

Creation of the subgenus *Testeria* Faust *subgen. nov.* *Protoberidinium* Bergh from the SW Atlantic Ocean: *Protoberidinium novella* sp. nov. and *Protoberidinium concinna* sp. nov. Dinophyceae

MARIA A. FAUST*

Department of Botany, US National Herbarium, Smithsonian Institution, 4210 Silver Hill Road, Suitland, Maryland 20746, USA

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Two new heterotrophic *Protoberidinium* species are described from oceanic waters from The Gulf Stream, SW Atlantic Ocean and Belizean Atlantic Barrier Reef Ecosystems, Caribbean Sea. *Protoberidinium novella* Faust *sp. nov.* and *P. concinna* Faust *sp. nov.* show a plate formula of 4', 1a, 7", 4C(3+t), 6S, 5"', and 2''', which is atypical for the genus. It is characterized by a miniscule flat closure on the extreme anterior of the pointed apical horn. Apical pore complex is absent. Apical ventral plate 1' disconnects from the tip of the apical horn and connects directly to the anterior sulcal plate (Sa) along a straight line of the longitudinal axis of the cell's sagittal plane. The shape and position of the intercalary plate (1a) is another distinctive feature for these species, the presence of only one 1a plate. The thecal plate features of *P. novella* and *P. concinna* justifies the establishment of the new subgenus, *Testeria* Faust *subgen. nov.* The relationship with other congeneric species and the position within the genus *Protoberidinium* are discussed.

KEY WORDS: Belize, coral reef-mangrove pond, dinoflagellates, Dinophyceae, ecology, Subgenus *Testeria* subgen. nov., *Protoberidinium novella* sp. nov., *Protoberidinium concinna* sp. nov., scanning electron microscopy, taxonomy

Dedicated to Dr Patricia A. Tester in recognition of her significant contributions to the dynamics of Karenina brevis (Davis) G. Hansen & Ø. Moestrup algal blooms.

INTRODUCTION

Protoberidinium Bergh is a large genus of armoured dinoflagellates usually without chloroplasts. Bergh (1881) realized that thecal plates always exhibit a certain basic pattern common within a genus. Schütt (1895) noted the importance of morphology identifying dinoflagellates. Jörgensen (1912) emphasized the importance of thecal plate pattern for species identification in the genus and presented new groups: e.g. *Peridinium*, whose divisions were based on the shape of the first apical 1' plate, and the position and shape of the dorsal epithelial plates, particularly the second intercalary 2a plate and the presence of three intercalary plates. Jörgensen (1912) established a second genus *Archaeoberidinium* with only two anterior intercalary plates. Balech (1974) transferred 231 species of marine *Peridinium* to the genus *Protoberidinium* which included species with ortho, meta and para apical plate 1', intercalary plate 2a with hexa, penta and quadra-type, four cingular plates, and six sulcal plates, and left *Peridinium* to include only those with ortho-type of 1' plate, five or six cingular plates and five or six sulcal plates. Balech (1980) recommended that the morphology of cingular and sulcal plates, the shape of apical plate 1', and the intercalary plates be used in species identification.

The use of a scanning electron microscope provided additional new information on the fine morphology of thecal plates (Gocht & Netzel 1974; Balech 1975, 1999; Taylor 1976; Andreis *et al.* 1982; Dodge 1983, 1985; Netzel & Dürr 1984; Lewis & Dodge 1990; Delgado & Fortuno 1991; Hansen & Larsen 1992; Toriumi & Dodge 1993). Toriumi & Dodge (1993) found that the apical pore structures of *Protoberidinium* species were species specific, enclosed by up to six collar plate sections and the narrow canal plate X, and varied in morphology. The genus *Protoberidinium* identified by the typical thecal plate formula: Po, X, 4', 2 or 3a, 7", 4C(3+t), 6S, 5"', and 2''' (e.g. Steidinger & Tangen 1996). Classification of *Protoberidinium* today are based on prominent morphological features including cell size and shape, horns or spines, apical plate 1', intercalary plates, the apical pore complex (APC), ornamentation of thecal plates, displacement of cingulum and six sulcal plates (e.g. Toriumi & Dodge (1993)).

The growing recognition that armoured dinoflagellates are important members of marine ecosystems has accentuated the need for detailed taxonomic studies (Yamaguchi & Horiguchi 2005). Information is limited on the biodiversity of *Protoberidinium* Bergh (1881) dinoflagellates from the SW Atlantic Ocean and the Belizean Atlantic barrier coral reef ecosystems. This paper illustrates the thecal plate morphology of two new *Protoberidinium* in recent marine collections. Both species are heterotrophic, exhibit similar cell shape, and a slender apical horn characteristic for the section *Oceanica* Jörgensen (1912), the genus *Protoberidinium*. *Protoberidinium novella* sp. nov. and *P. concinna* sp. nov., however, differ in thecal plate morphology by the absence of an APC, the apical ventral plate 1' that disconnects from the tip of the apical horn, and the pres-

* Corresponding author (faust.maria@nmnh.edu).

Table 1. Comparison of morphological features of *Protoperidinium novella* sp. nov. and *P. concinna* sp. nov. and selected *Protoperidinium* species.¹

Taxa	APC	Plate 1'	a*	Cell size (μm)		References
				Length	Width	
<i>P. novella</i>	no	ortho	1a	98–116	62–75	Present study
<i>P. concinna</i>	no	ortho	1a	103–137	52–67	Present study
<i>P. seidingerae</i>	no	ortho	3a	117–182	64–88	Balech 1988, p. 188, fig. 85:1–7
<i>P. oblongum</i>	yes	ortho	3a	130–147	70–80	Hansen & Larsen 1992, p. 124, figs 4.72a–c
<i>P. oceanicum</i>	yes	ortho	3a	155–165	78–80	Abé 1981, p. 324, fig. 300–2.
<i>P. claudicans</i>	yes	ortho	3a	105–50	76–48	Dodge 1982, p. 182, fig. 20D.
<i>P. carus</i>	yes	ortho	3a	85–93	45–48	Abé 1981, p. 271, figs 337–41
<i>P. complanatum</i>	yes	ortho	3a	110	110	Abé 1981, p. 276, figs 249–54
<i>P. depressum</i>	yes	ortho	3a	116–200	116–144	Dodge 1982, p. 177, fig. 20A
<i>P. valgus</i>	yes	ortho	2a	105–113	68–72	Abé 1981, p. 318, fig. 43:282–84
<i>P. consimilis</i>	yes	ortho	2a	53–58	45–50	Abé 1981, p. 316, fig. 42:276–81

¹ 1', apical plate; a*, refers to the numbers of intercalary plates; μm , micrometre.

ence of only one intercalary plate 1a. I report here, unusual thecal plate pattern on *P. novella* sp. nov. and *P. concinna* sp. nov. These *Protoperidinium* species exist in oceanic neritic waters in The Gulf Stream off shore Point Lookout, North Carolina, and off shore Fort Pierce Inlet, Florida, SW Atlantic Ocean, and the Atlantic Barrier Coral Reef Ecosystems, Belize, Central America.

MATERIAL AND METHODS

Sample collections

Dinoflagellates were collected in warm tropical ocean waters at three distant locations: (1) in The Gulf Stream off shore Point Lookout, North Carolina (34°23'N, 79°56'W) (2002 and 2003); (2) off shore Fort Pierce Inlet, Florida (27°32'N, 79°54'W) (2001 to 2003); and (3) outside Douglas Cay (16°43'N, 88°13'W) and Manatee Cay (16°39'N, 88°11'W) (1994 to 1996), Belize, Central America. Douglas Cay and Manatee Cay are oceanic mangroves, biologically diverse mangrove ponds within the Pelican Cays Archipelago, Belize (Macintyre & Ruetzler 2000). The cays are considered Holocene lagoon reefs (Purdy 1994) colonized by red mangroves, *Rhizophora mangle* Linnaeus. The biology and ecology of this ecosystem is complex; it supports an uncommonly rich fauna and flora of marine, benthic and planktonic organisms (D.S. Littler & M.M. Littler 1997; Faust 2000; Ruetzler *et al.* 2000). Two sampling methods were used: (1) a horizontal tow below the water surface using 20 m pore size plankton net towed by a boat at low speed for 5 min and (2) a vertical tow from 10

to 100 m depth between surface using the same 20 m pore size net with an attached weight. Water temperature ranged from 28.5°C to 33.9°C. Salinity levels ranged from 32.3 to 36.7 psu.

Sample preparation

For scanning electron microscopy (SEM) preparation, plankton samples were concentrated to 100 ml volume and fixed with 1% glutaraldehyde final concentration (Faust 1990). The fixed dinoflagellates were isolated using a capillary pipette under a Zeiss stereo microscope. Cells were concentrated onto a polycarbonate filter at room temperature, rinsed six to eight times with deionized water, dehydrated in a graded series of ethanol concentrations, and critical-point dried. The preparation was coated with carbon followed by a layer of gold-palladium (Faust 1990). Cell size estimated from SEM photographs (at least 10 cells); ranges of cell size shown in Table 1. Cell length estimated from anterior to posterior in girdle view, and cell width estimated from trans-diameter in lateral view. Kofoidian nomenclature was used for identification of species (Kofoid 1909). Dinoflagellate specimens used in this investigation are deposited in the US Dinoflagellate Type Collection, the US National Herbarium, Department of Botany, NMNH, Smithsonian Institution, Washington, DC 20013-7012, USA.

RESULTS

Phylum: Pyrrophyta Pasher, 1914

Class: Dinophyceae Fritsch, 1929

Figs 1–7. Scanning electron micrographs of *Protoperidinium novella* sp. nov. Faust.

Fig. 1. Ventral view. Cell has an apical horn and two antapical horns; ventrally depressed. Apical plate 1' ortho disconnected from the apical horn (arrows).

Fig. 2. Apical cell view illustrates the tip of apical horn as a miniscule flat closure (arrowhead), and 1a rectangular intercalary plate, and 1', 2', & 4' ridged apical plates.

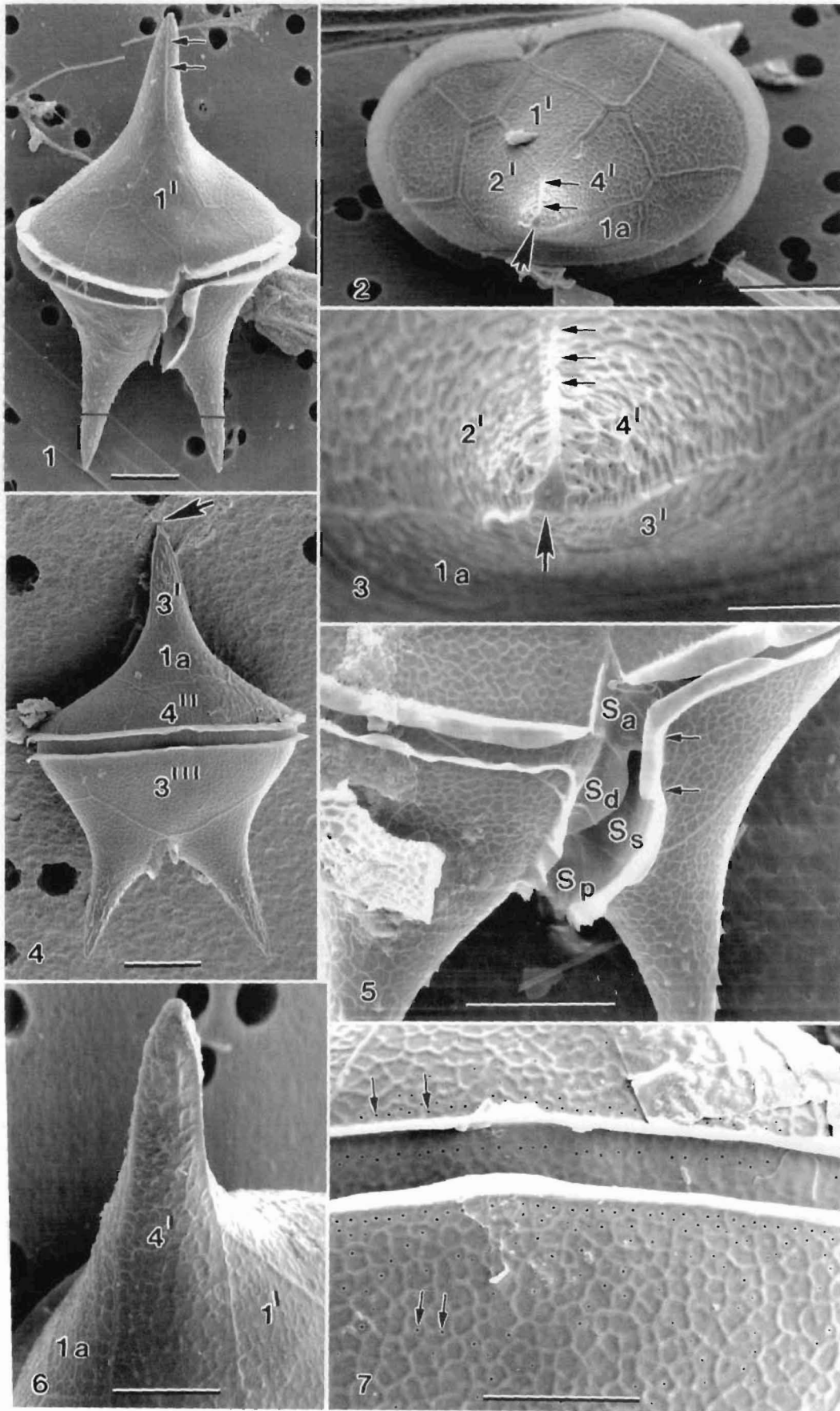
Fig. 3. Apical pore complex is absent (arrow) and surface sculptured. Anterior intercalary plate 1a is large and rectangular.

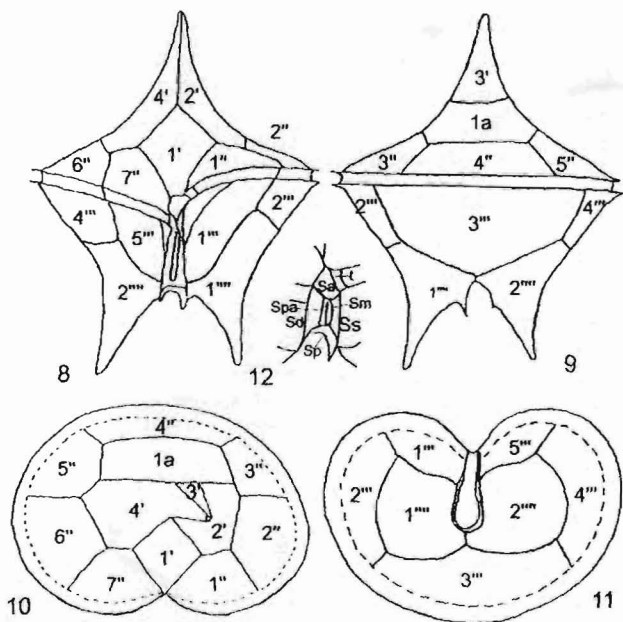
Fig. 4. Dorsal view. Cell is slender, two antapical horns pointed and equal in length and shape. Intercalary plate 1a is large. Postcingular plate 3'' is pentagonal and very large.

Fig. 5. Cingulum is equatorial, descending 1 × its width. Cingular list is smooth. Sulcus is deep and narrow, bordered by sulcal lists (arrowheads). Sulcal plates are: Sa, Sd, Ss, Sp. Apical plate 1' touches the sulcal Sa plate.

Fig. 6. Apical 4' plate is long and narrow situated between apical plate 1' and intercalary plate 1a.

Fig. 7. Thecal plate surface rugose, some plates with a small round pore situated in the centre (arrowheads) and pores in line on the cingulum.





Figs 8–12. Line drawing of *Protoperidinium novella* sp. nov. Faust. Morphology of thecal plates illustrated in various views.

- Fig. 8. Ventral.
 Fig. 9. Dorsal.
 Fig. 10. Apical.
 Fig. 11. Antapical.
 Fig. 12. Sulcal.

Order: Peridinales Haeckel, 1894

Family: Peridiniaceae Ehrenberg, 1832

Genus: *Protoperidinium* Bergh, emend Balech, 1974

Subgenus: *Testeria* subgen. nov. Faust, 2005

Subgenus: *Testeria* Faust, subgen. nov.

Dinoflagellatae armatae. Epithea unicornis et hypotheca bicornis aequalis. Valva apicalis (1') symmetricalis 'ortho' et cornu apicali separato. APC absque. Disci intercalaris (1a) quadra. Dispositio discorum: 4', 3a, 7", 4C(3+t), 6S, 5"', et 2'''. Pagina rugosa thecae.

Armoured dinoflagellate. Epithea one-horned are equal in size to the two-horned hypotheca. Apical plate (1') is symmetrical, ortho, and disconnected from the apical horn. APC is absent. With intercalary plates 1a quadra. Arrangement of plates: 4', 1a, 7", 4C(3+t), 6S, 5"', and 2'''. Thecal surface is rugose.

ETYMOLOGY: Named after Dr Patricia A. Tester in recognition of her significant contributions to understanding the dynamics of *Karenina brevis* (Davis) G. Hansen & Ø. Møstrup harmful algal blooms.

TYPE SPECIES: *Protoperidinium novella* sp. nov. Faust.

Subgenus: *Testeria* Faust

Protoperidinium novella Faust, sp. nov.

Figs 1–12

Cellulae armatae, 98–116 μm longae et 62–75 μm latae, pyriformes. Dispositio discorum: 4', 1a, 7", 4C(3+t), 6S, 5"', et 2'''. Valva apicalis 1' ortho. Valva apicalis (1') ortho et valva intreccalaris 1a hexa. APC absque. Cingulum equatoriale excavatum demotum 1 \times ejus latitudinis. Sulcus profundus et marginatus. Hypotheca ventraliter depressa. Spinae duae in valvibus antapicales 1''' et 2'''. Valvae rugosae. Chromatophores absque. Flagellae duae.

Cells armoured, 98–116 μm long (L), and 62–75 μm wide (W) shape pyriform. The plate tabulation: 4', 1a, 7", 4C(3+t), 6S, 5"', 2'''. Shape of apical plate 1' is ortho. Intercalary plate is 1a, and shape of plate 1a quadra. APC is absent. Cingulum equatorial with cingular lists, excavated, displaced 1 \times its width. Sulcus is deep, bordered by sulcal list. Hypotheca ventrally slightly, depressed. Two horns are present on antapical plates 1''' and 2'''. Thecal surface is rugose. Chromatophores are absent. Flagella are two.

TYPE: Fig. 1, collected by Faust. Sample no. 2398-2001.

ISOTYPES: Figs 2–6, sample no. 2488-2002; Fig. 7, sample no. 2367-2002.

TYPE LOCALITY: Gulf Stream off shore Fort Pierce Inlet, Florida (27°32'N, 79°54'W), Southwest Atlantic Ocean, USA. Sample preserved in glutaraldehyde, currently held in the US Dinoflagellate Type Collection, US National Herbarium, Department of Botany, NMNH, Smithsonian Institution, Washington DC, 20013-7012, USA.

ETYMOLOGY: Novella (Latin) means unfamiliar structure.

DISTRIBUTION: Known from waters The Gulf Stream oceanic current off shore from Point Lookout, North Carolina, and outside Manatee Cay (16°39'N, 88°11'W), and Douglas Cay (16°43'N, 88°13'W), Belize, and in The Gulf Stream off shore Point Lookout, North Carolina (34°23'N, 79°56'W), USA.

Cells of *P. novella* sp. nov. armoured, pyriform in shape with a pointed apical horn and two antapical horns (Figs 1, 4, 8–12). Epithea and hypotheca are equal in size. Cells are 98–116 μm long (L) (\bar{X} 104.2 \pm 8.3; n = 9) and 62–75 μm wide (W) (\bar{X} 70.3 \pm 5.6; n = 9), L/W = 1.48 (Table 2). The shape of apical plate 1' is ortho, and it is disconnected by 25 μm from the pointed and slender apical horn (Fig. 1), but apical 1' plate in direct contact with anterior sulcal (Sa) plate (Figs 1, 2, 5, 8). APC is absent; is characterized by a miniscule flat closure on the extreme anterior of the pointed apical horn (Figs 1–4, 6). The anterior intercalary plate 1a is quadra situated dorsally adjacent apical 2' to 4' plates (Figs 2–4, 9, 10). Surface of *P. novella* is rugose, covered by polygonal intricate ridged surface pattern and small unevenly distributed round pores (Figs 1–7). Cells ventrally depressed (Figs 1, 5, 10, 11). Chloroplasts are absent. Cell content is colourless or pale pink.

The cingulum is deep, equatorial, descending 1 \times its width (Figs 1, 5). It bears prominent smooth cingular lists (Figs 1, 2, 4, 5, 7). Four cingular plates are present 4C(3+t); cingular plates 1C plate is considered a transitional plate (t), C2 and C4 plates are rather narrow, whereas, C3 plate very broad and constitute a major part of the cingulum (Figs 8, 9). Row of small round pores line the cingulum surface (Fig. 7).

Table 2. Comparison of the morphological features of *Protoperidinium novella* and *P. concinna*.¹

Taxa	Cell size (μm)		Ratio L/W	Apical horn		Antapical horn		1a Plate	
	L	W		L	W	L	W	L	W
<i>P. novella</i>	104.2 \pm 8.3	70.3 \pm 5.6	1.48	19.5 \pm 2.2	9.3 \pm 0.8	28.9 \pm 1.2	15.8 \pm 1.0	21.9 \pm 1.1	6.5 \pm 0.3
<i>P. concinna</i>	124.0 \pm 11.4	58.2 \pm 5.0	2.13	24.9 \pm 2.2	7.4 \pm 0.7	30.5 \pm 1.9	13.4 \pm 0.8	26.5 \pm 2.0	7.3 \pm 0.3

¹ L, length; W, width; L/W, ratio of length/width; 1a, intercalary plate 1a.

The hypotheca is oblong and ventrally depressed (Figs 1, 5, 10, 11). Postcingular plates are short, but 3^m plate is the largest (Figs 4, 9). The postcingular list continuously follows along the deep and narrow sulcus (Figs 1, 5, 8). Sulcus bordered by widened postcingular sulcal list (Figs 1, 5, 8). It is bearing six sulcal plates, where Sa plate extends anteriorly into the epitheca (Fig. 5). Two dimorphic flagella present, but not shown. Two antapical horns are 40 µm, each with fins associated with antapical plates 1^m and 2^m (Figs 1, 4, 5, 8, 9, 11). Plate formula: 4', 1a, 7'', 4C(3+t), 6S, 5^m, and 2^m.

HABITAT: *Protoperidinium novella* sp. nov., is a neritic, heterotrophic dinoflagellate species, identified from The Gulf Stream oceanic current off shore from Point Lookout, and off shore Fort Pierce Inlet, Florida (27°32'N, 79°54'W), and SW Atlantic Ocean, USA; and the Belizean coral reef-mangrove ecosystems outside Manatee Cay and Douglas Cay, Caribbean Sea.

ASSOCIATED ALGAE: In the collections *P. novella* sp. nov. were associated with cosmopolitan and world wide distributed oceanic species. The biodiversity of dinoflagellate species varied in each collection. Photosynthetic species were: *Prorocentrum compressum* (Bailey) Abé ex Dodge, *P. gracile* Schütt, *P. lima* (Ehrenberg) Dodge & *P. micans* (Ehrenberg), *Pyrophacus steinii* (Schiller) Wall & Dale and *Pyrodinium bahamense* Plate var. *bahamense*. Heterotrophic species were: *Phalacrocoma rapa* Jörgensen and *P. rotundatum* (Claparède & Lachmann) Kofoid & Mitchener, *Dinophysis caudata* Saville-Kent, *Ornithocercus thumii* (Schmidt) Kofoid & Skogsberg and *O. splendidus* Schütt, *Ceratocorys horrida* Stein, and *Paleophalacrocoma uncinatum* Schiller.

Subgenus: *Testeria* Faust

Protoperidinium concinna Faust, sp. nov.

Figs 13–25

Cellulae armatae, 103–137 µm longae et 52–67 µm latae, pyriformes. Dispositio discorum: 4', 1a, 7'', 4C(3+t), 6S, 5^m, et 2^m. Valva apicalis 1' ortho. Valva apicalis (1') ortho et valva intrecalaris 1a quadra. APC absque. Cingulum equatoriale excavatum demotum 1X ejus latitudinis. Sulcus profundus et marginatus. Hypotheca ventraliter depressa. Spinae duae in valvibus antapicales 1^m et 2^m. Valvae rugosae. Chromatophores absque. Flagellae duae.

TYPE: Fig. 13, collected by Faust. Sample no. 2398-2001.

ISOTYPES: Figs 14–25, sample no. 2488-2002; Figs 7, 8, sample no. 2367-2002.

TYPE LOCALITY: The Gulf Stream off shore Point Lookout, North Carolina (34°23'N, 79°56'W), SW Atlantic Ocean, USA. Sample preserved in glutaraldehyde, currently held in the National Dinoflagellate Type Collection, US National Herbarium, Department of Botany, Smithsonian Institution, Washington DC, 20013-7012, USA.

ETYMOLOGY: *Concinna* (Latin) means harmony and proportion.

DISTRIBUTION: Known from waters outside Manatee Cay mangrove pond (16°39'N, 88°11'W), and Douglas Cay (16°43'N, 88°13'W), Belize, and Gulf Stream off shore Fort Pierce Inlet, Florida (27°32'N, 79°54'W), SW Atlantic Ocean, USA.

Protoperidinium concinna sp. nov. a heterotrophic marine species, slender in cell shape, dorso-ventrally compressed with characteristic long apical and antapical horns (Figs 13–15, 22, 23). Cell size ranges between 103–137 µm (L) (\bar{X} 124 ± 11.4; n = 9) and 52–67 µm (W) (\bar{X} 58.2 ± 5.0; n = 9; Table 2). Epitheca and hypotheca equal in length. Thecal surface polygonally ornamented with intricate shallow ridged polygonal surface pattern and small unevenly distributed round pores (Fig. 21), and recognized as faint striations under the light microscope. Cells lack the APC, characterized by a miniscule flat closure on the extreme anterior of the pointed apical horn (Figs 13–17, 22, 23). Apical plate 1' ortho (Figs 13, 16, 18, 22, 24), disconnected from pointed and slender apical horn but in direct contact with the anterior sulcal (Sa) plate (Fig. 20). Anterior intercalary plate 1a is quadra (Figs 14, 16, 23, 24) situated dorsally between apical plates 2' to 4' (Figs 16, 23, 24).

Cingulum is deep, equatorial and displaced about 1 × its width (Figs 13–15, 18, 19, 22). It bears prominent smooth cingular lists (Figs 13–16, 18–20). Four cingular 4C(3+t) plates are present: 1C is a small transitional plate (t) (Fig. 20), 2C and 4C plates are rather narrow, whereas, C3 plate is very broad and constitutes a major part of the cingulum (Figs 22, 23).

The hypotheca is oblong and ventrally depressed (Figs 13, 15, 18, 19). Postcingular plates are short and 3^m plate is the largest (Figs 14, 23). The post cingular list follows along the deep and narrow sulcus (Figs 13, 18, 19, 22). Two dimorphic flagella present, but not shown. Antapical horns each with fins, associated with antapical plates 1^m and 2^m (Figs 13–15, 19, 22, 23).

Sulcal list smooth (Figs 16, 18–20) bordered by widened postcingular sulcal list (Figs 13, 19). Sulcus is deep and narrow composed of six sulcal plates (Fig. 26). The postcingular list continuously follows along the narrow sulcus. The anterior sulcal plate Sa extends into the epitheca and touches the apical 1' plate at the cingular-sulcal junction (Figs 18–20). Nucleus situated centrally with radiating protoplasmic strands. Protoplasm is clear. Thecal plate formula of *P. concinna* is: 4', 1a, 7'', 4C(3+t), 6S, 5^m, and 2^m.

HABITAT: Populations of *P. concinna* cells were associated with neritic oceanic species present in coastal marine waters, coral reef-mangrove habitats in the western Caribbean Sea: pond Manatee Cay and Douglas Cay, Pelican Cays Archipelago, Belize (Faust 2000), The Gulf Stream off shore Point Lookout, North Carolina, and off shore from the Fort Pierce Inlet, Florida, SW Atlantic Ocean (Faust & Tester 2005). Balech (1988) recognized a morphologically similar species, *P. steidingerae* Balech, in samples collected in both warm waters of southern Brazil (33.2–35.4°C) and in cold waters of the Southeastern Atlantic Ocean (24.7–27.5°C).

ASSOCIATED ALGAE: In the collections *P. concinna* from Manatee Cay were associated with *Diplopsalis lenticula* Bergh, *Diplopsalopsis* sp., *Dinophysis rotundata* Claparède & Lachmann, *Diplopelta* sp., *Lingulodinium polyedrum* (Stein) Dodge, and *Prorocentrum elegans* Faust. Harmful species included: *P. lima* (Ehrenberg) Dodge, and *P. hoffmannianum* Faust.

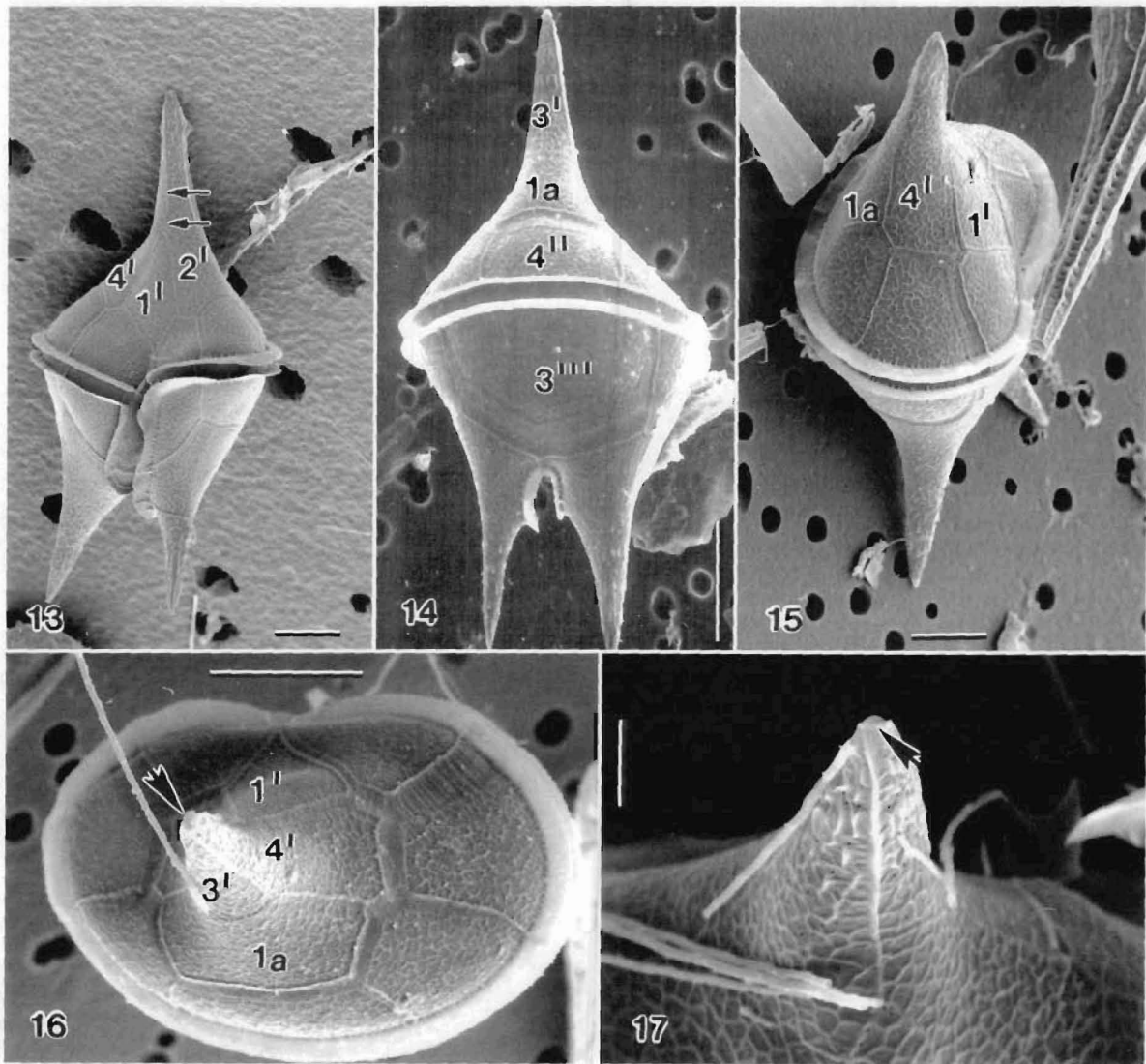
DISCUSSION

According to the subdivision of the genus proposed by Balech (1974, 1975, 1979, 1988, 1999) and Abé (1936, 1981), *Protoperidinium novella* and *P. concinna* can be attributed to section *Oceanica* in the genus *Protoperidinium*. Some unique morphological features, however, characterize *P. novella* and *P. concinna* different from species in the section *Oceanica*.

Morphology of two new species

Comparison of morphological features of *P. novella* and *P. concinna* and selected *Protoperidinium* species in the section *Oceanica* are listed in Table 1. *Protoperidinium novella* and *P. concinna* differ in morphology from other congeneric species based on three morphological characters: (1) the extreme anterior of the apical horn is pointed characterized by a miniscule flat closure, absence of APC (Figs 1–3, 16, 17; Table 1); (2) the apical ventral plate 1' disconnects from the tip of the apical horn; and (3) presence of two intercalary plate 1a. The plate formula of *P. novella* and *P. concinna* is as follows: 4', 1a, 7'', 4C, 6S, 5^m, and 2^m. The unique plate structures on the episome of *P. novella* and *P. concinna* are supported by SEM pictures (Figs 1–7, 13–21). The morphological features that characterize them are stable characters and not aberrant forms of certain 'normal *Protoperidinium*'.

Comparing the presence or absence of APC from line drawings is troublesome. Balech (1979, 1988) and Steidinger & Tangen (1996) reported no APC on *P. steidingerae*. All spe-

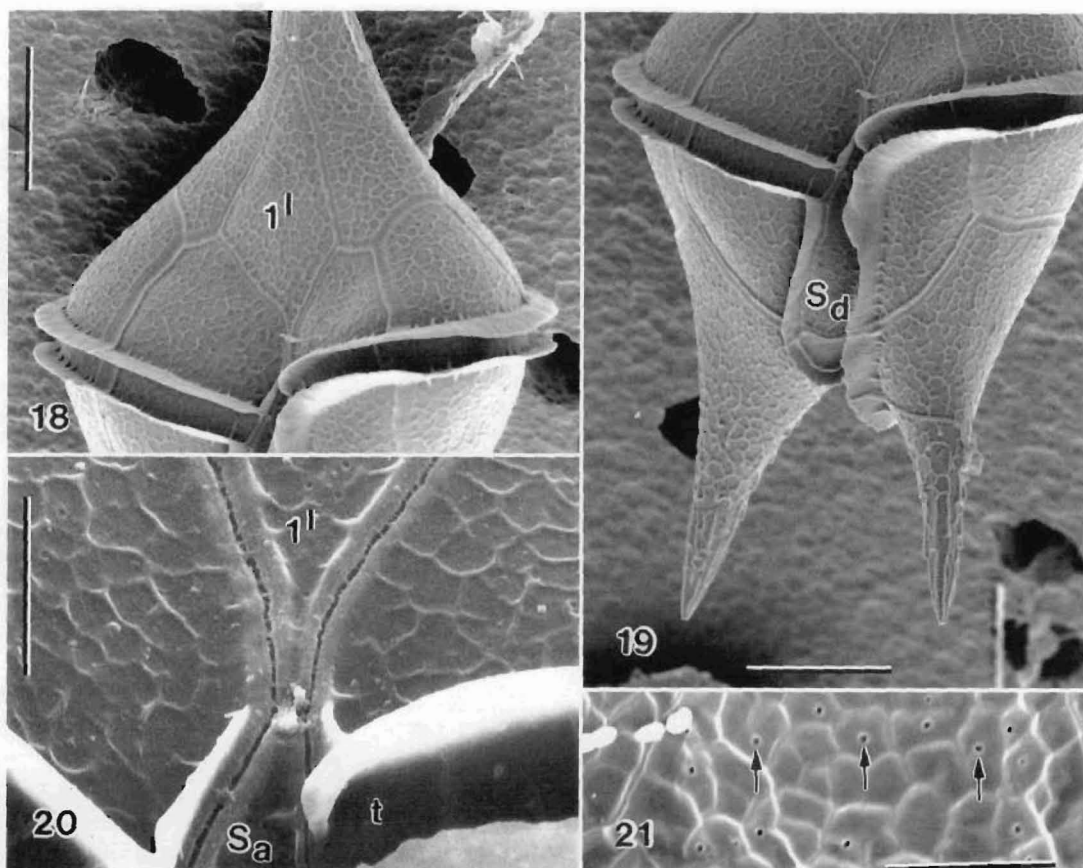


Figs 13–17. Scanning electron micrographs of *Protoperidinium concinna* sp. nov., Faust.
Fig. 13. Ventral view. Cell is slender with a long pointed apical horn and two antapical horns. Apical plate 1' ortho disconnected from the apical horn (arrowheads). Cingulum is displaced width by 1 ×. APC is absent. Left sulcal list is wide.
Fig. 14. Dorsal view. Apical horn pointed. Anterior intercalary (1a) plate is large. Cingulum is equatorial with smooth cingular lists. Postcingular plate (3''') is very large. Antapical horns equal in length, broad proximally, pointed distally.
Fig. 15. Epitheca excavated ventrally. Location of apical plate 4' is long and narrow, situated adjacent intercalary plate 1a and apical plate 1'.
Fig. 16. Apical view of epitheca. Apical horn pointed, and APC absent (arrowhead). Intercalary plate 1a is large. Intercalary band slightly striated.
Fig. 17. Tip of apical horn is a minuscule flat closure, void of an apical pore (arrowhead). Thecal surface rugose with scattered pores.

cies in the section *Oceanica* show typical thecal morphology. But some published figures are to some extent uncertain as to the exact feature of the APC or intercalary plates in apical views. Illustrations of the APC in SEM pictures are limited to a few species making comparison with other species difficult: *P. oblongum* (Hansen & Larsen 1992; Tourimi & Dodge 1993), *P. oceanicum* (Toriumi & Dodge 1993), and *P. depressum* (Abé 1981; Dodge 1985; Toriumi & Dodge 1993).

Distinctive recognizable feature of *P. novella* and *P. concinna* concerns the architecture of apical plate 1' which disconnects from the tip of the apical horn situated directly along a straight line of the longitudinal axis at the cell's sagittal plane. Plate 1' maintains its typical elongated ortho-

shape and seems totally disconnected from the apical horn on whole thecae. Such a feature of plate 1' has only been discovered on *P. steidingerae* (Balech 1979). The plate 1' not only disconnects from the pointed apical horn but is in direct contact with anterior sulcal plate Sa and a very narrow 1C plate located in the cingulum-sulcus juncture; 2C and 4C plates are also rather narrow, whereas 3C is very broad and constitutes the major part of the cingulum. Although *P. steidingerae* shares a few important characteristics with these two new species, it can be distinguished from my species by possessing three apical intercalary plates. The apical plate 1' of the section *Oceanica* and other species in *Protoperidinium* (Table 1), extend from the tip of the apical horn and ends at



Figs 18–21. Scanning electron micrographs of *Protopteridinium concinna* sp. nov., Faust.

Fig. 18. Ventral apical view of epitheca. Apical plate 1' is ortho and disconnected from the apical horn. Cell ventrally depressed.

Fig. 19. Cingulum is descending $1 \times$ its width. Cingular list is smooth. Sulcus deep, narrow, and bordered by smooth sulcal lists.

Fig. 20. Apical plate 1' touches the sulcal Sa platelet extended into the epitheca and touches transition plate (t) at the cingulum-sulcus junction.

Fig. 21. Thecal plate surface rugose with small unevenly distributed round pores (arrows).

the sulcus on the ventral surface of the episome (Abé 1981; Toriumi & Dodge 1993).

Protopteridinium novella and *P. concinna* possess only one dorsal intercalary plate (Tables 1, 2). This small dorsal 1a plate is only recognizable on tilted SEM stub (Figs 2, 16; Abé 1981). The morphology of plates on the episome on these new species is distinct but comparable in line drawings of species in the section *Oceanica* (Steidinger & Williams 1970; Balech 1979, 1988; Abé 1981). SEM pictures of species in the section *Oceanica* are few and often very small to recognize thecal details (Delgado & Fortuno 1991) and at times inconsistent with some features of species. In fact, *P. oblongum* is illustrated with only one 1a intercalary plate plus an APC (Hansen & Larsen 1992), in other publications the same species exhibiting 3a plates plus an APC (Dodge 1985; Toriumi & Dodge 1993).

In conclusion, I demonstrated unequivocally that *P. novella* and *P. concinna* lack contact between apical plate 1' and the tip of apical horn, APC absent, and only 1a intercalary plate present. I conclude that *P. novella* and *P. concinna* are different from all other *Protopteridinium*.

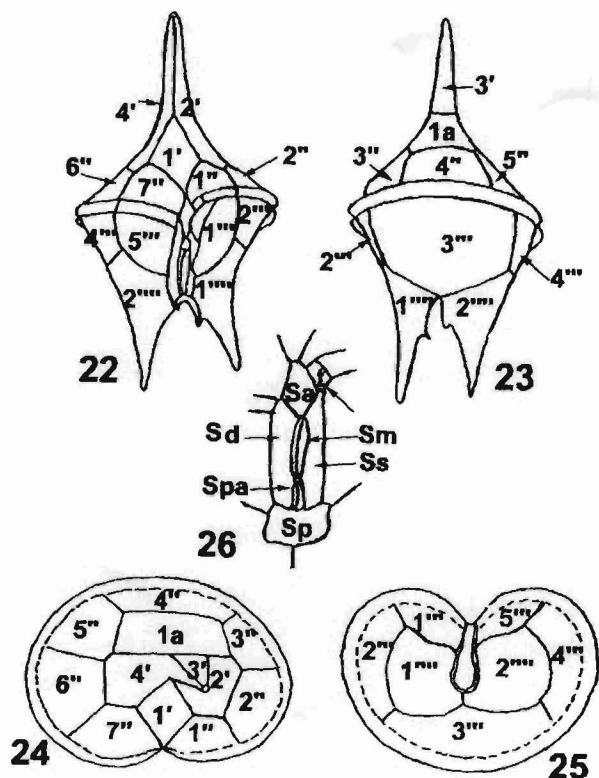
Separation of new species

Protopteridinium novella and *P. concinna* are separable from each other based on cell shape and size, length of apical and

antapical horns and dimension of 1a intercalary plate (Table 2). Both species exhibit polygonal intricate ridged surface pattern (Figs 5, 19). The differences are (1) cell shape of *P. novella* is broader with a ratio L/W 1.48 [cell length (L) and cell width (W)], including a shorter and wider apical and hypothecal horns and a smaller 1a intercalary plate; (2) shape of *P. concinna* is slender, cell size longer with a ratio of L/W of 2.13, including narrower apical and antapical horns and larger 1a intercalary plate; (3) sulcal Sa plate of *P. concinna* extends into the epitheca and touches apical 1' plate and 'transitional' t-plate in the cingulum-sulcus juncture (Figs 13, 20); (4) engenders change in the plate relationship of the ventral area causing a distinct depression ventrally (Figs 13, 15, 18, 19); and (5) these features are different from each other in structural relations within the ventral area and also in the relations between the ventral area and the forward bending apical horn on *P. concinna* (Fig. 15) when compared to upward apical horn on *P. novella* (Fig. 6).

Placement of new species in genus *Protopteridinium*

The morphology and plate tabulation of *P. novella* and *P. concinna* suggest that these species might be closely related to members of the section *Oceanica*. The situation is, however, problematic for *P. novella* and *P. concinna* because these two species are characterized by three distinct features, which



Figs 22–26. Line drawings of *Protoperidinium concinna* sp. nov. Faust. Morphology of plates illustrated in several views.

- Fig. 22. Ventral.
 Fig. 23. Dorsal.
 Fig. 24. Apical.
 Fig. 25. Hypothecal.
 Fig. 26. Sulcal.

are quite uncommon in the genus *Protoperidinium*. Whether or not to place these two species in the genus *Protoperidinium* is a worthwhile discussion (Figs 1–3, 16, 17). The variations of epithecal plate arrangement, deviation from the typical plate formula, have been well known in the genus *Protoperidinium*. These variant forms are classified in the distinct subgenus such as *Archaeperidinium* based on the position and shape of the first 1' apical plate and those species with only two 2a anterior intercalary plates, e.g. *P. consimilis* and *P. valgus* (Table 1; Abé 1981). Sournia (1986) considered *Archaeperidinium* to be taxonomic junior synonym of *Protoperidinium*. Fensome *et al.* (1993) prefer to retain *Archaeperidinium* at generic rank to enable practical subdivision of the large genus *Protoperidinium*.

In the freshwater genus *Peridinium*, the presence or absence of APC has been regarded as difference at subgeneric level rather than the generic separation (Balech 1988). Balech (1995) separated species in the genus *Alexandrium* into two subgenera, *Alexandrium* and *Gessnerium* by recognizing synonymy in APC architecture and ventral plate 1' morphology of species. Furthermore, among *Protoperidinium* species, absence of an APC is few, and recognized only in other related dinoflagellate genera, e.g. *Gotoius mutsuensis* (Abé 1981) and *G. abei* (Matsuoka 1988) in the diplopsalid group. The situation in *P. novella* and *P. concinna* is comparable to this practice.

In conclusion, I believe that *P. novella* and *P. concinna* are distinct enough from all other species of the genus *Protoperidinium* and it is appropriate to classify them as a new subgenus. I, therefore, propose to establish the subgenus *Testeria* Faust, *subgen. nov.* in the genus *Protoperidinium*. I have selected *P. novella* sp. nov. Faust as the type species, and *P. concinna* sp. nov. Faust a second species.

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REFERENCES

- ABÉ T.H. 1936. Report of the biological survey of Mutsu Bay, 30. Notes on the protozoa fauna of Mutsu Bay. Genus *Peridinium* III. Subgenus *Protoperidinium*. *Science Report Tohoku Imperial University Series* 11: 19–48.
- ABÉ T.H. 1981. Studies on the family Peridiniidae. An unfinished monograph of the armored dinoflagellata. *Seto Marine Biological Laboratory. Special Publication Series* 6: 1–413.
- ANDREIS C., CIAPI M.D. & RODINDI G. 1982. The thecal surface of some Dinophyceae: a comparative SEM approach. *Botanica Marina* 25: 225–236.
- BALECH E. 1974. El genero "*Protoperidinium*" Bergh, 1881. ("*Peridinium*" Ehrenberg, 1831, partim). *Revista del Museo Argentino de Ciencia Naturales "Bernardino Rivadavia."* *Hidrobiologia* 4: 1–79.
- BALECH E. 1975. Estructuras de *Protoperidinium* en microscopia electronica de banido. *Neotropica* 21: 20–25.
- BALECH E. 1979. Tres dinoflagelados nuevos o interesantes de aguas Brasilenas. *Biologi Instituto de Oceanografia, S. Paulo* 28: 55–64.
- BALECH E. 1980. On thecal morphology of dinoflagellates with special emphasis on circular and sulcal plates. *Annales Centro Ciencia del Marina y Linnology* 7: 57–68.
- BALECH E. 1988. *Los Dinoflagelados del Atlantico Sudoccidental*. Publication Especiales No. 1, Instituto Espanol de Oceanografia, Madrid, Spain. 310 pp.
- BALECH E. 1995. *The genus Alexandrium Halim (Dinoflagellata)*. Sherkin Island Marine Station. Sherkin Island Co, Cork, Ireland. 151 pp.
- BALECH E. 1999. *Protoperidinium* (Dinoflagellata) nuevos o interesantes de la Bahia de Manila (Filipinas). *Revista del Museo Argentino Ciencia Naurales* 1: 165–171.
- BERGH R.S. 1881. *Der Organismus der Cilioflagellaten*. Eine phylogenetische Studie. *Morphologisches Jahrbuch* 7(2): 177–288, pls 12–16.
- DELGADO M. & FORTUNO J.-M. 1991. Atlas de fitoplankton del Mar Mediterraneo. *Scienza Marine* 5: 1–133, 66 pls.
- DODGE J.D. 1983. Ornamentation of thecal plates in *Protoperidinium* (Dinophyceae) as seen by scanning electron microscopy. *Journal Plankton Research* 5: 119–127.

- DODGE J.D. 1985. *Atlas of Dinoflagellates: a scanning electron microscope study*. Ferrand Press, London, 119 pp.
- FAUST M.A. 1990. Morphologic details of six benthic species of *Pro-rocentrum* (Pyrrophyta) from a mangrove island, Twin Cays, Belize, including two new species. *Journal of Phycology* 26: 548–558.
- FAUST M.A. 2000. Dinoflagellate associations in a coral reef-mangrove ecosystem: Pelican and associated Cays. In: *Natural history of the Pelican Cays, Belize* (Ed. by I.G. Macintyre & K. Ruetzler), *Atoll Research Bulletin* No. 473: 135–152.
- FAUST M.A. & TESTER P.A. 2005. Harmful dinoflagellates in the Gulf stream and Atlantic barrier coral reef, Belize. In: *Harmful Algae 2003. Proceedings of the Xth International Conference of Harmful Algae* (Ed. by K.A. Steidinger, J.H. Landsberg, C.C. Tomas & G.A. Vargo), pp. 326–328. Florida Fish and Wildlife Conservation and IOC of UNESCO, St. Petersburg, Florida.
- FENSOME R.A., TAYLOR E.J.R., NORRIS G., SARJEANT W.A.S., WHARTON D.L. & WILLIAMS G.L. 1993. "A classification of living and fossil dinoflagellates." Sheridan Press, Hanover, PA, USA, 351 pp.
- GOCHT H. & NETZEL H. 1974. Scanning electron microscopy studies on the theca of *Peridinium* (Dinoflagellata). *Archiv Protisten Kunde* 116: 381–410.
- HANSEN G. & LARSEN J. 1992. Dinoflagellater (Dinophyceae) i danske farvande. In: *Plankton i de indre danske farvande*, Nr. (Ed. by H.A. Thomsen), vol 11, pp. 45–155. Havforskning fra Miljøstyrelsen, Miljøministeriet, København, Denmark.
- JÖRGENSEN E. 1912. Bericht über die von der schwedischen hydrographisch biologischen Kommission in den schwedischen Gewässern in den Jahren 1909–10 Eingesammelten Planktonproben. *Svenska hydrografisk-biologiska kommissionens Skrifter* 4: 1–20.
- KOFOED C.A. 1909. On *Peridinium steinii* Jörgensen, with a note on the nomenclature of the skeleton the Peridinidae. *Archiv Protisten Kunde* 16: 25–47, pl. 1.
- LEWIS J. & DODGE J.D. 1990. The use of SEM in dinoflagellate taxonomy. In: *Scanning electron microscopy in taxonomic and functional morphology*. (Ed. by D. Claugher), *Systematic Association Special Volume* 41: 128–148.
- LITTLER D.S. & LITTLER M.M. 1997. An illustrated marine flora of the Pelican Cays, Belize. *Bulletin of the Biological Society of Washington* 9: 1–149.
- MACINTYRE I.G. & RUETZLER K. 2000. *Natural history of the Pelican Cays, Belize. Atoll Research Bulletin* Nos. 466–480. Smithsonian Institution Press, Washington, DC, USA, 333 pp.
- MATSUOKA K. 1988. Cyst-theca relationship in the Diplopsalid group (Peridiniales, Dinophyceae). *Review of Paleobotany and Palynology* 56: 95–122.
- NETZEL H. & DÜRR G. 1984. Dinoflagellate cell cortex. In: *Dinoflagellates*. (Ed. by D.L. Spector), pp. 43–105. Academic Press, Orlando, FL, USA.
- PURDY E.G. 1994. Karst-determined facies patterns in British Honduras: holocene carbonate sedimentation model. *American Association of Petroleum Geologists* 58: 825–855.
- RUETZLER K., DIAZ M.A., VON SOEST R.W.M., SWEN Z., SMITH K.P., ALVAREZ B. & WULFF J. 2000. Biodiversity of sponge fauna in mangrove ponds, Pelican Cays, Belize. In: *Natural history of the Pelican Cays, Belize*. (Ed. by I.G. Macintyre & K. Ruetzler), *Atoll Research Bulletin* 476: 231–250.
- SCHÜTT F. 1895. Die Peridineen der Plankton Expedition. 1. Teil. Studien über die zellen der Peridineen. *Ergebnisse der Plankton-Expedition der Humboldt-Stiftung* 4: 1–170, 27 pls. A. Kiel, Lipsius and Teicher.
- SOURNIA A. 1986. "Atlas du Phytoplankton Marin." Volume I: Introduction, Cyanophycées, Dictyochophycées, Dinophycées et Raphidophycées." Editions du Centre National de la Recherche Scientifique, Paris, France, 219 pp.
- STEIDINGER K.A. & TANGEN K. 1996. Dinoflagellates. In: *Identifying marine diatoms and dinoflagellates* (Ed. by C.R. Tomas), pp. 387–584. Academic Press, San Diego, CA, USA.
- STEIDINGER K.A. & WILLIAMS J. 1970. Dinoflagellates. *Memoirs of the Hourglass Cruises. Marine Research Laboratory Contributions* 148: 1–251.
- TAYLOR E.J.R. 1976. Dinoflagellates from the international Indian ocean expedition. A report on material collected by the R.V. 'Anton Brunn' 1963–64. *Bibliotheca Botany* 132: 1–234, pls 1–46.
- TORRUMI S. & DODGE D.J. 1993. Thecal apex structure in the Peridinaceae (Dinophyceae). *European Journal of Phycology* 28: 39–45.
- YAMAGUCHI A. & HORIGUCHI T. 2005. Molecular phylogenetic study of the heterotrophic dinoflagellate genus *Protoferidinium* (Dinophyceae) inferred from small subunit rRNA gene sequences. *Phycological Research* 53: 30–42.

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