suture lines bordered these thecal plates. The Kofoidan tabulation formula for the thecal plates was APC (Po), 3', 3a, 6", 6c, 6s, 6"', 2"", thus conforming to the original description of L. polyedra. This study demonstrated that the use of light microscope is still highly practical for correct morphological characterization of marine microalgae, including potentially harmful, thecate dinoflagellate species.

Key Words: light microscopy, *Lingulodinium polyedra*, microalgae, morphology.

Introduction. Among many microalgae species, about 200 of them are harmful (Lundholm et al 2009; Lassus et al 2016) due to their ability to form massive cell proliferation (i.e. harmful algal bloom (HAB)) or produce phycotoxins linked to numerous fish and shellfish poisoning incidents (Hallegraeff 2003; Lassus et al 2016; Hallegraeff et al 2021). Approximately 68% of these potentially harmful microalgae belong to the dinoflagellate (Lundholm et al 2009; Lassus et al 2016). This monophyletic group of mainly unicellular eukaryotes is distinguished from other microalgae by a set of unique features such as the presence of two distinct flagella (i.e. transverse and longitudinal flagella), specific pigmentations (e.g. peridinin, dinoxanthin, etc.), distinctive organelles (e.g. the pusule, numerous types of eyespots and nucleus (i.e. the dinokaryon), among other features (Fensome et al 1993; Hackett et al 2004; Taylor et al 2008).

Several harmful dinoflagellate species are armored with cellulosic thecal plates (Taylor 1987). Unlike their unarmored counterparts that are harder to distinguish at the species level, the thecal plates of armored dinoflagellates are arranged in species-specific patterns that serve as a reliable identification criterion when observed using microscopes (Taylor 1987; Fensome et al 1993). One such species is *Lingulodinium polyedra* (F. Stein) J. D. Dodge (Syn. *Gonyaulax polyedra*) (Lassus et al 2016). This thecate and planktonic marine dinoflagellate is a bloom-forming species with some isolates or strains that have been reported to produce toxic compounds (Bruno et al 1990; Paz et al 2004; Armstrong & Kudela 2006; Lassus et al 2016). Taxonomically, the species was initially described as

G. polyedra from the Baltic Sea in northern Germany (Stein, 1883) before being reclassified into the genus *Lingulodinium* by Dodge (1989). Currently, two accepted taxonomic identities are used for this species: one assigned to its motile form (i.e L. polyedra) and another for its fossil or cyst form (i.e. L. machaerophorum) (Tillmann et al 2021). The earliest reports of harmful microalgae in Indonesia date back to the 1980s (Dahril 1981; Adnan 1989; Sidharta 2005). Subsequent studies have primarily focused on the ecological aspects of HAB events (Thoha & Rachman 2013; Thoha et al 2019; Nasution et al 2021; Rachman et al 2021). Accurate identification of potentially harmful marine microalgae is essential for biological risk monitoring, particularly in coastal regions with prominent aquaculture and tourism activities. Although molecular-based identification is becoming the standard method for species delimitation, morphological identification using light microscopy is often preferred as the tools required are easily available and accessible to both the general public and scientific communities. Yet, taxonomic and systematic studies of harmful microalgae in Indonesia, particularly those providing detailed morphological descriptions and illustrations of the reported species are rare (Sidharta 2005), thus, making it difficult to confirm the correct identity and occurrence of the taxa in subsequent inventory studies. Moreover, with more than 17,000 islands and over 80,000 km long coastline (Lestari et al 2008), information on the biodiversity and distribution records of harmful microalgae is still lacking. With regard to L. polyedra, only a few reports have been published from Indonesia.

In the current study, two cells of a thecate marine planktonic dinoflagellate were found in seawater samples collected from the mangrove area in Tanjung Api-Api, South Sumatra, Indonesia in 2021. Upon morphological examination using a light microscope, we identified the species as *L. polyedra*. This study reports for the first time the occurrence record, morphological description, and illustrations of this potentially harmful dinoflagellate from this area.

Material and Method

Sample origin and processing. Seawater sample was originally obtained in June 2021 during the low tide from five locations (i.e MW4, MW7, MW11, MW13-E, MW17 and MW21) in the mangrove area of Tanjung Api-Api, South Sumatra, Indonesia (Figure 1, Table 1).



Figure 1. Sampling in the mangrove area of Tanjung Api-Api, South Sumatra, Indonesia. Red triangles indicate the sampling points. Yellow circles above the triangles indicate where specimens of *Lingulodinium polyedra* were encountered in the samples. Source: Google Earth.

Water samples were collected from the surface layer (with a depth of ca. 0.3 m) using a plankton net (20 μm mesh size) and stored in 50 mL Falcon tubes. Each sample was subsequently enriched by adding a few drops of Daigo's IMK medium (Wako, Tokyo,

Japan) before storing them in a cool box and sent to the Laboratory of Phycology at the Indonesian Culture Collection (InaCC) in Cibinong, West Java, Indonesia. Water temperature, salinity and pH were measured using an EZ-9909 5-in-1 water quality tester (Thincol). Temperatures at the sampling points ranged between 31.5–34°C, while the salinity was 13.0–18.5 PSU (Table 1), indicating a typical brackish estuary environment with primary freshwater input from the Musi River.

Table 1

Sample	Date	Lat. (S)	Lon. (E)	Т (°С)	Salinity (PSU)	pН
MW4	1 June 2021	2.35	104.91	34.0	13.0	6.7
MW7	2 June 2021	2.38	104.89	33.2	18.5	6.7
MW11	3 June 2021	2.27	104.92	33.0	16.0	6.8
MW13-E	5 June 2021	2.29	104.93	33.0	17.5	6.4
MW17	5 June 2021	2.37	104.81	32.5	17.5	7.9
MW21	5 June 2021	2.37	104.82	31.5	16.0	7.2

Sampling information including the collection date, the GPS position and the measured environmental parameters

Once arrived at the laboratory (~5 days after sampling), each sample was immediately transferred into clean plastic Petri dishes and incubated under 25°C with about 30 µmol m⁻² s⁻¹ fluorescent illumination (12:12 hours light-dark cycles) for the duration of sample observation. The samples were routinely checked daily for microalgal cells using an Olympus CKX 53 inverted microscope (Olympus Corp., Tokyo, Japan), during another 5 days.

Morphological characterization and taxonomic identification. The samples contained very few microalgal cells or their remains, with the diatoms (e.g. *Coscinodiscus* sp., *Chaetoceros* sp., and *Navicula* sp.) as the dominant components. A small number of unidentified filamentous cyanobacteria were also present. As for dinoflagellate, the cell remains of *Tripos* sp. (syn. *Ceratium* sp.) and *Protoperidinium* sp. were observed, but could not be used for accurate species-level identification.

Regarding L. polyedra, only two intact cells were encountered in samples from MW17 and MW21, respectively. Although both cells were morphologically identical, the specimen found at MW21 were selected for further morphological characterization, mainly because of its larger cell size. Due to the scarcity of available specimens, we took utmost care when handling this cell for light microscopy (LM) observation and photomicrography, by following a similar method reported in Prabowo et al (2023). The cell was carefully isolated from the Petri dish sample using a glass capillary micropipette and gently transferred into a drop of IMK medium previously adhered to a clean glass microscope slide. Morphological observation was carried out using an Olympus CKX 53 inverted light microscope (Olympus Corp., Tokyo, Japan) equipped with an Olympus EP50 (Olympus Corp., Tokyo, Japan) digital camera under various objective magnifications (i.e. $4\times$, $10\times$, $20\times$, $40\times$). For photomicrography, a fine glass capillary micropipette was used to adjust the position of the cell specimen under the inverted microscope. Acquired micrographs were digitally enhanced (i.e. adjusting the brightness, exposure, contrast, orientation and trimming) using Adobe Photoshop 21.1.1 (Adobe). All photographed cells were positioned on a clean background using the same software. Subsequently, line drawings of the cell and its thecal plate arrangement were illustrated by hand using Affinity Designer ver. 1.10.1 (Serif Europe Ltd). The Kofoidan plate tabulation system was adopted (Kofoid 1909; Kofoid 1911) to enumerate and map the thecal plate arrangement of the specimen. Finally, the identity of the species was determined based on the taxonomic description of Dodge (1989) with comparative information provided by Faust & Gulledge (2002), Kim et al (2005), Tillmann et al (2021) and other relevant references.

Results and Discussion

General morphology. This study presents the first occurrence record, morphological description and illustrations of L. polyedra from the Tanjung Api-Api, South Sumatra, Indonesia. The cell specimens of *L. polyedra* were angular, somewhat pentagonal in ventral view or polyhedral-shaped, slightly pointed at the apical end, but blunt or flat at the antapical end (Figures 2a and 2b). The cell of MW17 specimen measured 44.34 µm wide and 44.94 µm long (Figure 2a), whereas the cell of MW21 specimen was 48.78 µm wide and 50.12 µm long (Figure 2b). Both specimens lacked apical horn or antapical spine. The epitheca was slightly larger than the hypotheca and separated by a deeply excavated cingulum positioned in the middle and descended leftward towards its right end (Figure 2b). The sulcus was also deeply excavated, narrowing at the anterior where it slightly invaded the epitheca but broadened towards the posterior of the hypotheca (Figure 2c). The right and left cingulum end (RCE and LCE) were displaced by about one to two cingulum-widths and both converged at the sulcal area where the transverse and longitudinal flagella were supposed to emerge (Figures 2b and 2c). The anterior and posterior rims of the cinqulum were extended by a thin and narrow list (Figure 2c). Cells were armored with thick and well-defined thecal plates, also bordered by strong suture lines (Figures 2b and 2c). The surface of the plates was coarsely areolated with numerous trichocyst pores (Figure 2d).



Figure 2. Light micrographs of *Lingulodinium polyedra* specimens found in Tanjung Api-Api, South Sumatra, Indonesia: MW17 (a) and MW21 (b) specimens from showing the general cell outline. Magnified area of the cingulum and sulcus (c) and the trichocyst pores (d) decorating the surface of the thecal plates. White arrowheads indicate the cingulum position. The right and left cingulum ends were abbreviated as RCE and LCE, respectively. Scale bars = 10 μ m.

Thecal plates arrangement. A total of 33 thecal plates were enumerated for the MW21 cell specimen and its Kofoidan tabulation formula was defined as APC (Po), 3', 3a, 6", 6c, 6s, 6", 2"". The complete thecal plates arrangement and tabulation of L. polyedra are illustrated in Figures 3 and 4. The epitheca comprised a total of 13 plates: one pore plate (Po), three apical plates (i.e. plate 1'-3'), three anterior intercalary plates (i.e. plate 1a-3a) and six precingular plates (i.e plate 1''-6'') (Figures 3a-3i). The pore plate (Po) was small, oblong and bordered by the three apical plates (i.e. plate 1", 2" and 3") (Figure 3a and 3f). The 1' plate was long and narrow at the anterior part where it connected with the posterior of the Po, widening at its upper median part before slightly narrowing towards its rear where it touched the anterior sulcal plate (Sa) (Figures 3a, 3b, 3f and 3g). The 3' plate was rectangular and it was the smallest among the apical plates (Figures 3a and 3g). All three anterior intercalary plates (i.e. plate 1a, 2a, 3a) were roughly similar in size and positioned on the right apical side of the epitheca (Figures 3a and 3f). The precingular plates (i.e. plate 1''-6'') were guadrangular or pentagonal and more prominent than any other plates of the epitheca (Figures 3a-3e and 3f-3j). The third precingular plate (3") was five-sided, with its posterior margin being the widest. An opening gap observed at the anterior end of this 3" plate likely indicated the ecdysis route of the cell content from its encasement (Figures 3c, 3d, 3h, and 3j).



Figure 3. Epithecal plates tabulation of *Lingulodinium polyedra* specimen from MW21, based on light micrographs (a–e) and line drawings (f–j). The orientations of the micrographs are as follows: apical view (a, f), right ventral view (b, g), left lateral view (c, h), dorsal view (d, i), and right lateral view (e, j). Note the partially detached third precingular plate (3''') indicating the ecdysis route of this species. The cingulum and sulcal plates are highlighted in yellow and red, respectively. Scale bars = 10 μm.



Figure 4. The cingulum, sulcal, and epithecal plates tabulation of *Lingulodinium polyedra* specimen from MW21, based on light micrographs (a–d) and line drawings (e–f). The orientations of the micrographs are as follows: cross-sectional view through the antapex (a, e), lower ventral views (b, c, f, g), and antapical view (d, h). The cingulum and sulcal plates are highlighted in yellow and red, respectively. Scale bars = 10 μm.

The cingulum consisted of six plates (i.e. plate c1-c6) (Figures 3g, 3h, 3j, 4e, and 4f). Similarly, the sulcal plates also comprised six plates (6s): i.e the sulcal anterior (Sa) plate, the right sulcal anterior (Sda), the left sulcal anterior (Ssa), the right sulcal posterior (Sda), the median sulcal plate (Sm) and the sulcal posterior plate (Sp). (Figure 4f). Finally, the hypotheca consisted of eight thecal plates: i.e. six postcingular plates (plate 1'''-6''') and two antapical plates (1''' and 2'''') (Figure 4). The first postcingular

plate (1"") was four or five-sided and the smallest among the hypothecal plates (Figures 4f and 4q). The 1"" antapical plate was ventrally located at the left side of the sulcus, elongated and polygonal-shaped. In contrast, the 2"" plate was six-sided with a concave ventral margin and positioned at the center of the antapex (Figures 4a, 4b, 4c, 4e, and 4f). No spine was observed on the antapical end of the hypotheca. Based on these morphological features and following the revised species description of Dodge (1989), we were able to identify our specimen as *L. polyedra*, with a few accessory observations. First, a ventral pore (Vp) was supposedly present at the upper right side of the first apical plate (1') (Kofoid 1911; Dodge 1989; Lewis & Hallet 1997; Tillmann et al 2021). However, due to the limitation of the LM used in this study, the Vp could neither be observed nor differentiated from the numerous trichocyst pores in our specimen. Observation using a scanning electron microscope (SEM) is needed to confirm the presence of the Vp on our specimen, including other fine morphological features that could not be detected by LM alone (e.g. the structure of the apical pore complex (APC), the ornamentations of the sulcus and the suture lines, the reticulated pattern of the thecal plates, etc.). Secondly, the number of sulcal plates of L. polyedra was not enumerated in the original and revised description, either by Stein (1883) or Dodge (1989). Faust & Gulledge (2002) mentioned a total of seven sulcal plates (7s), although the micrographs and illustrations provided in their report did not indicate the position of these seven sulcal plates. Our observation revealed a total of six sulcal plates (6s) (Figure 4), which agreed with the reports of Kim et al (2005) and the detailed morphological illustration of *L. polyedra*, published by Tillmann et al (2021), based on high-resolution SEM and fluorescence microscopy observation.

Concerning the thecal plate arrangement of L. polyedra, several tabulation formulas have been proposed, mainly adopting the Kofoidan (Kofoid 1909) and Taylor-Evitt systems (Fensome et al 1993) with some variations (Kim et al 2005). However, the differences in the tabulation formulas could be a subjective interpretation of how thecal plates are arranged and enumerated. Thus, when an armored dinoflagellate is described with different tabulation systems, all of the described formulas must be compared to normalize the dissimilarity of notations between them, like those shown in Tillmann et al (2021). In our study, due to the limited magnification capabilities of the LM, we could only locate the Po plate in the APC of our specimen. Using SEM, Tillmann et al (2021) recognized two other APC components in L. polyedra (i.e. the cover plate (cp) and the X plate), which have not been illustrated in other reports due to the difficulty in observing them. The remaining epithecal plates composition of our specimen agreed with other reports (e.g. Dodge 1989; Steidinger & Tangen 1996; Faust & Gulledge 2002; Tillmann et al 2021). Similarly, the number of thecal plates at the hypotheca also conform to these studies, although different notations were used. In our study, the posterior intercalary (1p) and one antapical (1"") plates reported by Dodge (1989) were regarded as the first and second antapical plates (1"" and 2""), respectively, following the Kofoidan tabulation system. The same tabulation notation was also used by Steidinger & Tangen (1996), Faust & Gulledge (2002) and Tillmann et al (2021) to enumerate these two antapical plates of *L. polyedra*.

Nomenclatural remark. Although the name "*L. polyedrum*" is frequently used for this dinoflagellate in other publications, the correct species epithet is actually "*polyedra*" instead of "*polyedrum*". The reason is due to the fact that the epithet "polyedra" is a noun in apposition and is non-declinable, so use of the epithet "polyedrum", supposedly to agree with the gender of the genus name, is incorrect. ICN Art 23.5 [Melbourne Code] (Guiry & Guiry 2021).

Distribution record in Indonesia and challenges. *L. polyedra* has a wide geographic distribution, from the cold and temperate regions (Stein 1883; Okolodkov 1998; Caroppo et al 2017; Tillmann et al 2021) to the warm and tropical seas (Zonneveld et al 2010; Gárate-Lizárraga et al 2014; Abbass et al 2018; Prabowo & Agusti 2019). In Indonesia, *L. polyedra* have been reported from several locations with different taxonomic identities (Table 2). Unfortunately, from the eight reports found in digital archives or online public

repositories, only three palynological studies provided illustrative evidence or comprehensive information on its geographical distribution, albeit only in its non-motile form (i.e. cyst; L. machaerophorum) (Hessler et al 2013; Poliakova et al 2017; Likumahua et al 2021). The validity of the remaining records should be interpreted with caution due to the lack of reliable illustrative evidence provided. Furthermore, the possibility of misidentification with other morphologically similar species could also be considered. For instance, Suryani & Arya (2017) reported L. polyedra and Peridiniella catenata (syn. Gonyaulax catenata) from the freshwater Lake Beratan in Bali. However, both species exclusively inhabit the marine environment (Okolodkov 1999; Tillmann et al 2021). In such a case, there is a possibility that cells of thecate freshwater dinoflagellates, like *Peridinium* spp. or other armored species occupying this lake (such as those listed in Suwangsa 2006), were misdiagnosed as *L. polyedra* because of their roughly similar cell outline when observed under LM. Without the determination of the thecal plate tabulation, it is highly challenging to identify such armored dinoflagellates at the species level, correctly. Unfortunately, no illustrative information was provided to validate the correct identity of the species identified as L. polyedra reported from Lake Beratan or other locations (Table 2). Hence, we highlight the importance of including morphological descriptions and illustrative evidence (i.e. micrographs or hand drawings) for every microalgal species identified, instead of only providing an inventory species checklist, to validate the identity and occurrence record of every microalgal taxon encountered in biodiversity surveys, particularly in understudied areas in Indonesia or elsewhere.

Table 2

Taxonomic ID	Location	References	
	Selabih Beach, Bali	Da Costa (2017)	
	Beratan Lake, Bali	Suryani & Arya (2017)	
Gonyaulax polyedra	Padang Pariaman, West Sumatra	Agusta & Ihwan (2019)	
	Ujung Pandang (Makassar)	brindonews.com	
Lingulodinium polyedrum	Western coast of Lampung	kompas.com	
	Simeuleu Island, Aceh		
	Enggano Island, Bengkulu	Hessler et al (2013)	
Lingulodinium	South Java Sea		
macnaeropnorum	Timor Sea		
(Cyst; non-motile form)	Mouth of Pembuang River,	Poliakova et al (2017)	
	Central Borneo		
	Ambon Bay	Likumahua et al (2021)	

Records of Lingulodinium polyedra in Indonesia

Despite the scanty occurrence records of *L. polyedra* in Indonesia, when considering its global distribution, this species is likely present in many coastal regions of this country and may play a role in the formation of microalgal bloom at the local scale. For instance, in 2019, a microalgal bloom in Lampung Bay (Table 2) drew the attention of local communities due to the resulting bioluminescence phenomenon. Among other microalgae species thought to be responsible for this bloom, L. polyedra was also identified in the seawater samples acquired from this locality and most likely contributed to the bioluminescence events like those reported in other areas (Lewis & Hallet 1997; Latz & Rohr 1999; von Dassow et al 2005). Even though frequent microalgal blooms and their impacts have been reported from Lampung Bay (Muawanah et al 2013; Irawan et al 2017; Aditya et al 2015; Barokah et al 2016; Sari 2018; Thoha et al 2019), to the best of our knowledge, no formal reporting concerning the impacts of L. polyedra blooms is published from this area. Moreover, the small number of intact cell specimens found in our study do not necessarily indicate that the cell abundance of *L. polyedra* in the area of sampling (i.e. Tanjung Api-Api) was low. Considering the natural condition of the sampling location (i.e. at the mouth of the Musi River; Figure 1) and the sampling timing (i.e. low tide), it was possible that most *L. polyedra* cells could have been washed away by the freshwater inflow of the Musi River or transported by the tidal currents to the open coastal waters, reducing the cell abundance in the water column during the sampling.

Harmful status. The inclusion of L. polyedra within the taxonomic reference list of harmful marine microalgae by IOC-UNESCO (Lundholm et al 2009) was most likely due to its bloom forming ability and potential toxicity reported in several studies. The presence of yessotoxins (YTX) or their analogues have been detected from few isolates or strains of L. polyedra (Paz et al 2004; Armstrong & Kudela 2006; Karlson et al 2021), but no significant health impact to human was known. It is worth noting that the presence of toxic and non-toxic strains within a dinoflagellate species have been reported in other taxon as well, for example within Alexandrium minutum (Touzet et al 2007; Yang et al 2010). Hence, it is advised that for such species, including *L. polyedra*, toxicity analyses or assays be included in the exact determination of their harmful status. Unfortunately, no viable cells of L. polyedra were obtained from the seawater samples collected in Tanjung Api-Api. The lengthy transporting time (~ 5 days) and storage condition of the samples could have deteriorated many microalgal cells leading to their loss before they could be properly characterized, identified, and isolated at the laboratory. Consequently, the toxicity of *L. polyedra* from Tanjung Api-Api could not be verified in the present study. Nevertheless, considering the potentiality of this species to produce YTX in other studies, we suggest that more sampling efforts be conducted in this area (also in other coastal regions in Indonesia) in order to obtain viable isolates of not only L. polyedra, but also of other potentially harmful marine microalgae species.

Conclusions. This study demonstrated the use of light microscopy (LM) to successfully identify a new record of *L. polyedra* in South Sumatra based on its general cell morphology and thecal plate tabulation. With proper training, LM can still serve as a reliable method for accurate identification of microalgae, particularly dinoflagellates. The presence of *L. polyedra* specimens, albeit scarce, could anticipate that the coastal area of Tanjung Api-Api in South Sumatra is susceptible to HAB events. However, more biodiversity surveys on harmful microalgae are required before any monitoring program can be implemented in this essential ecosystem area.

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Conflict of interest. The authors declare no conflict of interest.

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