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RESEARCH NOTE

Observations on *Ceratoperidinium* (Dinophyceae)

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Until now, the rare dinoflagellate genus *Ceratoperidinium* Margalef has been recorded only from the Mediterranean Sea. For the first time, photomicrographs (bright field, Nomarski and epifluorescence) are reported from Lugol-fixed samples collected from the Sulu and Celebes Seas and the western Equatorial Pacific Ocean. The specimens showed high variability in the relative size of the flexible extensions. Several specimens corresponded to the type species, *Ceratoperidinium yeye,* and lacked the apical extension. Other specimens showed an apical extension of variable size that corresponded to the description of *C. mediterraneaum.* This taxon is considered to be a morphological variety of *C. yeye* on the basis of the high interspecimen variability in the length of the extensions; specimens intermediate between *C. yeye* and *C. mediterraneaum* occur where both forms coexist. The ventral view is proposed (confirmed by 4,6-diamidino-2-phenylindole staining) to be that with the nucleus located in the left side of the cell. Thecal plates were not observed in specimens stained with Fluorescent Brightener 28. Consequently, the placement of this genus in the order Peridiniales on the basis of the initial description from a single cell should be reconsidered.

Ceratoperidinium Margalef is a genus of planktonic marine dinoflagellate rarely reported in the literature. Margalef (1969) described the type species *Ceratoperidinium yeye* Margalef from a single individual collected in coastal waters of the Spanish Mediterranean Sea. Margalef reported a cell body that was pentagonal in outline and compressed dorsoventrally. The cingulum was weakly impressed and a sulcus was not observed. The cell surface was rigid and lacked sculpture or relief. No thecal plates were observed. The hypotheca (hyposoma) was drawn out into two long, slightly curved, rigid, cylindrical appendices with a row of three swellings at their extremities. The tips of the antapical extensions presented a tentacle-like shape. One large pusule, plastids and small drops of lipid occur in the cytoplasm and the nucleus is centrally located. Later, Loeblich (1982, p. 108) and Sournia (1986, p. 96) translated the description by Margalef (1969) to English and French, respectively.

The type species was redescribed as *C. margalefii* by Loeblich (1980) because of the absence of a Latin diagnosis. As reported by Sournia (1982, p. 153), Loeblich only added the Latin diagnosis, but instead of retaining the name with a new authority, *C. yeye* Margalef *ex* Loeblich III, he proposed the new name *C. margalefii* Loeblich III. The case of *C. yeye* is comparable to that of taxa such as *Petalodinium porcelio* J. Cachon & M. Cachon, in which the original publication of the type species lacked the Latin diagnosis; under the International Code of Botanical Nomenclature (Greuter *et al.* 2000; article 45.5 ex. 5), the name should retain its original authorship and date.

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After the initial record by Margalef (1969), Abboud-Abi Saab (1989) reported one specimen of *C. yeye* from Lebanese coastal waters. She further reported a new species, *C. mediterraneum* Abboud-Abi Saab (Abboud-Abi Saab 1989), that differs from the type species by the presence of a rounded tubular apical (capitate) process. The description of *C. mediterraneum* lacked a Latin diagnosis, line drawings and good-quality illustrations. This almost inaccessible publication goes unnoticed in or omitted from later literature.

Velásquez (1997) reported *C. yeye* in the Gulf of Lions (NW Mediterranean Sea) and more recently Gómez & Abboud-Abi Saab (2003) reported new records of *C. yeye* from the Alborán and Balearic Seas. These authors also reported a *Ceratoperidinium* sp. with a distinctive curved apical process more elongate than that in *C. mediterraneum* (Gómez & Abboud-Abi Saab 2003). There are no other records, either for the Mediterranean Sea (Gómez 2003) or for the rest of the world, to the best of our knowledge.

Ceratoperidinium has been placed in the family Ceratoperidiniaceae Margalef (Loeblich 1982) or *incertae sedis* (Sournia 1986), both in the order Peridiniales Haeckel, and later tentatively as an unarmoured taxon of the order Ptychodiscales Fensome, Taylor, Norris, Sarjeant, Wharton & Williams (Fensome *et al.* 1993).

This study presents photographic records of the genus for the first time. We tried to elucidate the presence of cellulose thecal plates by using the Fluorescent Brightener staining technique. The position and the shape of the nucleus were studied by using a DNA fluorochrome. The orientation of the cell is proposed for the first time. The morphological variability in the relative size of the extensions is emphasized.

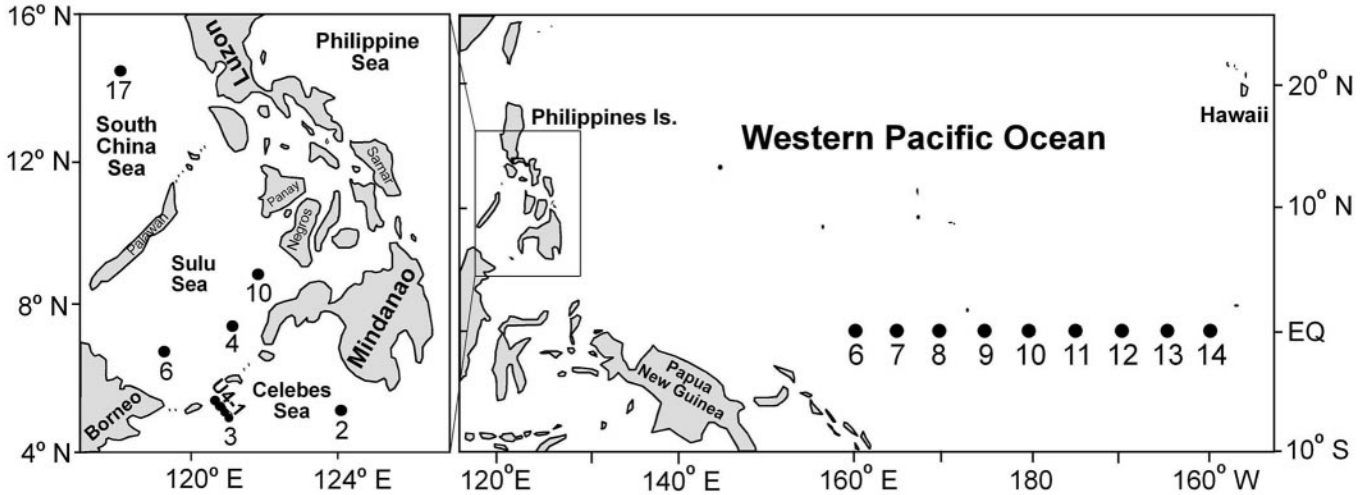


Fig. 1. Locations of the sampling stations in the tropical and western Equatorial Pacific Ocean.

Specimens were collected during two cruises: (1) aboard R/V Hakuho-maru (7 November–18 December 2002) in the Celebes, Sulu and South China Seas (Fig. 1). Sea water samples were collected by using Niskin bottles in 10 stations at six discrete depths from 0 to 150 m; and (2) aboard R/V Mirai (15–28 January 2003) along the equator from 160°E to 160°W. Sea water samples were collected by using Niskin bottles in nine stations at 14 discrete depths from 5 to 200 m. Samples were preserved with acidified Lugol's solution (Hasle & Syvertsen 1997) and stored at about 5°C. Samples of 400 ml were concentrated by settling in glass cylinders; concentrates were left to settle in standard sedimentation chambers and examined in a Diaphot inverted microscope (Nikon, Tokyo, Japan) using bright field optics. Cells were photographed on an inverted light microscope connected to a Nikon digital camera (Coolpix 4500).

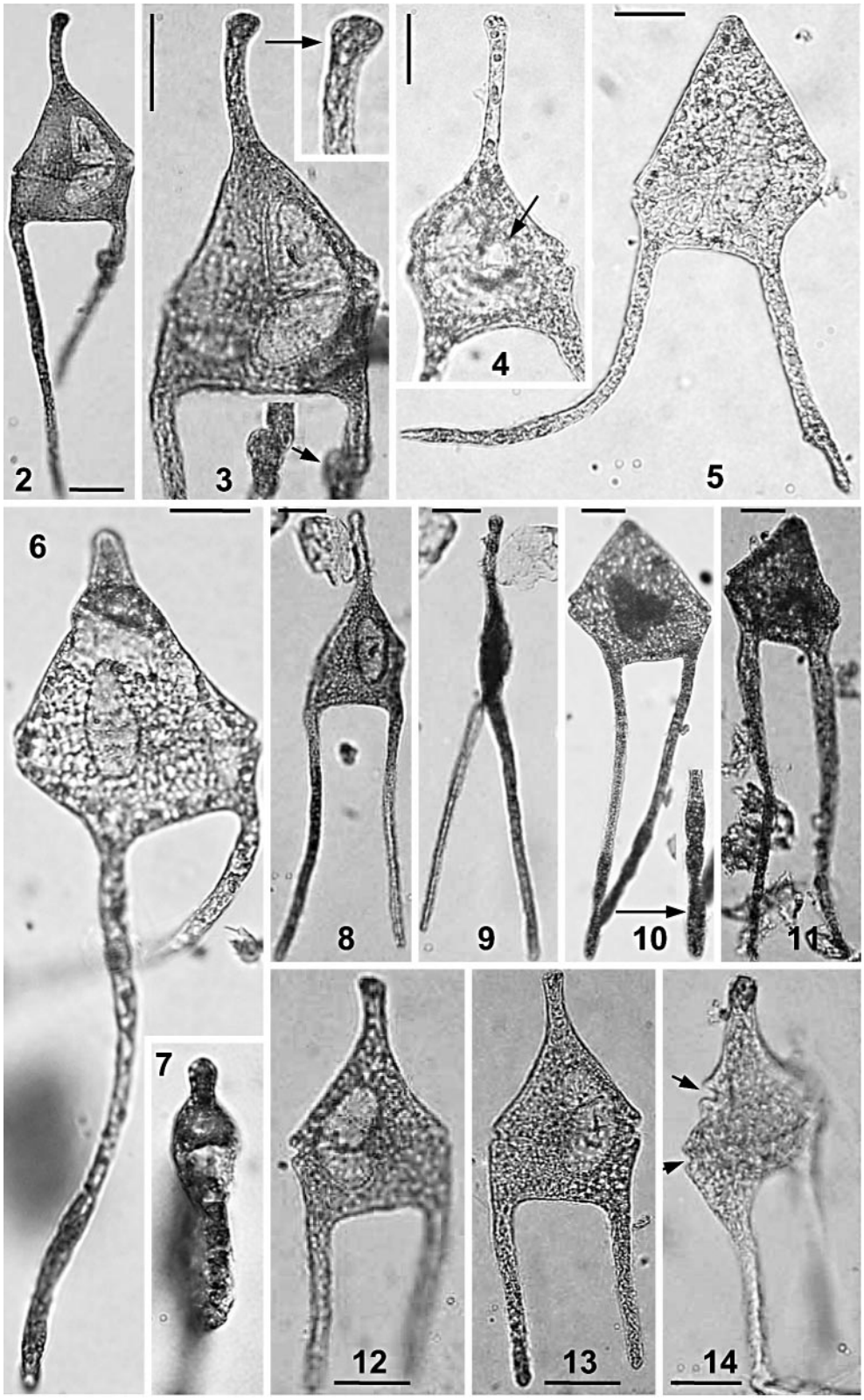
Several specimens were isolated using a capillary tube from the chambers, transferred to a glass slide and observed with an Olympus microscope (BX51; Tokyo, Japan) equipped with Nomarski differential interference contrast (DIC) optics. High-magnification microphotographs (×600 or ×1000) were obtained with an Olympus digital camera (C3040ZOOM). One specimen was stained by adding Fluorescent Brightener 28 (Sigma, St Louis, MO, USA) following the protocol of Fritz & Triemer (1985). Three specimens (one under division) were stained by adding a mix containing 4,6-diamidino-2-phenylindole (DAPI; Sigma) and Fluorescent Brightener. The DAPI specifically binds to double-stranded DNA, and when excited with ultraviolet (UV) light the DAPI–DNA complex fluoresces a bright blue (Porter & Feig 1980). Epifluorescence microscopy was done with Olympus (BX60) and Zeiss Axiophot2 microscopes (Zeiss, Jena, Germany) to excite with UV light for DAPI and Fluorescent Brightener stains.

Eight specimens were observed from the Sulu and Celebes Seas and five from the western Equatorial Pacific Ocean. The maximum occurrence was in the Sulu Sea (station 4; 7°25′N, 121°12.5′E), with four specimens (10 cells l^{-1}) at 30 m depth (Fig. 1; Table 1). Nine specimens had an apical protuberance that differed from the type species; they were closer to *C. mediterraneum*, here considered to be a morphological variety of the type species (Figs 2–4, 8, 9, 12–14). Three specimens corresponded to the type species, lacking the apical process (Figs 5, 10, 11). One specimen was intermediate between these two taxa (Fig. 6), with a wider section at the base of

Table 1. Stations, depth, geographic coordinates (latitude, longitude) and dimensions: W, width at the level of the cingulum; L, total length of each record of *Ceratoperidinium yeye*.

Station	Depth (m)	Latitude	Longitude	W (μm)	L (μm)	Figs
4	−30	7°25.3′N	121°12.5′E	43	205	2–3
4	−30	7°25.3′N	121°12.5′E	37	165	
4	−30	7°25.3′N	121°12.5′E	48	180	8–9, 18–20
4	−30	7°25.3′N	121°12.5′E	38	150	15
4	−50	7°25.3′N	121°12.5′E	38	180	
2	−30	5°10.8′N	124°04.9′E	39	105	12–13
6[1]	−30	6°54.1′N	119°11.1′E	37	100	14, 16–17, 21–24
6[2]	−20	6°54.1′N	119°11.1′E	52	200	11
6[2]	−60	0°	160°E	68	225	10
6	−100	0°	160°E	45	172	
8	−70	0°	170°E	48	185	4
9[2]	−120	0°	175°E	52	145	5
11[3]	−120	0°	175°W	65	230	6–7

[1] Specimen undergoing division.
[2] No apical process.
[3] Apical process scarcely developed.



Figs 2–14. *Ceratoperidinium yeye*, bright field optics. See Table 1 for location of the records and the size of the specimens. Scale bars = 20 μm.

Figs 2, 3. Ventral views of one specimen. The arrow in Fig. 3 indicates a knob on one of the antapical extensions, and the arrow in the inset the extremity of the apical process.

Fig. 4. Dorsal view of a specimen showing a hole (arrow).

Fig. 5. Ventral view of another specimen lacking the apical process.

Figs 6, 7. A specimen intermediate between *C. yeye* and the morphological variety *C. mediterraneum.* Fig. 7 shows a lateral view of the cell body with a wider section at the base of the short apical extension.

the apical tip seen in lateral view (Fig. 7). One of the specimens was observed under division, with two contours of groove observed in one side of the cells (Figs 14, 16, 17, 21–24). The size of the extensions relative to the cell body varied between the specimens (Figs 2–14). The antapical appendices were highly flexible. One of the specimens had a protuberance in one of the antapical appendices that we named 'the knob' (Fig. 3). As general trend, the antapical extension was slightly shorter in the side where the cingular groove was more apical (near the nucleus). From Lugol-fixed specimens, the maximum length ranged from 100 to 230 μm and the width at the cingulum level was 37–68 μm; specimens lacking the apical extension were larger than the others (Table 1). The cingulum was weakly impressed, and inclined relative to the base of the cell body. Neither flagellum nor sulcal groove was observed (Figs 18, 19). A slight irregularity, perhaps pores, appeared near the basis of the hyposoma (Fig. 20).

The DAPI staining reveals the nucleus to be kidney-shaped and located laterally, glowing brightly under UV excitation (Fig. 17); under light microscopy, it appears as a pale area (Fig. 22) and microfilaments (chromosomes) can sometimes be seen (Fig. 15).

Cellulose thecal plates were not observed in specimens stained with Fluorescent Brightener and a mixture of DAPI–Fluorescent Brightener illuminated with UV light. However, cellulose thecal plates were observed in cells of *Prorocentrum* added to the samples as a positive control. The same protocol has been successfully used previously with other thecate dinoflagellates.

Dinoflagellates have been divided into naked (or unarmoured) and thecate (or armoured). However, the distinction is not clear-cut (Dodge & Crawford 1970). The scarce information on our genus is based on the single record by Margalef (1969). The systematic position of this genus remains uncertain; the pentagonal shape of the cell body is reminiscent of peridinialeans, but the presence of extensions suggests the brachydiniaceans. Loeblich (1982, p. 108), based on Margalef (1969), reported 'the thecal tabulation is unknown; however, the presence of a large apical pore indicates that a thecal layer is present'. We have not observed any apical pore. Loeblich (1982) placed this genus in the family Ceratoperidiniaceae Margalef of the order Peridiniales. Sournia (1986, p. 96) placed *Ceratoperidinium* in an undetermined position – *incertae sedis* – in the order Peridiniales. Fensome *et al.* (1993) interpreted that the rigid wall that might be evidence of a pellicle and tentatively placed the genus as an athecate dinoflagellate of the order Ptychodiscales. According to Fensome *et al.* (1993, p. 54) the ptychodiscacean cell wall tends to be very flexible, due to the presence of a well-developed pellicle with cellulose as principal component (Morrill & Loeblich 1981). Fluorescent Brightener specifically stains cellulose, the main component of the dinoflagellate theca (Fritz & Triemer 1985). According to our results, *Ceratoperidinium* lacks the thecal plates that are characteristic of members of the order Peridiniales.

The orientation of the genus is unresolved. Neither flagellum nor sulcal groove was observed. The description by Margalef reported one large pusule and that the nucleus was located centrally (see also Loeblich 1982, p. 108). However, the use of DAPI staining in this study reveals that the nucleus is located laterally (Fig. 17) with microfilaments (chromosomes) visible under DIC microscopy (Fig. 15).

The cingulum is left-handed and weakly impressed (Figs 18–20). Observation at different focus levels reveals that a discontinuity in the cingulum occurs in the side opposite the nucleus (Fig. 19). We consider that this view, with the nucleus in the left side of the cell, is the ventral position (Figs 25, 26).

The specimens collected in the Pacific Ocean were very variable in the relative size of the antapical extensions (Figs 2–14). At the same stations were found specimens with and without an apical extension (Table 1). In the Mediterranean waters, Gómez & Abboud-Abi Saab (2003) reported the presence of *C. yeye* and *Ceratoperidinium* sp. (with an elongate and curved apical extension) at the same location. Consequently, *C. mediterraneum* was reported as intermediate between *C. yeye* and *Ceratoperidinium* sp. Athecate dinoflagellates such as *Pseliodinium vaubanii* Sournia are very variable with respect to the size of their flexible extensions (Sournia 1972). Recently, Konovalova (2003) reported that *P. vaubanii* constitutes one stage in the life history of *Gyrodinium falcatum* Kofoid & Swezy. Within this context, the relative size of the apical extension of *Ceratoperidinium* should not be considered as a criterion for the differentiation of species. Until further research, taxa such as *C. mediterraneum* or *Ceratoperidinium* sp. (Gómez & Abboud-Abi Saab 2003) should be considered as a morphological variety of the type species. Cell division occurs in specimens with short apical extensions. Specimens lacking the apical extension showed a larger size than those with the apical extension.

Despite the distinctive morphology and the relatively large size (> 200 μm), records of *Ceratoperidinium* are extremely rare. Even distinctive taxa remain insufficiently known, especially in open waters of the subtropical and tropical oceans.

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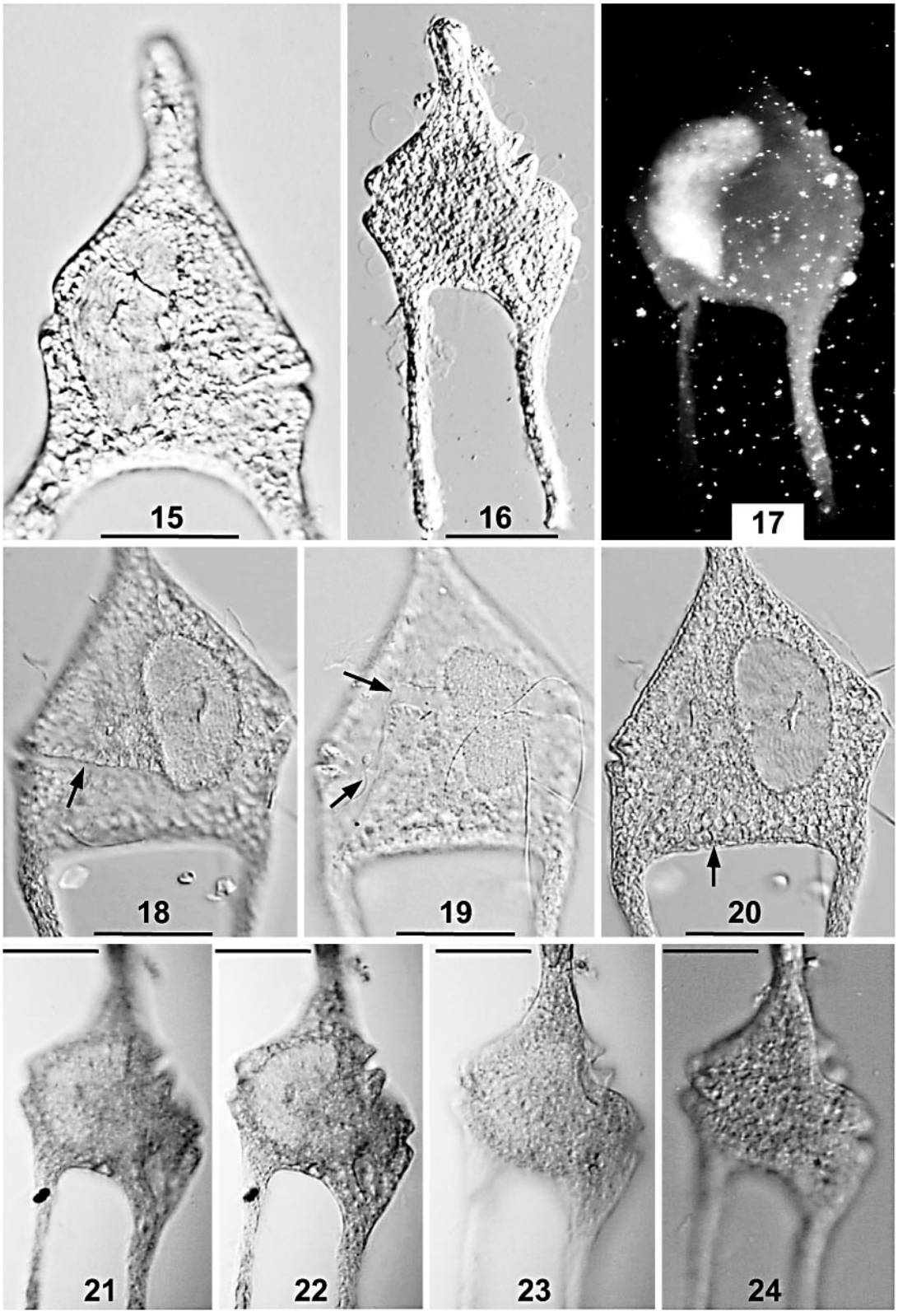
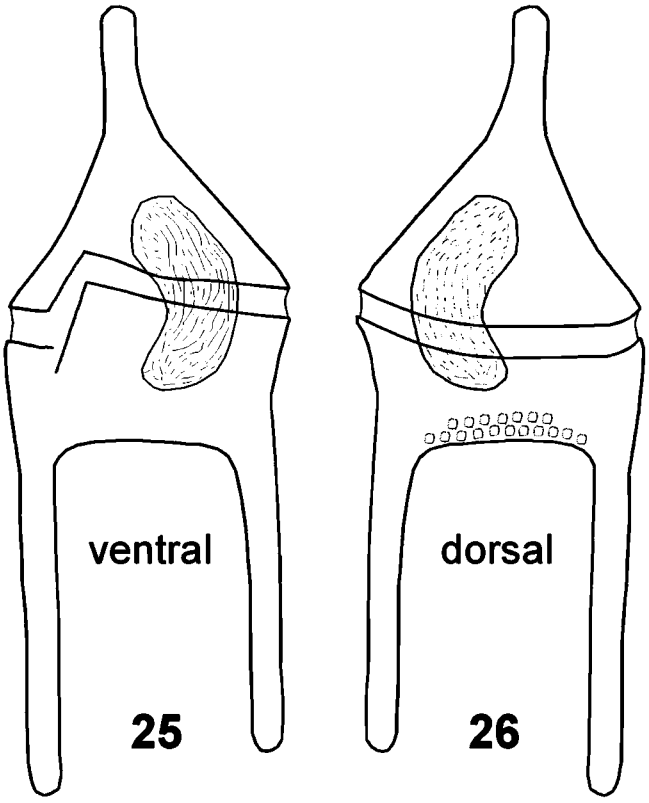
Figs 8, 9. Ventral and lateral views, respectively, of another specimen.
Figs 10, 11. Two different specimens lacking an apical extension; dorsal view. The arrow in Fig. 10 (inset) indicates the extremity of the antapical extension.
Figs 12, 13. Dorsal and ventral views, respectively, of the same specimen.
Fig. 14. Specimen undergoing division. The arrows indicate the cingular grooves.



Figs 15–24. *Ceratoperidinium yeye*, DIC (except Fig. 17, epifluorescence). Scale bars = 20 μm.
Fig. 15. Specimen in dorsal view showing microfilaments within the kidney-shaped nucleus.
Fig. 16. Specimen in dorsal view undergoing division.
Fig. 17. The DAPI-Fluorescent Brightener–stained specimen showing the nucleus glowing brightly in a lateral location under UV excitation. No Fluorescent Brightener–stained cellulose (blue) was observed that would indicate the presence of thecal plates.
Figs 18–20. Detail of the cingulum.
Fig. 18. Ventral view. The arrow indicates the cingulum.
Fig. 19. Ventral view. The arrows indicate the discontinuity in the cingular groove (the fibres are not related to the specimen).
Fig. 20. The arrow points to pores in the surface of the base of the hyposoma.
Figs 21–24. Specimen undergoing division. Note the shape of the nucleus in Fig. 22 (also Fig. 17).



Figs 25, 26. Schematic line drawings of the orientation (ventral and dorsal, respectively) of a *Ceratoperidinium* cell.

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REFERENCES

ABBOUD-ABI SAAB M. 1989. Les dinoflagellés des eaux côtières libanaises – espèces rares ou nouvelles du phytoplancton marin. *Lebanese Science Bulletin* 5: 5–16.

DODGE J.D. & CRAWFORD R.M. 1970. A survey of thecal fine structure in the Dinophyceae. *Botanical Journal of the Linnaean Society* 63: 53–67.

FENSOME R.A., TAYLOR F.J.R., NORRIS G., SARJEANT W.A.S., WHARTON D.I. & WILLIAMS G.L. 1993. *A classification of living and fossil dinoflagellates.* American Museum of Natural History, Sheridan Press, Hanover, Pennsylvania. 351 pp. [*Journal of Micropaleontology*, special publication 7.]

FRITZ L. & TRIEMER R.E. 1985. A rapid, simple technique utilizing calcofluor white M2R for the visualization of dinoflagellate thecal plates. *Journal of Phycology* 21: 662–664.

GÓMEZ F. 2003. Checklist of Mediterranean free-living dinoflagellates. *Botanica Marina* 46: 215–242.

GÓMEZ F. & ABBOUD-ABI SAAB M. 2003. Records of *Ceratoperidinium* Margalef (Dinophyceae) from the Mediterranean Sea. *Vie et Milieu* 53: 43–46.

GREUTER W., MCNEILL J., BARRIE F.R., BURDET H.M., DEMOULIN V., FILGUEIRAS T.S., NICOLSON D.H., SILVA P.C., SKOG J.E., TREHANE P., TURLAND J. & HAWKSWORTH D.L. 2000. *International Code of Botanical Nomenclature (Saint Louis Code).* Koeltz Scientific Books, Königstein, Germany. 474 pp. [*Regnum Vegetabile* 138.]

HASLE G.R. & SYVERTSEN E.E. 1997. Marine diatoms. In: *Identifying marine phytoplankton* (Ed. by C.R. Tomas), pp. 5–385. Academic Press, San Diego.

KONOVALOVA G.V. 2003. The life history of *Gyrodinium falcatum* and validity of *Pseliodinium vaubanii* (Dinophyceae). *Russian Journal of Marine Biology* 29: 167–170.

LOEBLICH A.R. III. 1980. Dinoflagellate nomenclature. *Taxon* 29: 321–324.

LOEBLICH A.R. III. 1982. Dinophyceae. In: *Synopsis and classification of living organisms* (Ed. by S.P. Parker), pp. 101–115. McGraw-Hill, New York.

MARGALEF R. 1969. Composición específica del fitoplancton de la costa catalano-levantina (Mediterráneo occidental) en 1962–1967. *Investigaciones Pesqueras* 33: 345–380.

MORRILL L.C. & LOEBLICH A.R. III. 1981. The dinoflagellate pellicular wall layer and its occurrence in the division Pyrrhophyta. *Journal of Phycology* 17: 315–323.

PORTER K.G. & FEIG Y.S. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnology and Oceanography* 25: 943–948.

SOURNIA A. 1972. Une période de poussées phytoplanctoniques prés de Nosy-Bé (Madagascar) en 1971. Espèces rares ou nouvelles du phytoplancton. *Office de la recherche scientifique et technique d'Outre-mer. Cahiers, série océanographique* 10: 151–159.

SOURNIA A. 1982. Catalogue of the species and infraspecific taxa of the recent marine dinoflagellates (Dinophyceae) published since the revision by J. Schiller IV. *Archiv für Protistenkunde* 126: 151–168.

SOURNIA A. 1986. *Atlas du phytoplancton marin: introduction, Cyanophycées, Dictyochophycées, Dinophycées et Raphidophycées,* vol. 1. Editions du Centre National de la Recherche Scientifique, Paris. 219 pp.

VELÁSQUEZ Z.R. 1997. *Fitoplancton en el Mediterráneo Noroccidental.* PhD thesis. Universitat Politècnica de Catalunya, Barcelona. 272 + 55 pp.

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