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New species and combination in section Oceanica, genus Protoperidinium (Peridiniales, Dinophyceae) from Vietnamese waters

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Abstract

A new heterotrophic dinoflagellate, Protoperidinium vietnamicum sp. nov. was described from the coastal waters of Viet Nam. It is characterized by its elongation, pentagonal to pyriform body in ventral or dorsal view, and moderately dorsoventrally compressed. Epitheca is tapering into a robust apical horn and shorter than antapical horns. Cingulum equatorial, slightly descending. Sulcus wide, shallow, and slightly oblique. Plate 1' is ortho-type, and intercalary 2a and 3a plates are pentagonal. Plate 1a is quadrangular, and its area is approximately triple times smaller than plate 3a. A new combination is proposed, the Protoperidinium curvicorne (Böhm) comb. nov. has distinct morphological features from other members of the section Oceanica. It presents a large cell-sized species with a round, broad body, and strong dorsoventral compression. The epitheca abruptly transitions into a slender apical horn strongly tilted towards the ventral side. Cingulum is narrow, shallow, descending, and strongly inclined relative to the equatorial plane of the cell. The plate 1' is ortho-type, and 2a and 3a are asymmetrical and pentagonal. Both species are provided with detailed descriptions and are illustrated with line drawings, as well as with light microscopy images and SEM micrographs. In-depth discussion and comparison with similar species are presented.

Introduction

Protoperidinium Bergh (1881) is one of the largest and most abundant genera of thecate dinoflagellates inhabiting marine and brackish waters across the globe (Balech 1988). Despite its long and extensive history of investigation, there has always been a great deal of taxonomic uncertainty surrounding the genus (Gribble and Anderson 2006). Balech (1974) reestablished the genus *Protoperidinium* , transferring 231 marine *Peridinium* species Ehrenberg (1830) to*Protoperidinium* . His demarcation of the two genera was largely based on the following characteristics: *Protoperidinium* species have 4C or (in terms of the interpretation) 3C and 1 transitional plate (t) configuration, with their distribution covering marine habitats, whereas *Peridinium* members have 5C and occur in freshwater habitats. Furthermore, Balech (1974) introduced the division of the genus *Protoperidinium* into three subgenera, in accordance with the number of anterior intercalary and precingular plates:*Minusculum* (6 precingular and 3 intercalary plates),*Archaeperidinium* (7 precingular, 2 intercalary plates) and*Protoperidinium* (7 precingular, 3 intercalary plates). Later, Faust (2006) erected the subgenus *Testeria* Faust for*Protoperidinium* species with seven precingular plates, apical plate 1' disconnected from the tip of the apical horn, one anterior intercalary plate, and no apical pore complex. In contrast, Abé (1981) subdivided the genus *Peridinium* into three subgenera (*Protoperidinium* ,

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Mesoperidinium , and *Veroperidinium*) based on the shape of the posterior sulcal plates. He simultaneously proposed the subdivision of the subgenera into groups and sections.

Formerly, Jörgensen (1912) divided the genus *Peridinium* into two subgenera – *Orthoperidinium* (ortho–type) and *Metaperidinium* (meta and para–types). The author further subdivided *Orthoperidinium* into three sections (*Tabulata* , *Conica* , and *Oceanica*) based on the pattern of the 2a plate (quadra, penta, and hexa) and the characteristics of antapical horns. The classification system of Jörgensen was generally accepted by the later authors (Lebour 1925, Paulsen 1931_1930, Schiller 1937) with some amendments. For the present, the section *Oceanica* Jörgensen is placed in the subgenus *Protoperidinium* (Gran) Balech (1974).

Members of the section *Oceanica* are characterized by well-developed apical and antapical horns and ortho–quadra plate arrangement. However, some species were reported as penta or hexa–type (Taylor 1976, Throndsen et al. 2007, Phan-Tan et al. 2017). In addition, the cingulum is often markedly oblique relative to the equatorial plane of the cell (Phan-Tan et al. 2017). Even though a few studies pertaining to the section *Oceanica* had been carried out (such as Paulsen 1908, Lebour 1925, Schiller 1937, Taylor 1976, Abé 1981), most of the published illustrations did not reveal clear features of the anterior intercalary plates (Phan-Tan 2020). In these publications, observations were mainly based on cell outline and size, as well as on the morphology of the apical and antapical horns. The mentioned limitations cause notable confusion when it comes to the identification of *Oceanica*species. Phan-Tan et al. (2017) presented detailed descriptions and photomicrographs of seven species (ten intraspecific taxa) belonging to the section *Oceanic* a, including a new species and two new combinations, from Vietnamese waters. The micromorphology of the studied specimens, together with the shape and configuration of intercalary plates, was thoroughly documented and illustrated. The paper was the first in the series aimed at presenting the true diversity of *Protoperidinium* species found in Vietnamese waters. In 2020, *Protoperidinium carum* (Abé) Balech was first recorded in Southeast Asia and the waters of Viet Nam (Phan Tan Luom 2020). The species was re-described in detail in terms of its cell morphology and thecal plate pattern.

The present paper describes the new species, *Protoperidinium vietnamicum* sp. nov., from Vietnamese coastal waters. *Protoperidinium curvicorne* (Böhm) comb. nov. is proposed as a new combination. Detailed descriptions, accompanied by line drawing, light, and scanning electron microscopy micrographs are provided for both species.

Materials and methods

Sampling

Phytoplankton samples were collected with vertical net hauls (mesh size – 25 μm and net diameter – 30 cm) in multiple locations along Vietnamese coastal waters (Fig. 1a). The samples were fixed with formaldehyde to a final concentration of approximately 5% and then stored in 25 ml dark glass bottles at the Institute of Oceanography (Nha Trang, Viet Nam). Additionally, dinoflagellate samples collected in waters surrounding Hormuz Island in the Strait of Hormuz (Persian Gulf) were examined during the study. In total, ten samples were collected with a plankton net (mesh size – 55 μm) from five sites (Fig. 1b). At first, the material was studied in vivo at the laboratory of the Department of Marine Biology of Tarbiat Modares University (Iran), and then it was preserved in formaldehyde (4% final concentration). Subsequent study of the material was carried out at the Institute for Evolutionary Ecology of the National Academy of Sciences of Ukraine (Kiev, Ukraine).

Analyses

Samples from Viet Nam were examined under a Leica DMLB (Solms, Germany) microscope using phase-contrast (PC), differential interference contrast (DIC), and epifluorescence (Epi) optics. In order to visualize thecal plate patterns, dinoflagellate cells were stained with Calcofluor White M2R according to Fritz and Triemer (1985). The digital camera Olympus DP71 was used for light and epifluorescence microphotography. Microscopy study of the samples from the Persian Gulf was conducted at the Institute for Evolutionary Ecology of the NAS of Ukraine using an Olympus BX51 light microscope with UPlanFLN 40x/0.67 and

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UPlanFLN 100x/1.30 (Oil) lenses. Dinoflagellates were examined in transmitted light, DIC, and in an epifluorescence mode with preliminary staining of cells using Calcofluor White M2R (Fritz and Triemer, 1985). Pictures were taken with a Canon EOS 5D Mark II digital camera.

For scanning electron microscope (SEM) examination, cells of*Protoperidinium* were isolated by Pasteur pipettes from preserved samples under a stereomicroscope and placed on a 5 µm carbon membrane filter, rinsed with distilled water, dehydrated through an ethanol series (15, 30, 50, 70, 90, 99, and 100) of absolute ethanol, and air-dried. The filter was mounted onto an aluminum stub with carbon tape and finally coated with gold. The stubs were studied under a Hitachi FE-SEM (Field Emission Scanning Electron Microscope) model S4800 at the Institute of Materials Science, Viet Nam Academy of Science and Technology (Ha Noi, Viet Nam).

Thecal plate terminology follows the Kofoid tabulation system (Kofoid 1909). The identification of *Protoperidinium* species, as well as the use of biometric terminology and abbreviations, was based on Schiller (1937), Graham (1942), Taylor (1976), Abé (1981), Balech (1974, 1988), Okolodkov (2008), and Phan-Tan et al. (2017). The taxonomic position was specified in accordance with the classification of dinoflagellates by Fensome et al. (1993), taking into account Balech (1988).

Results and Discussion

Species description and diagnosis

Division Dinoflagellata (Butschli 1885) Fensome et al. (1993)

Class Dinophyceae Pascher (1914)

Order Peridiniales Haeckel (1894)

Family Protoperidiniaceae Balech (1988) nom. cons.

Genus *Protoperidinium* Bergh (1881)

Subgenus *Protoperidinium* (Gran 1902) Balech (1974)

Section *Oceanica* Jörgensen (1912)

Protoperidinium vietnamicum **sp. nov. (Figs 2a–h, 4a–d)**

Diagnosis

Cells elongated, with a pentagonal to pyriform shape in ventral view, and moderately dorsoventrally compressed. The lateral sides of the epitheca and hypotheca are slightly convex. The epitheca is tapering into a robust apical horn. The first apical plate (1') is orthogonal and symmetrical. The second apical intercalary plate 2a and the third intercalary plate 3a are asymmetrical and pentagonal, plate 1a is quadrangular and has approximately triple times smaller area than plate 3a. The cingulum is equatorial, displaced, descending by about 0.5–0.75 of its width, not excavated, and bears lists. The sulcus is wide, shallow, with lists, and slightly oblique. The hypotheca possesses two long slender antapical horns, which are slightly divergent (the right one slightly longer than the left). Dimensions: length 143 ± 2 µm (min. 140 µm, max. 145 µm, n = 30), width 61 ± 1 µm (min. 60 µm, max. 63 µm, n = 30), and depth 37 ± 2 µm (min. 35 µm, max. 40 µm, n = 30).

Holotype

Figs 2a–d, the specimen was collected on August 1st, 2013, at Station DHN-ST1, Ninh Thuan Province, Viet Nam.

Paratype

The phytoplankton sample (ID: NThVN2013) was preserved in formaldehyde (5% final concentration) and is stored in the Herbarium at the Institute of Oceanography (VMO), Nha Trang City, Viet Nam.

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Type locality

Ninh Thuan Province, Viet Nam; DHN-ST1 (11.39210° N, 109.02203° E).

Etymology

The epithet "*vietnamicum* " refers to the coastal waters of Viet Nam, where the species was originally recorded.

Distribution

The *Protoperidinium vietnamicum* sp. nov. has been observed in the coastal waters of south-central and south of Viet Nam from April to June, August, and October.

Description

Cells armored, elongated, with a pentagonal to pyriform shape in ventral and dorsal views, moderately dorsoventrally compressed, and with slightly convex lateral sides of the epitheca and hypotheca (Figs 2a, b, d–f, and 4a–b). The epitheca is tapering into a robust apical horn, which is slightly shorter than antapical horns (Figs 2a, e–f, and 4a–b). The first apical plate 1' is symmetrical and orthogonal, with its proximal margins being the longest (Figs 2a–b, e, and 4a). The quadrangular 1a plate is the smallest apical intercalary plate (Figs 2h, and 4b, d). The plate 2a is asymmetrical and pentagonal, slightly shifted to the left (Figs 2d, h, and 4b, d). The pentagonal 3a plate is about triple the area of plate 1a (Figs 2d, h, and 4b, d), and the fourth precingular plate (4") is trapeziform (Figs 2d, h, and 4b, d). The cingulum is equatorial, displaced, descending by about 0.5–0.75 cingular width (Figs 2a–b, e, and 4a), not excavated, moderately inclined relative to the equatorial plane of the cell, and bears prominent smooth cingular lists (Figs 2e–f). The broad sulcus is shallow and somewhat oblique, bordered by sulcal lists (Figs 2a, e, and 4a). The hypotheca possesses two long antapical horns, slender and slightly divergent (the right one slightly longer than the left) ending in sharp tips (Figs 2a, e–f, and 4a–c). The distance between the tips of the antapical horns is narrower than the cell's width (Figs 2a, e–f, and 4a–b). The theca is faintly reticulated with incomplete meshes and two kinds of pore sizes (Fig. 2g). *Dimensions* : length 143 ± 2 µm (min. 140 µm, max. 145 µm, n = 30), width 61 ± 1 µm (min. 60 µm, max. 63 µm, n = 30), and depth 37 ± 2 µm (min. 35 µm, max. 40 µm, n = 30).

Protoperidinium curvicorne **(Böhm) comb. nov. (Figs 3a–l, 4i-l)**

Basionym : *Peridinium oceanicum* f. *curvicorne* Böhm (1931, p.194, Fig. 12)

References

Böhm (1931, p.194, Fig. 12; 1936, p.49, fig. 21 e, f; 1976, p.105, Figs 142-143), Taylor 1967 (Pl. 34, Fig. 380; Pl. 45, Figs 522-523).

Description

Large cell-sized species, with round and broad bodies in ventral and dorsal views (Figs 3a–b, d–e, i–j, and l), strongly dorsoventrally compressed (Figs 3c, k, and 4k). The epitheca abruptly transitions into a long and slender apical horn (Figs 3a–b, e, h, i–j, and 4i–j), which is strongly tilted towards the ventral side (Figs 3c, k, and 4k). The 1' plate is orthogonal, wide, and almost symmetrical, with extended proximal margins (Figs 3b, e, g, j, and 4i). The plate 2a is asymmetrical and pentagonal, shifted to the left (Figs 3h and 4j, l). The plate 3a is asymmetrical and pentagonal, about 2.5–3 times the area of 1a, which is quadrangular, and the plate 4" is about double times wider than long (Figs 3h and 4j, l). The cingulum is very narrow, displaced, descending by about 1.5–2.0 cingular widths, not excavated (Figs 3a–b, e, i–j, and 4i), strongly inclined relative to the equatorial plane of the cell (Figs 3c, k, and 4k), and bears smooth lists (Fig. 4e). The sulcus is deep and very narrow in the anterior part, and reaches the antapex, with lists (Figs 3a–b, e, i–j, and 4i). The hypotheca possesses two long antapical horns (the right one is longer than the left), which are slender, pointed, almost parallel, and nearly equal in length to the apical horn (Figs 3a, c, e, i, and 4i–k). The thecal surface is smooth, with pores (Figs 3f–g). Dimensions: length 215.6 ± 2.7 µm (min. 210 µm, max.

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220 μm, n = 26), width 115 ± 2.9 μm (min. 110 μm, max. 120 μm, n = 27), and depth 40.6 ± 1.3 μm (min. 38 μm, max. 42 μm, n = 22).

Distribution

The taxon was described from the Persian Gulf and the Gulf of Oman (Böhm 1931), later it was reported from the coastal waters of the Western Pacific (Böhm 1936) and the Indian Ocean (Taylor 1976 as *P. murrayi*). It is also widespread in the coastal waters of Viet Nam.

Discussion

Protoperidinium vietnamicum closely resembles *P. oceanicum*(Vanhöffen) Balech (sensu Vanhöffen 1897: p. 268, 298, 312, 382, Tafel V, 2; Paulsen 1908: p. 54, Fig. 69a–c; Graham 1942: p. 24, Fig. 30; Dodge 1985: p. 60) by cells outline, especially from the ventral side, location and structure of apical and antapical horns, cingulum and sulcus morphologies, and the first apical plate 1' (ortho–type). At the same time, the new species is clearly distinct from *P. oceanicum*by the morphology of the intercalary plates 1a–3a, and the plate 2a in particular, which in case of *P. vietnamicum* is penta, whereas in*P. oceanicum* 2a – quadra (sensu Jörgensen 1912: p. 5; Leboure 1925: p. 120, Fig. p. 36, b; Graham 1942: 24, Fig. 30; Balech 1974: 57; Hermosilla 1973: p. 26, Lám. 9, Figs 1–15; Dodge 1982: p. 180; Balech 1988: p. 85, Lám. 23, Figs 7–10; Okolodkov 2008: p. 119, Pl. 6, Figs 4–7; Al-Yamani and Saburova 2019: p. 316, Pl. 175). Abé (1981: p 324, Fig. 46(300–302) considered that the plate 2a in *P. oceanicum* could be quadra or penta. However, it should be noted that the illustrations of *P. oceanicum* in the mentioned study are more reminiscent of *P. murrayi* (sensu Kofoid 1907: p. 176, Pl. 5, Fig. 29), which is reported to exhibit the 2a plate of penta–type (Phan-Tan et al. 2017: p. 136, Figs 6a–e). *Protoperidinium vietnamicum*shows similarity with *P. murrayi* (Kofoid) Hernandez-Becerril (1991) in some morphological characteristics, including developed apical and antapical horns, shape, and ortho–penta plate arrangement (Figs 4a-f). However, *P. vietnamicum* is distinguished from *P. murrayi* by the following features: (1) the epitheca swiftly transitions into the shorter apical horn; (2) the fourth precingular plate (4") is trapezoidal, with the up-side being about equal to the two lateral sides; (3) the antapical horns are slightly divergent (the angle between the horns is about 24–26?); (4) the wide cingulum slightly descending by 0.5–0.75 cingular width. In contrast, *P. murrayi* possesses a long apical horn arising abruptly from the epitheca; strongly diverged antapical horns (Figs 4e-f), with the angle between the horns being around 38–42? (Phan-Tan et al. 2017); the plate 4" approximately two times wider than long (Fig. 4h); the narrow cingulum descending about 2.0–2.5 times its width (Fig. 4e). The length of examined *P. vietnamicum* specimens ranges from 140 to 145 μm, and the width – from 60 to 63 μm. In comparison, the registered length of *P. oceanicum*varies from 220 to 300 μm (Leboure 1925), 110–200 μm (Wood 1968), 156–167 (Hermosilla 1973), 160 μm (Dodge 1985), 127–160×87–95 μm (Al-Yamani and Saburova 2019); and the registered length of *P. murrayi* varies from 250 μm (Kofoid 1907), 205 μm (Matzenauer 1933), 190–208 μm (Hernández-Becerril 1991), and 170–185 μm (Phan–Tan et al. 2017). Over thirty cells of *P. vietnamicum* from net samples were examined by means of light microscopy and SEM, showing that it has distinct morphological features from other members of the section *Oceanica* .

Böhm (1931) presented a new form – *Peridinium oceanicum* f.*curvicorne* from the Persian Gulf and the Gulf of Oman.*Protoperidinium curvicorne* , which is proposed here as a new combination, possesses a unique set of morphological characteristics and is easily distinguished from other members of the genus*Protoperidinium* by its round, broad, strongly dorsoventrally compressed body with strongly inclined cingulum relative to the equatorial plane of the cell, and a pronounced tilt of a slender apical horn towards the ventral side. The taxon is insufficiently studied. Prior to the current work, there have been no data in the literature regarding the structure of the dorsal part of its epitheca, and the plates 1a–3a, in particular. The SEM micrographs provided in the monograph of Taylor (1976: Plate 45, Figs 522 b, 523), which was devoted to the dinoflagellates of the Indian Ocean, probably presented the best illustration of *Protoperidinium curvicorne* . We believe that the author erroneously determined the examined specimens as "*Peridinium murrayi* Kofoid". However, the line drawings of*P. murrayi* , which were also provided by Taylor (1976: Pl. 34, Figs 379 and 380), do resemble the latter species.

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Furthermore, in order to prove the identity of *Protoperidinium curvicorne* specimens (= *P. oceanicum* f. *curvicorne*) found in the coastal waters of Viet Nam with the specimens collected from the type locality of this taxon (Böhm 1931), we conducted a comparative morphological study between *P. curvicorne* from the plankton of the Persian Gulf (Strait of Hormuz, coastal waters of Hormuz Island) and material from Vietnamese waters. We established the complete morphological identity of *P. curvicorne* specimens from these two populations (Fig. 3), despite the difference in environmental conditions and their remoteness from each other (about 6000 km). Dimensions of*P. curvicorne* cells from the Persian Gulf showed a wider range of values compared to the specimens from the coastal waters of Viet Nam: length 178.9–224.6 µm, width 112.8–140.7 µm, depth 46.5–53.7 µm; and length 210–220 µm, width 110–120 µm, depth from 38–42 µm, respectively.

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Illustrations

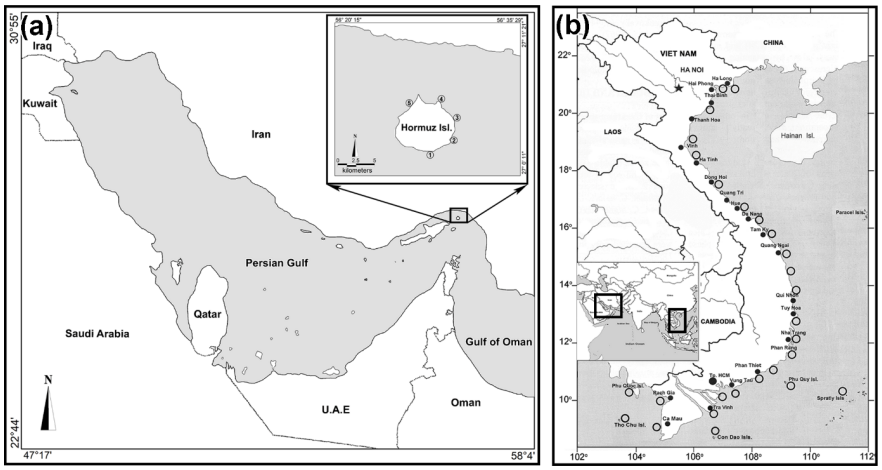

Figure 1. The schematic map of the study area with the sampling sites around Hormuz Island (Persian Gulf) (a). The position of sampling areas in Vietnamese coastal waters (b): Provincial names (filled circles) and sampling areas (open circles).

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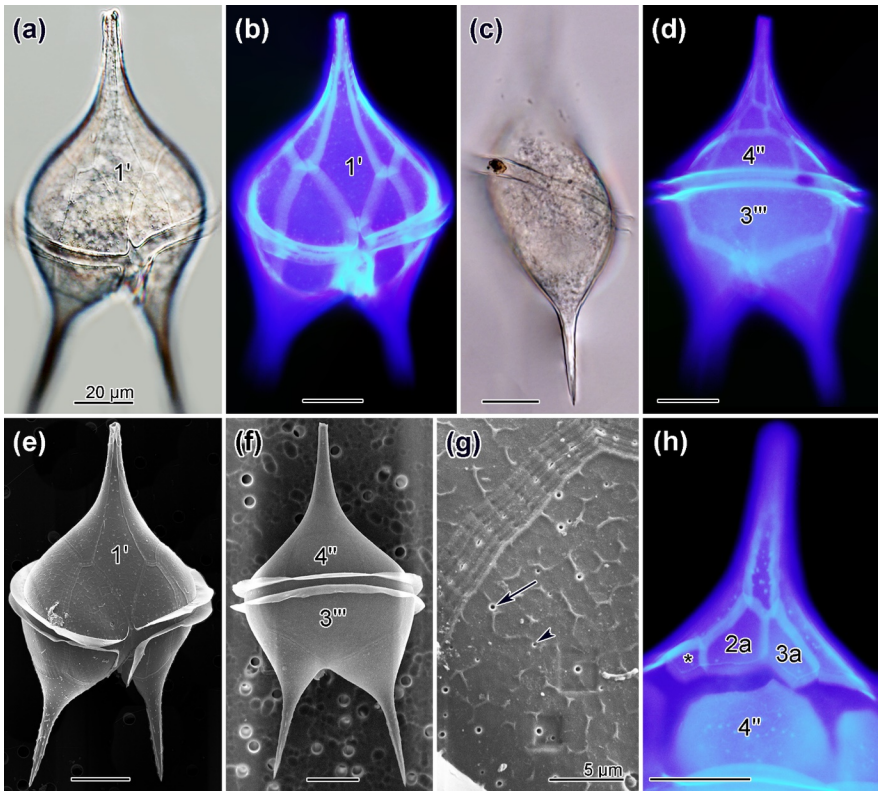

Figure 2. a–h. *Protoperidinium vietnamicum* sp. nov.: – a (PC), b (Epi), and e (SEM): cell in the ventral view showing the epithecal plates, descending cingulum, and the cingular list is smooth; – c (PC): cell in right lateral view showing the oblique cingulum; – d (Epi) and f (SEM): cell in dorsal view; – g (SEM): a part of the epitheca in ventral view shows theca's surface with incomplete meshes and two kinds of pore sizes (arrow and arrowhead); – h (Epi): a part of the epitheca in the dorsal view showing the three intercalary plates (asterisk: 1a) and the plate 4". Scale bar in Figs a-f, and h = 20 µm.

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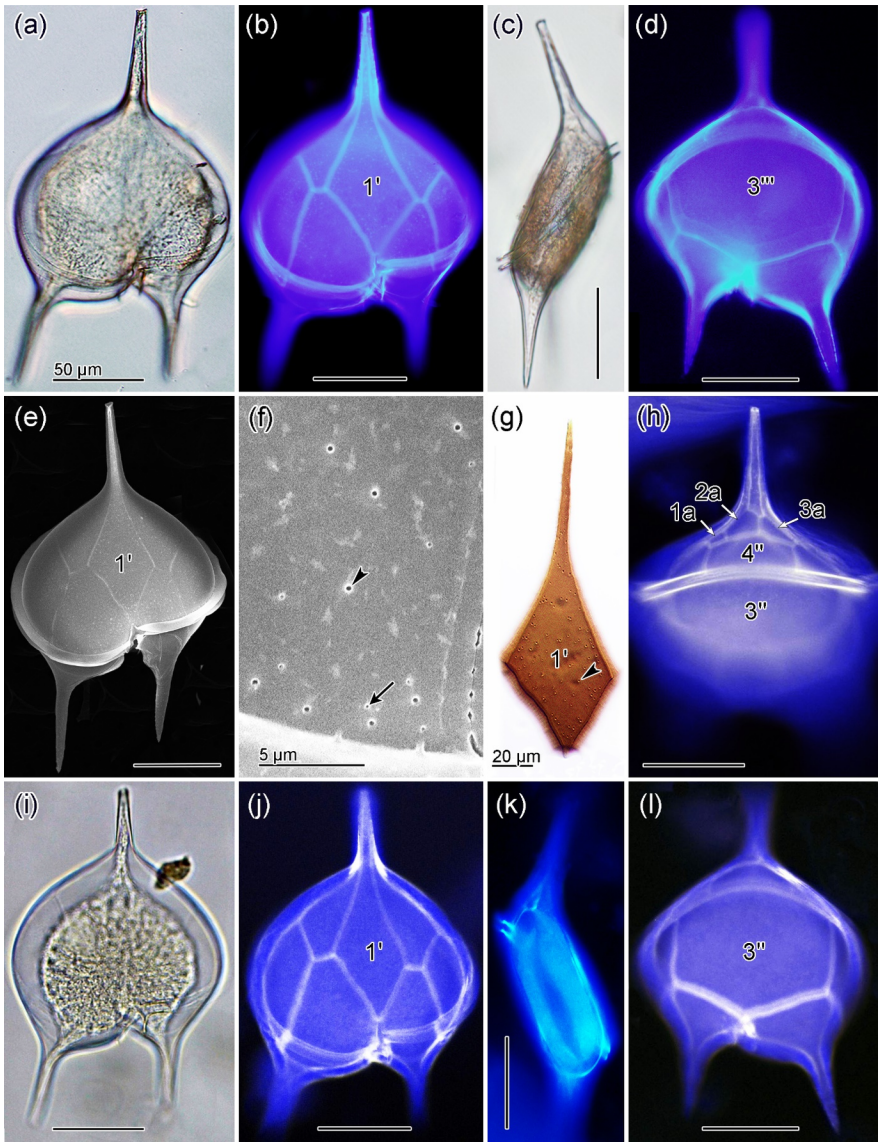

Figure 3. a–l. *Protoperidinium curvicorne* comb. nov. – a and i (PC), b and j (Epi), and e (SEM): cell in ventral view showing round and broad body with the long apical and antapical horns, the wide 1' plate, descending cingulum, the deep sulcus with two sulcal lists; – c (PC) and k (Epi): cell in left and right lateral view respectively, showing curved cell relative to the longitudinal axis by the apical horn strongly tilted forward, dorso-ventrally compressed cell and strongly oblique cingulum; – d and l (Epi): cell in dorsal view; – f (SEM): a part of the epitheca in ventral view showing the surface of the theca is slightly smooth and two kinds of pore sizes (arrow and arrowhead); – g (DIC, Lugol's stain): the 1' plate with pores (arrowhead); – h (Epi): cell in the dorsal view showing the epithecal plates. The scale bar in Fig. a = 50 µm applies to Figs b–e and h–l. Figs a–g were collected from Vietnamese coastal waters, and Figs h–l were collected around Hormuz Island (Persian Gulf, Iran).

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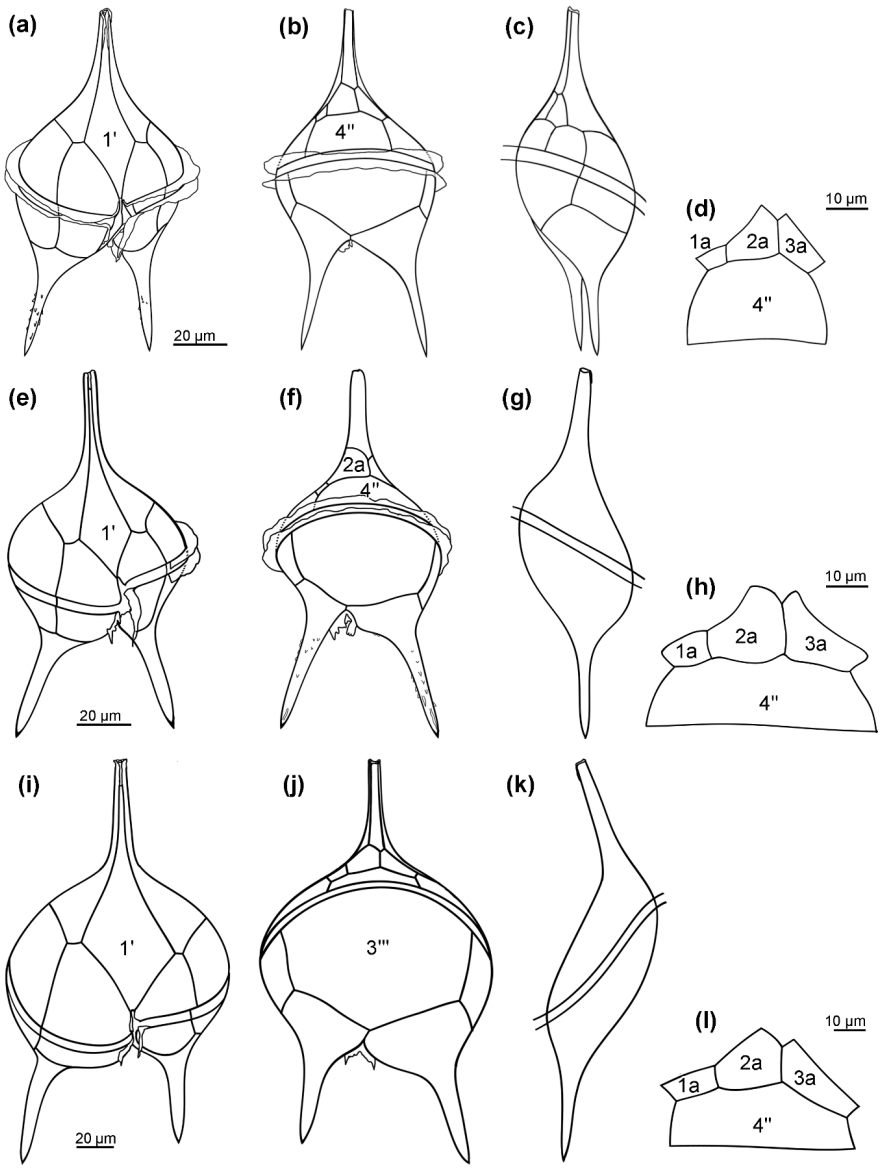

Figure 4. a–l. Line drawing of the thecal morphology of some*Protoperidinium* species in section *Oceanica* . Fig. a–d.*Protoperidinium vietnamicum* sp. nov.: cell in ventral view (a), dorsal view (b), right lateral view showing the oblique cingulum (c), and the patterns of the three intercalary plates and the plate 4" (d); Fig. e–h. *Protoperidinium murrayi* (Kofoid) Hernández-Becerril (1991, p. 79): cell morphology in ventral view (e), dorsal view (f), right lateral view (g), the patterns of the three intercalary plates and the plate 4" (h). Fig. i–l. *Protoperidinium curvicorne* comb. nov.: cell morphology in ventral view (i), dorsal view (j), left lateral view showing curved cell relative to the longitudinal axis by the apical horn strongly tilted forward, dorso-ventrally compressed cell and strongly oblique cingulum (k), the patterns of the three intercalary plates and the plate 4" (l). Scale bar in Fig. a applies to Figs b–c; the scale bar in Fig. e applies to Figs f–g, and the scale bar in Fig. i applies to Figs j–k.