

STUDY OF MARINE SAND-DWELLING DINOFLAGELLATE WITH FOUR NEW RECORDS FROM THE CENTRAL SOUTH COAST OF VIET NAM

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Keywords: Marine sand-dwelling; Dinoflagellates taxonomy; Vietnamese coast.

Abstract

In this study, marine sand samples were collected from the shallow intertidal zone of Nha Trang bay, Viet Nam. Taxonomic observations were conducted for the sand-dwelling dinoflagellates using light, epifluorescent and scanning electron microscopy. Morphology characterizations of the four species namely, *Amphidiniopsis hexagona*, *Thecadinium pseudokofoidii*, *Amphidinium herdmanii* and *Fukuyoa ruetzleri* have been reported as new records for Vietnamese coastal waters. The genus *Amphidiniopsis* and *Thecadinium* are a new addition to the dinoflagellates flora of Viet Nam. Taxonomic descriptions and photographs of these species have been provided.

Introduction

Benthic dinoflagellates (including epiphytic and sand-dwelling species) consist of known potentially toxic species which produce toxins that cause ciguatera fish poisoning (CFP), a common illness associated with fish consumption in tropical areas. Benthic dinoflagellates have been found to be associated with sandy bottoms, dead corals, seagrasses, macroalgae, detrital aggregates and even artificial substrate in tropical and subtropical waters (Faust, 1995; Tester *et al.*, 2014). Over the past decade in Viet Nam, surveys of the benthic dinoflagellates have mostly focused on epiphytic toxic species (Nguyen and Larsen, 2004; Ho *et al.*, 2010; Ho and Nguyen, 2014; Ho and H'Yon, 2019), whereas sand-dwelling dinoflagellates have been poorly studied and information on them is insufficient. The aim of this study is to document the occurrence of marine sand-dwelling dinoflagellates from the intertidal zone of Nha Trang Bay contribute to a basis for better understanding of their global distribution. Toward said goal, four marine sand-dwelling dinoflagellate species *viz.*, *Amphidiniopsis hexagona*, *Thecadinium pseudokofoidii*, *Fukuyoa ruetzleri* and *Amphidinium herdmanii* have been described in detail and illustrated herein.

Materials and methods

Samples were taken monthly (July - November 2013) from the shallow intertidal zone of Nha Trang bay (109°13.218' E, 12°12.75' N) (Fig. 1). Surface sand samples were collected by snorkeling divers using plastic bags at a depth of 1–1.5 m and from the upper layer of sandy flat using a spoon during low tide. Water temperature (°C) and salinity (psu) were measured every month during sampling collection in the water column.

In the laboratory, the sand samples were mixed and lightly shaken with filtered seawater taken from the same site and the material was passed through consecutive smaller sieve sizes (125 µm,

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64 μm , 32 μm and 20 μm) to remove large particles. Material on the 64 μm , 32 μm and 20 μm sieves were examined and observed using a Leica MZ 12 stereo microscope. Cells of dinoflagellates were sought and isolated by pipette. The wild cells were observed alive or preserved with lugol-iodine solution using light microscopy (LM). Cell dimensions were measured by light microscopy using a calibrated ocular micrometer. Thecal plate patterns were observed after staining with calcofluor white M2R with a Leica LDMB microscope equipped with phase and differential interference contrasts, epifluorescence microscopy. Photographs were recorded with a digital camera (Olympus DP-71). For scanning electron microscopy (SEM), fixed specimens were isolated and placed on 5 μm carbon membrane in a filter-holder (Millipore), rinsed three times with distilled water and dehydrated through an ethanol series of 15%, 30%, 50%, 70%, 90% and 99.99%, then air dried. The filter was mounted on an aluminium stub with carbon tape and coated with gold in a vacuum sputter coater. Specimens were observed using a JEOL JSM-5410 LV scanning electron microscope.

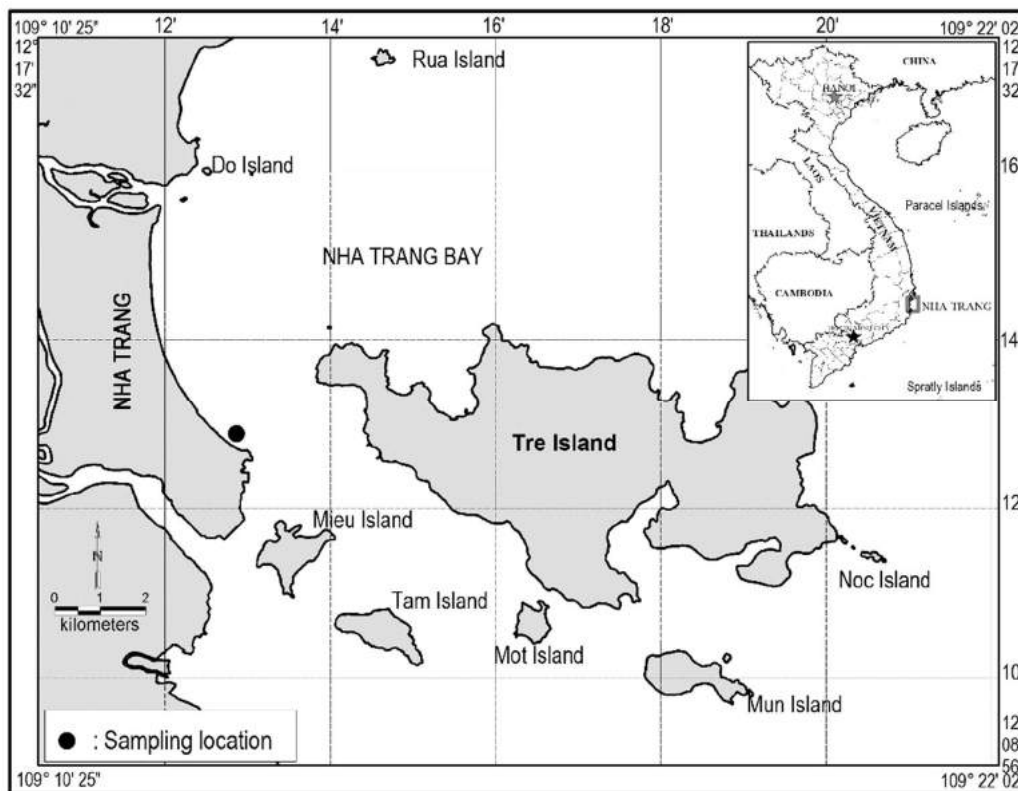


Fig. 1. Map showing the sampling location in Nha Trang bay, Viet Nam.

Results and Discussion

Based on an examination of cell size shape, cell surface morphology and architecture of thecal plates by light and scanning electron microscopy, the marine sand-dwelling dinoflagellates namely, *Amphidiniopsis hexagona*, *Thecadinium pseudokofoidii*, *Amphidinium herdmanii* and *Fukuyoa ruetzleri* have been described and documented with illustrations.

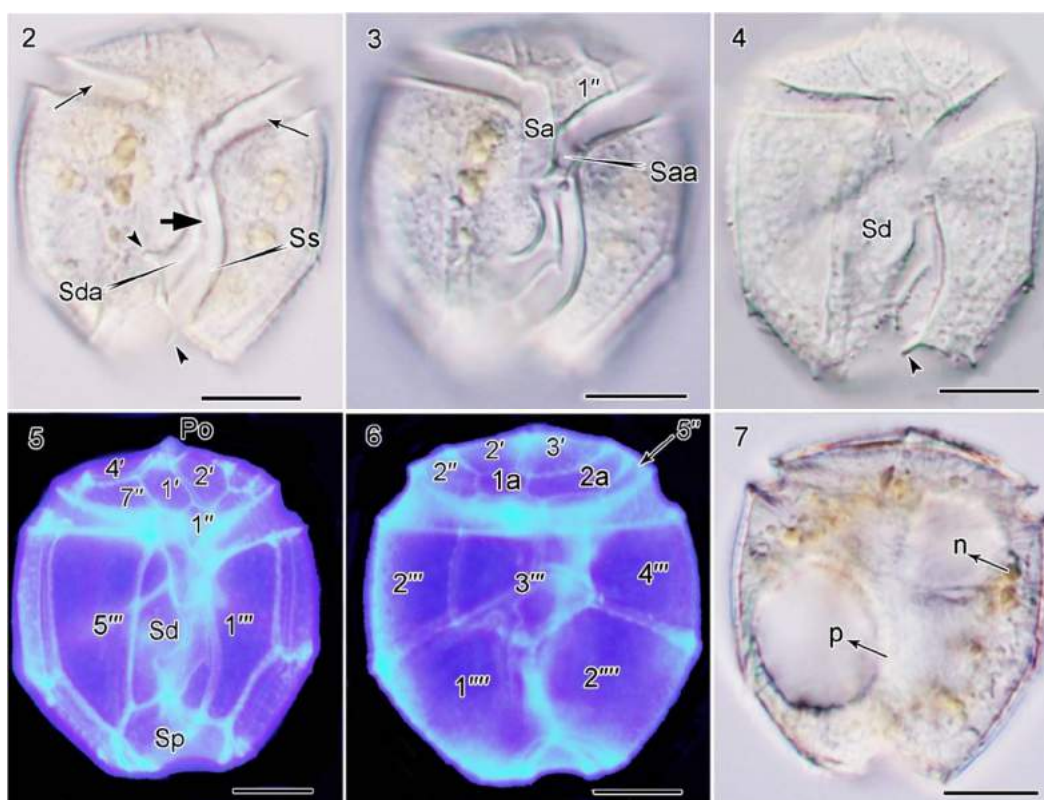
**Class: Dinophyceae West & Fritch 1927; Order: Peridiniales Haeckel 1894;
Family: Thecadiniaceae Balech 1956; Genus: *Amphidiniopsis* Woloszyńska 1929**

Amphidiniopsis hexagona Yoshimatsu, Toriumi *et Dodge*

(Figs 2–7)

(Yoshimatsu *et al.*, 2000a: figs 10–17, figs 18a–d.)

Taxonomic remarks: Cells of *A. hexagona* are hexagonal in ventral view, slightly dorso-ventrally flattened (Figs 2–4, 7). Thecal surface is slightly thick and coated with nipple-like processes (Figs 3–4). Cell dimensions range from 34.5–37 μm long and 30.8–31.7 μm wide. Measurements of our specimens were smaller than those reported in the document for *A. hexagona* (44–59 μm long and 40–53 μm wide, Yoshimatsu *et al.*, 2000a). The plate formula is Po, 4', 2a, 7'', 4s, 5''', 2'''' (The cingulum plates (c) were not visible in the specimens of our study). The epitheca is short, 6.5–7.5 μm long. The epitheca consists of thirteen plates: an apical pore plate (Po), four apical (1', 2', 3' and 4'), two anterior intercalary (1a, 2a), and seven precingular plates (1'', 2'', 3'', 4'', 5'', 6'' and 7'') (Figs 5–6). Four apical plates are of equal size. The anterior intercalary plate 1a is pentagonal and the plate 2a is long and narrow. Plate 1'' is five-sided, plates 2'', 3'', 4'', 5'', 6'' are four-sided and plate 7'' is trapezoidal. The cingulum is deep and equatorial. It



Figs 2–7. Micrographs of *Amphidiniopsis hexagona* under light and epifluorescent microscopy. Fig. 2. Ventral view showing a small epitheca and large hypotheca, cingulum (long arrows) and sulcus (arrow) and two spines emerged at end of the plates Sda and Ss (arrowheads). Figs 3–5. Ventral view. Fig. 6. Dorsal view showing the epithecal and hypothecal plates. Fig. 7. Dorsal view showing a pusule (marked p) and nucleus (marked n) dorsally situated in hypotheca (arrow). Scale bars: 10 μm .

is sinistral torsion and displaced by a deeply as it is wide. The sulcus includes six plates: anterior, right, left and posterior plates (Sa, Sd, Ss and Sp) (Figs 3–5), as well as to two anterior and right accessory sulcus plates (Saa and Sda) (Figs 2–3). Two spines are present at the end of plates Sda and Ss (Figs 2, 4).

Hypotheca is about 28–30 µm long and notched at the antapex (Figs 5–7). The hypotheca contains seven plates: five postcingular plates (1''', 2''', 3''', 4''' and 5'''), and two antapical plates (1''', 2'''). The first and fifth postcingular plates are wide and their surface covers most of the ventral side of the cell (Figs 5–6). The second and fourth plates are medium in sized and positioned dorsally. The third plate, the largest in the series of postcingular plates, is five-sided and situated at the posterior part of hypotheca. The first and second antapical plates are equally large and arranged symmetrically (Fig. 6).

At present, twenty-four *Amphidiniopsis* species have been identified and described (Yoshimatsu *et al.*, 2000a; Murray and Patterson, 2002a; Toriumi *et al.*, 2002; Hoppenrath, 2000a; Hoppenrath *et al.*, 2009, 2012, 2014; Reñé *et al.*, 2000; Selina and Morozova, 2017; Selina, 2016; Selina and Hoppenrath, 2013). Among them, *A. aculeata*, *A. konovalovae*, *A. strita*, *A. swedmakii*, *A. hirsuta* and *A. hexagona* are similar in shape, size and arrangement of epithecal and hypothecal plates. *A. hexagona* is distinguished from the five former by the shape and size of 3''', 5''', 2'''' plates, the number and shape of anterior intercalary plates (a), the presence or absence of two spines at the end of plates Sda and Ss and the presence or absence of spines at antapical margin.

Distribution: *A. hexagona* was found on sandy beach, Shirarahama, Wakayama Prefecture in Japan (Yoshimatsu *et al.*, 2000a). Lately, it has been reported from sandy sediment samples in Botany Bay, Sydney, Australia (Murray, 2003). This is the first record of *A. hexagona* in Viet Nam and genus *Amphidiniopsis* is a new addition to the dinoflagellate flora of Viet Nam. This species could be frequently observed and never in high abundance.

Class: Dinophyceae West & Fritch 1927; Order: Peridinales Haeckel 1894;

Family: Thecadiniaceae Balech 1956; Genus: *Thecadinium* Kofoid & Skogsberg 1928

Thecadinium pseudokofoidii Selina, Efimova & Hoppenrath

(Figs 8–13)

(Selina *et al.*, 2019: figs 8–12, 32–57)

Taxonomic remarks: Cells of *T. pseudokofoidii* have golden-brown chloroplasts (Figs 8–9). Cells are flattened laterally, broadly oval, slightly posteriorly point antapex, as illustrated in lateral view. Cells range in size from 25–32 µm long and 24.5–35 µm deep. The nucleus is dorsally located in the lower of the hypotheca (Fig. 9). The plate formula is Po, Pt, 4', 1a, 3'', 5c, 8s, 4''', 1'''' (Selina *et al.*, 2019). The epitheca is very short. All epithecal plates are ornamented with rough ribs and large pores (Fig. 12). The epithecal plates were not visible in our specimens of present study. The hypotheca consists of four postcingular and one antapical plates (1''', 2''', 3''', 4''' and 1'''). The second and fourth postcingular plates are the largest and cover most of the right and left sides of the hypotheca. Their thecal surface is smooth and perforated by numerous small pores, these pores are found mainly at the margins of the lateral plates (2''' and 4''') and are lacking at plate centers (Figs 11–13). The narrow cingulum is deep and does not displace.

The genus *Thecadinium* currently includes nine species described (Yoshimatsu *et al.*, 2000b; Hoppenrath, 2000a,b; Hoppenrath *et al.*, 2014; Selina *et al.*, 2019). Of the nine species, only two *T. pseudokofoidii* and *T. kofoidii* are similar in shape, size, numbers and arrangement of epithecal and hypothecal plates. Cells of *T. kofoidii* were 27.5–32.5 µm long, 22.0–26.0 µm wide (Hoppenrath, 2000b); 27.0–34.0 µm long, 21–27 µm deep (Selina *et al.*, 2019). Cells of *T. pseudokofoidii* were 23–37 µm long, 19–32 µm deep (Selina *et al.*, 2019), 25–32 µm long and 24.5–35 µm deep (present study). *T. pseudokofoidii* could be distinguished from *T. kofoidii* by the

ornamentation of thecal pores on the lateral hypothecal plates (2''' and 4'''). In *T. kofoidii*, pores were scattered over the entire surface of the lateral plates. Whereas, pores were mainly concentrated along the margins of these plates and were devoided in the central area of plates in *T. pseudokofoidii*. Selina *et al.* (2019) showed that the pores on the lateral hypothecal plates (2''' and 4''') of *T. pseudokofoidii* had fewer than (37 ± 4.2) those of *T. kofoidii* (48 ± 11). In contrast, *T. pseudokofoidii* in present study seem to have more pores (65 ± 20 , $n = 3$) than those of *T. kofoidii* as described in previous study (Selina *et al.*, 2019).

Distribution: So far, this species has only been reported in Japan, Russia and Viet Nam. *T. pseudokofoidii* was first discovered in sublittoral sands in Peter the Great Bay (northwest Sea of Japan), in Vostok Bay (Russia), with water temperature ranging from -1.5°C (in February) to 20°C (in September) and salinities from 30–34 psu and 30–32 psu, respectively (Selina *et al.*, 2019). This is the first reporting of *T. pseudokofoidii* species in Viet Nam and genus *Thecadinium* is a new addition to the dinoflagellate flora of Viet Nam, where the local waters are warmer than in either the sea of Japan or Russia. At the collection site, seawater temperature value ranged between 25°C and 29°C and the value of salinity had narrow range (32.5–33 psu). This species was rare during this sampling period.

Class: Dinophyceae West & Fritch 1927;

Order: Gymnodiniales Lemmermann 1910; Family: Gymnodiniaceae Lankaster 1885;

Genus: *Amphidinium* Claparède & Lachmann 1859

***Amphidinium herdmanii* Kofoid and Swezy**

(Figs 14–16)

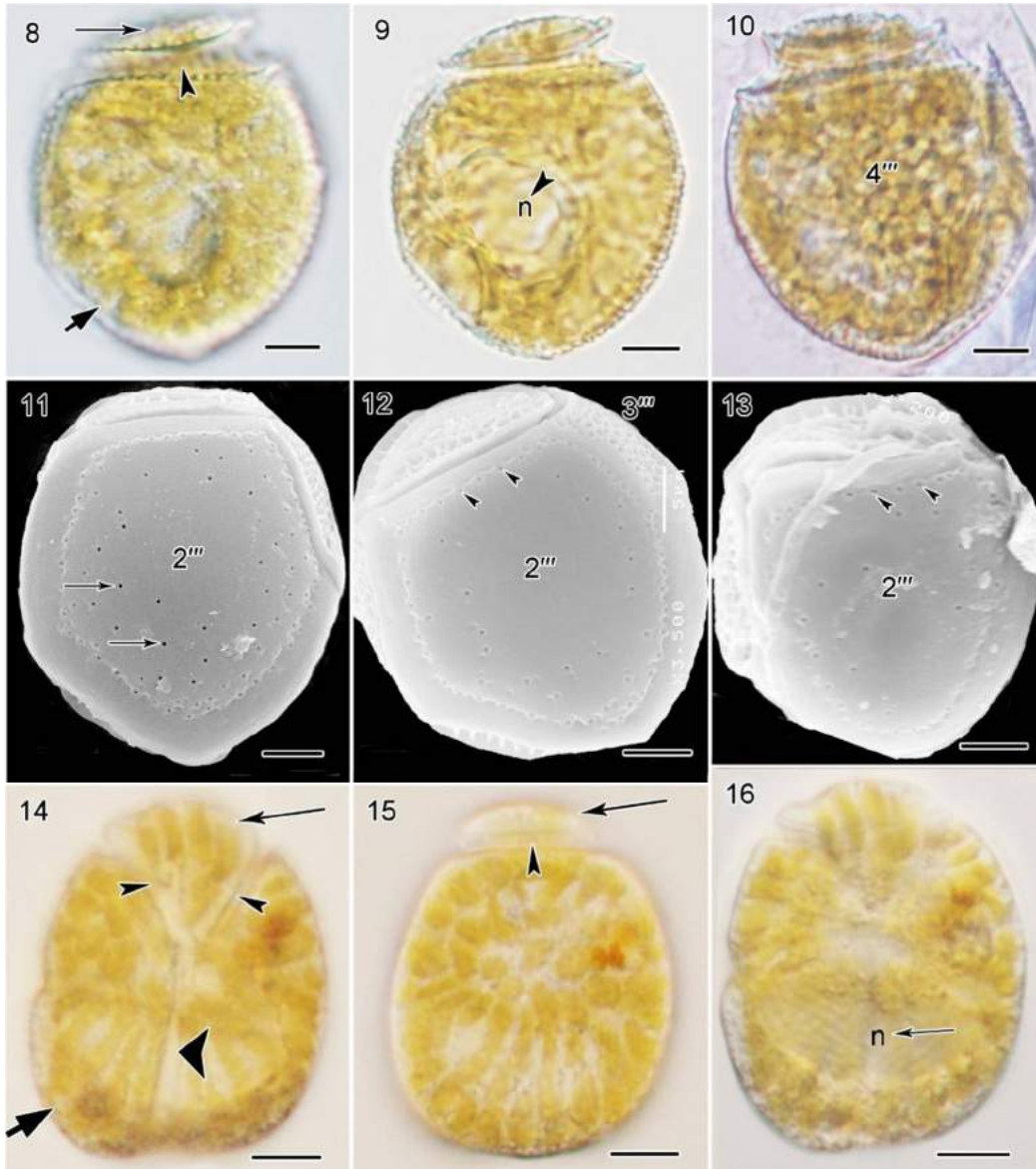
Synonym: *Amphidinium operculatum* Herdman

(Selina, 2016: fig. 3g; Hoppenrath *et al.*, 2014: figs 14I–K; Al-Yamani and Saburova, 2010: Pl. 22a–f; Jørgensen *et al.*, 2004: fig. 1C; Murray and Patterson, 2002b: figs 22–25, 79; Larsen and Patterson, 1990: figs 43a–b, 44e; Larsen, 1985: figs 20–25, 91)

Taxonomic remarks: Cell is quadrangular-broadly oblong, without thick theca, $26.5\ \mu\text{m}$ long and $23.5\ \mu\text{m}$ wide. Measurements in present study resemble those of previous descriptions for *A. herdmanii* at $26\text{--}33\ \mu\text{m}$ long and $18\text{--}23\ \mu\text{m}$ wide (Larsen, 1985), $27\text{--}32\ \mu\text{m}$ long and $14\text{--}21\ \mu\text{m}$ wide (Larsen and Patterson, 1990), $20\text{--}31\ \mu\text{m}$ long and $15\text{--}25\ \mu\text{m}$ wide (Murray and Patterson, 2002b) and $28\text{--}31\ \mu\text{m}$ long and $22\text{--}24\ \mu\text{m}$ wide (Al-Yamani and Saburova, 2010).

In ventral view, the epicone is short with a bent triangular shape, with $9\ \mu\text{m}$ long and slightly right deflection, protruding from the ventral side of the hypocone apex and extending dorsally (Fig. 14). From the dorsal side, the epicone is symmetrical, rising over the hypocone (Fig. 15). The narrow cingulum is short and V-shaped. The sulcus is narrow, beginning near the cell's center, then slightly widened at its posterior. The hypocone is quadrangular-broadly, notched at the antapex, and slightly asymmetrical, the left side being longer than the right side. The ratio of the epicone relative to total cell length is approximately one-third. The nucleus is crescent-shaped and positioned mainly in the posterior of the hypocone (Fig. 16). Living cells of *Amphidinium herdmanii* have gold-brown chloroplasts, cell swam freely. Chloroplasts appear single, with numerous lobes radiating from the center.

Distribution: This species is common world-wide, in temperate to tropical waters. This species was found in the UK (Lebour, 1925). Later, it has been recorded in Danish Wadden Sea (Jørgensen *et al.*, 2004), Japan, Russia (Selina, 2016) and from tropical marine sediments in Australia (Murray and Patterson, 2002b) and Kuwait (Al-Yamani and Saburova, 2010). *Amphidinium herdmanii* is now recorded for the first time in Viet Nam. As observed in this study, this species does not appear to be common.



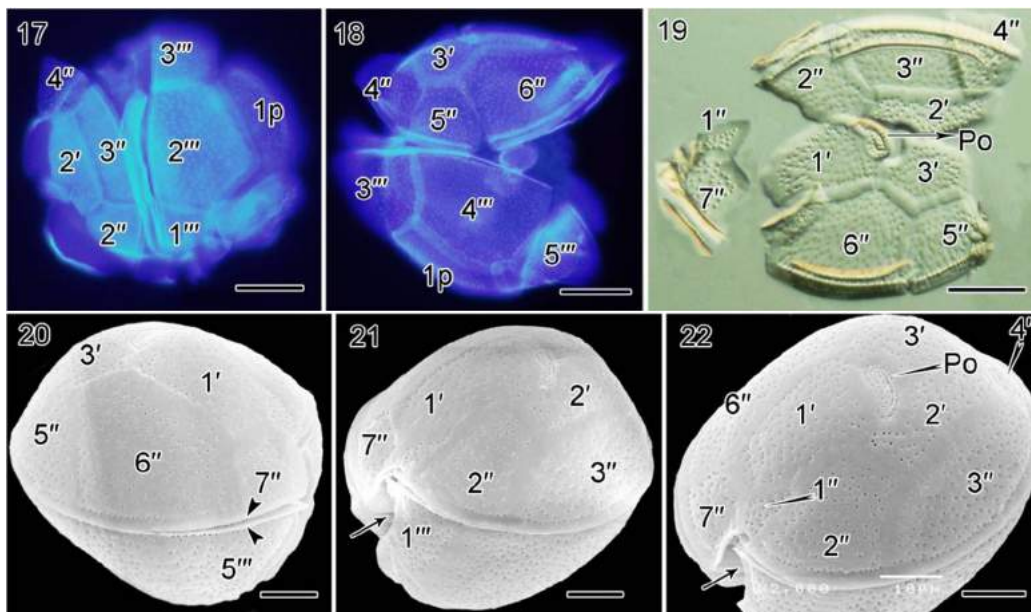
Figs 8–16. LM and SEM of *Thecadinium pseudokofoidii* (figs 8–13) and *Amphidinium herdmanii* (figs 14–16: the same cell). Fig. 8. Left lateral view showing a small epitheca (long arrow), large hypotheca (arrow) and cingulum (arrowheads). Fig. 9. Left lateral view with nucleus (marked n) located in hypotheca (arrowhead). Fig. 10. Right lateral view showing the large postcingular plate (4'''). Figs 11–13. Left lateral view showing the thecal pores (arrows) and marginal pores (arrowheads) on smooth large postcingular plate (2'''). Fig. 14. Ventral view showing a small epicone (long arrow) over a large hypocone (arrow) with elongated gold-brown chloroplasts; the V-shaped cingulum (arrowheads) and the sulcus (wide arrowhead). Fig. 15. Dorsal view showing an epicone (arrow) and cingulum groove (arrowhead). Fig. 16. Dorsal view showing nucleus (marked n) located in posterior part of hypocone (arrow). Scale bars: 5 μ m.

Class: Dinophyceae West & Fritch 1927; **Order:** Gonyaulales F.J.R. Taylor 1980;
Family: GoniDOMATAceae Lindem 1928; **Genus:** *Fukuyoa* Gómez, Qiu, Lopes & Lin 2015

Fukuyoa ruetzleri (Faust, Litaker, Vandersea, Kibler, Holland & Tester) Gómez, Qiu, Lopes & Lin (Figs 17–28)

Basionym: *Gambierdiscus ruetzleri* Faust, Litaker, Vandersea, Kibler, Holland & Tester (Litaker *et al.*, 2009: figs 43–59; Gómez *et al.*, 2015; Leung *et al.* 2018: figs 5 A–J)

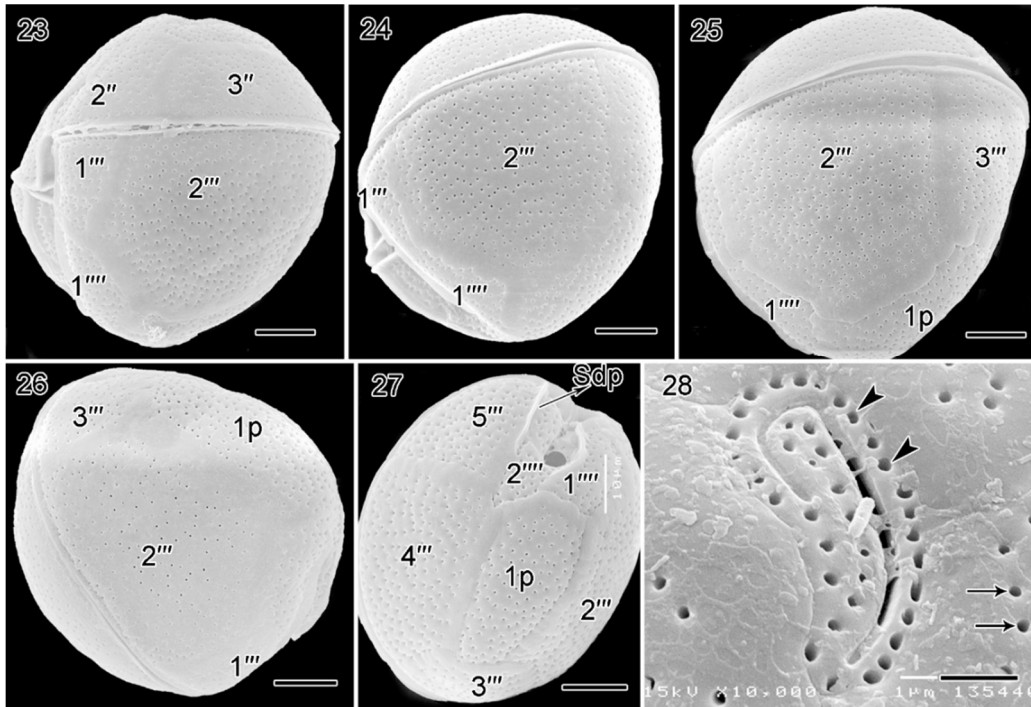
Taxonomic remarks: Cells have brown-yellow chloroplasts. Cells of *F. ruetzleri* are globular to obovoid shaped in apical or antapical view, varying from 54–57 µm long, 39–41 µm wide and 45–48 µm deep. Along the vertical axis, the apex is deflected dorsally and the antapex is deflected ventrally (Figs 20–21). The epitheca is slightly shorter than the hypotheca. The thecal surface is thick, smooth and covered with numerous round pores of about 0.32–0.38 µm in diameter, the thecal pores are evenly distributed (Fig. 28).



Figs 17–22. LM and SEM micrographs of *Fukuyoa ruetzleri*. Fig. 17. Right lateral view of the left of the cell. Fig. 18. Left lateral view of the right of the cell showing epithecal and hypothecal plates. Fig. 19. Apical view showing all epithecal plates and Po (arrow). Fig. 20. Left lateral view of the right of the epitheca showing epithecal plates and cingulum (arrowheads). Figs 21–22. Right lateral view of the left of the epitheca showing epithecal plates, apical pore plate (Po) and sulcus (arrows). Scale bars: 10 µm.

Thecal plate arrangement is Po, 3', 7'', 5''', 1^p and 2'''' (The cingulum (c) and several sulcus (s) plates were not visible in the specimens of our study). The epitheca consists of eleven plates: Po, 3' and 7'' (Figs 17–22). The apical pore plate is elongated and centrally positioned in the epitheca, 9–11 µm long, its largest width 3.5–4.0 µm, with a fish-hook-shaped surrounded by 38–39 round pores (Fig. 28). The apical plates contain three plates (1', 2' and 3'). Plate 1' is the largest,

plate 2' is long, narrow and five-sided, plate 3' is pentagonal and medium in size. The precingular plates comprise seven plates (1'', 2'', 3'', 4'', 5'', 6'' and 7''). Plate 1'' is pointed four-sided and the smallest in the series of precingular plates, followed by the trapezium-shaped 7'' plate. Plates 3'' and 6'' are the largest of the epitheca. Plates 2'' and 3'' are quadrangular, whereas plates 4'', 5'' and 6'' are pentagonal (Figs 20–22). The cingulum is narrow and descended by twice its own width. The sulcus is deeply excavated (Figs 20–21).

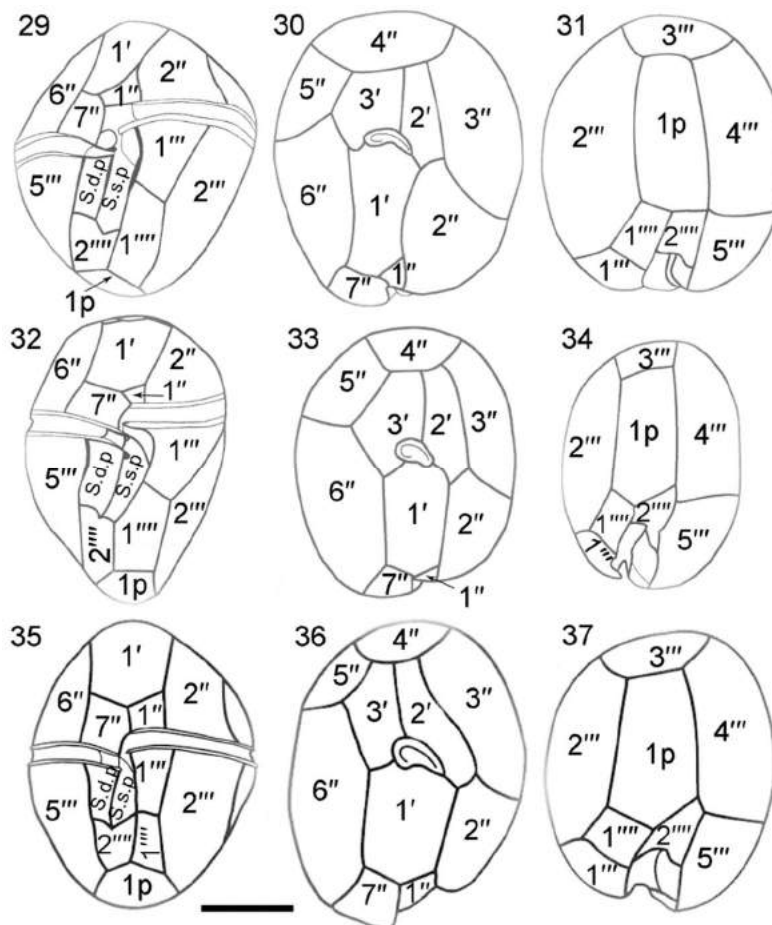


Figs 23–28. SEM micrographs of *Fukuyoa ruetzleri*. Figs 23–25. Right lateral view of the left of the hypotheca showing three postcingular (1''', 2''', and 3'''), one antapical (1''') and posterior intercalary (1p) plates. Fig. 26. Right lateral view of the left of the hypotheca showing three postcingular and posterior intercalary plates. Fig. 27. Antapical view showing hypothecal plates. Fig. 28. Detailed thecal surface showing numerous round pores (arrows) and apical pore plate (Po) with a fish-hook-shaped surrounded by many round pores (arrowheads). Scale bars: 10 μ m, except Fig. 28: 2 μ m.

The hypotheca is made up of eight plates: five postcingular plate (1''', 2''', 3''', 4''', and 5'''), one posterior intercalary plate (1p) and two antapical plate (1''', 2''') (Figs 23–27). Plates 1''', 2''', and 5''', are triangular and the smallest in the series of postcingular plates. Plate 3''' is quadrangular and medium in size, plates 2''' and 4''' are quadrangular and the largest (Figs 23, 27). The posterior intercalary plate (1p) is long and narrow, 27 μ m long, its largest width 15.8 μ m and positioned between plates 2''' and 4''' (Fig. 27). The antapical plate 1''' is small, four-sided, and situated on the left side of the sulcus. The antapical plate 2''' is forked, pointed and six-sided, it occupies the base of the sulcal hollow (Fig. 27).

At present, three *Fukuyoa* species viz., *F. yasumotoi*, *F. ruetzleri* and *F. paulensis* have been identified and described (Homles, 1998; Litaker *et al.*, 2009; Gómez *et al.*, 2015; Leung *et al.*, 2018). The morphology of *F. ruetzleri* is highly similar to those of *F. yasumotoi* and *F. paulensis*

(Figs 29, 32, 35). However, three species could be distinguished by a number of morphological characteristics. According to Litaker *et al.* (2009), the cell dimensions of *F. ruetzleri* are 52 ± 5 μm long (range 45–59), 37 ± 3 μm wide (31–42), and 45 ± 3 μm deep (42–45) and for *F. yasumotoi* is 62 ± 4 μm long (range 54–68), 52 ± 5 μm wide (43–60) and 57 ± 5 μm deep (49–67). Gómez *et al.* (2015) reported that the dimensions of *F. paulensis* are 56 ± 3 μm long (range 51–62), 45 ± 2 μm wide (41–48) and 50 ± 3 μm deep (45–56). Of the three species, cell dimensions of *F. yasumotoi* are the biggest, followed by *F. paulensis* and *F. ruetzleri* smallest. Besides, plate 1' is nearly rectangular in *F. yasumotoi* (Fig. 30) or broadly pentagonal in *F. paulensis* (Fig. 33) whereas plate 1' is narrow pentagonal in *F. ruetzleri* (Fig. 36). Plate 2''' of *F. yasumotoi* and *F. paulensis* is more larger and broader than those in *F. ruetzleri*. The posterior intercalary plate 1p is elongated, narrow and pentagonal in *F. ruetzleri* and broader than in *F. paulensis* and *F. yasumotoi* (Figs 31, 34, 37). From the previous descriptions and above comparisons, the specimens of our present study were described and identified as *F. ruetzleri*.



Figs 29–37. Thecal plate tabulation of *Fukuyoa* species. Figs 29–31: *F. yasumotoi*. Figs 32–34: *F. ruetzleri* (Redrawn from Litaker *et al.*, 2009). Figs 35–37: *F. paulensis* (Redrawn from Gómez *et al.*, 2015). Figs 29, 32 & 35: Ventral. Figs 30, 33 & 36: Epitheca. Figs 31, 34 & 37: Hypotheca. Scale bar: 20 μm .

Distribution: Previously, *F. ruetzleri* has been reported in Caribbean Sea, Belize, Central America to North Carolina, it was associated with macroalgae (Litaker *et al.*, 2009). Recently, this species has been found to be associated with surface of rocks and dead corals from the coast of Hong Kong, it can produce a compound putatively assigned as MTX-3 (Leung *et al.*, 2018). This is the first description of *F. ruetzleri* in Viet Nam, with water temperature ranging from 25°C to 29°C and salinities of 32.5–33 psu during collection time. This species does not appear to be common in our study.

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