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Loïc Ten-Hage, Jean-Pascal Quod, Jean Turquet, Alain Couté. Bysmatrum granulosum sp. nov., a new benthic dinoflagellate from the southwestern Indian Ocean. European Journal of Phycology, 2001, vol. 36, pp.129-135. 10.1080/09670260110001735278 . hal-00906298

HAL Id: hal-00906298

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To link to this article : DOI:10.1080/09670260110001735278
URL : http://dx.doi.org/10.1080/09670260110001735278

To cite this version :
Ten-Hage, Loïc and Quod, Jean-Pascal and Turquet, Jean and Couté, Alain *Bysmatrum granulosum sp. nov., a new benthic dinoflagellate from the southwestern Indian Ocean.* (2001) European Journal of Phycology, vol. 36 (n° 2). pp. 129-135. ISSN 0967-0262

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Bysmatrum granulosum sp. nov., a new benthic dinoflagellate from the southwestern Indian Ocean

LOÏC TEN-HAGE[1], JEAN-PASCAL QUOD[2], JEAN TURQUET[2] AND ALAIN COUTÉ[1]

[1] *Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, 12, rue Buffon, 75005 Paris, France*
[2] *Agence pour la Recherche et la Valorisation Marine, 14, rue du stade de l'Est, 97490 Ste Clotilde, France*

A new benthic marine dinoflagellate, *Bysmatrum granulosum* Ten-Hage, Turquet, Quod & Couté, sp. nov., was obtained from sediment and coral samples from sites of La Réunion Island (SW Indian Ocean). This new species is described and illustrated by light and scanning electron micrographs. Cells are 40–50 μm long and 40–46 μm wide. The epitheca is conical and smaller than the hypotheca, which is trapezoidal with convex sides. Plate tabulation is typical for the genus (P_0, X, 4′, 3a, 7″, 6c, 5s, 5‴, 2⁗); epithecal plates 3′ and 4″ separate the intercalary plates 2a and 3a. Thecal plates are perforated by pores and covered by both small and minute wart-like projections, linearly arranged, radiating from the apical pore. This new species differs from the three others of the genus *Bysmatrum* in the following characters: cell shape and size, size of the apical pore complex and features of thecal plates (plate shapes and ornamentations).

Key words: benthic dinoflagellates, *Bysmatrum granulosum* sp. nov., Dinophyceae, Indian Ocean, taxonomy

Introduction

Benthic dinoflagellates are important components of subtropical and tropical marine coral reef-lagoonal environments. In these environments, identified species mainly belong to the genera *Gambierdiscus* Adachi & Fukuyo, *Ostreopsis* Schmidt, and *Prorocentrum* Ehrenberg (Fukuyo, 1981; Faust 1995*a*, 1996*a*); less known genera in these habitats include *Bysmatrum* Faust & Steidinger (1998).

The tropical benthic scrippsielloid genus *Bysmatrum* was recently established by Faust & Steidinger (1998) for three species: *B. arenicola* Horiguchi & Pienaar (Horiguchi & Pienaar, 2000), *B. caponii* (Horiguchi & Pienaar) Faust & Steidinger and *B. subsalsum* (Ostenfeld) Faust & Steidinger, the type species (Faust & Steidinger, 1998). These species, previously placed in the genus *Scrippsiella* Balech (Steidinger & Balech, 1977; Horiguchi & Pienaar, 1988*a*, *b*), are distinguished from planktonic scrippsielloids mainly by thecal reticulation and the lack of contact between intercalary plates 2a and 3a, which are separated by apical plate 3′ (Faust & Steidinger, 1998). *B. subsalsum* was reported in association with floating detritus, coral rubble and sediment at Twin Cays, Belize (Faust & Steidinger, 1998), and from Florida west coastal waters (Steidinger & Balech, 1977), the Atlantic Barrier Reef (Belize) (Faust & Steidinger, 1998) and tropical coral reefs in the East China Sea (Faust, 1996*b*). The two other species, *B. arenicola* and *B. caponii*, were described from tidal pools, respectively along the eastern coast of South Africa (Horiguchi & Pienaar, 1988*b*) and in southern California (Horiguchi & Pienaar, 1988*a*).

During a study on the diversity of benthic dinoflagellates from La Réunion Island (SW Indian Ocean, France) among which many species are known to be toxic, a new *Bysmatrum* species, *Bysmatrum granulosum* sp. nov., was recorded. In this paper we describe the morphology of this new species isolated from coral and sediments.

Materials and methods

The material studied here was collected in the coral reef habitat of St Leu, La Réunion Island (21°09′12″ S, 55°17′40″ E, French Indian Ocean). The water temperature ranged from 23·3 to 29·0 °C and salinity from 34·7 to 35·2 psu. The samples were collected in this area in April 1999. The new species was present also in samples from La Saline reef complex, La Réunion Island (21°04′87″ S, 55°13′10″ E).

Dead coral branches, covered by an algal turf consisting of red algae in the Ceramiales and Gelidiales, and sediment samples from the study area, were collected by diving and placed in plastic containers. Samples were fixed with 4% glutaraldehyde.

Cell dimensions were determined by measuring the length and width of 20 cells using an ocular micrometer on a light microscope. Fixed cells were isolated with a

capillary pipette under a compound dissecting microscope. Cells were rinsed with distilled water to remove salt and fixatives, dehydrated in a series of graded ethanol concentrations, and critical-point dried. For scanning electron microscope (SEM) observations, the preparations were coated with a layer of gold-palladium. Micrographs were taken on a JEOL 840 SEM of the Life Sciences Laboratories of the Muséum National d'Histoire Naturelle of Paris.

Results

Bysmatrum granulosum Ten-Hage, Quod, Turquet Couté, sp. nov.

Figs 1–27

Cellula in ventrale visu pentagona, in apicale visu sub-circularis. Longitudo: 40–50 μm; latitudo: 40–46 μm. Epitheca minor quam hypotheca. Cinguli positio supra-aequatorialis, 1–1·5-plo latitudine sui descendens. Sulcus profundus cum suo sinistro latere lato et in sui terminali parte acuto. Thecae pagina projecturas parvas et minutissimas verruciformes longitudinaliter ordinatas fert. Parvae projecturae saepe cum uno poro. Formula thecae laminarum P_0, X, 4′, 3a, 7″, 6c, 4s, 5‴ et 2⁗. 2a et 3a laminae separatae. Intercalares suturae subtiliter striatae. Chloroplasti multi aurei brunei et stigma praesens.

Cell pentagonal in ventral view, subcircular in apical view, 40–50 μm long, 40–46 μm wide. Epitheca smaller than hypotheca. Cingulum in supraequatorial position, descending 1–1·5 times its width. Sulcus deep, left sulcal list large and pointed in its terminal part. Thecal surface covered by both small and minute wart-like projections, linearly arranged. Small projections often associated with one thecal pore. Thecal formula P_0, X, 4′, 3a, 7″, 6c, 4s, 5‴, 2⁗. Plates 2a and 3a separated. Intercalary bands finely striated. Chloroplasts many, golden brown. Stigma present.

HOLOTYPE: Fig. 9.

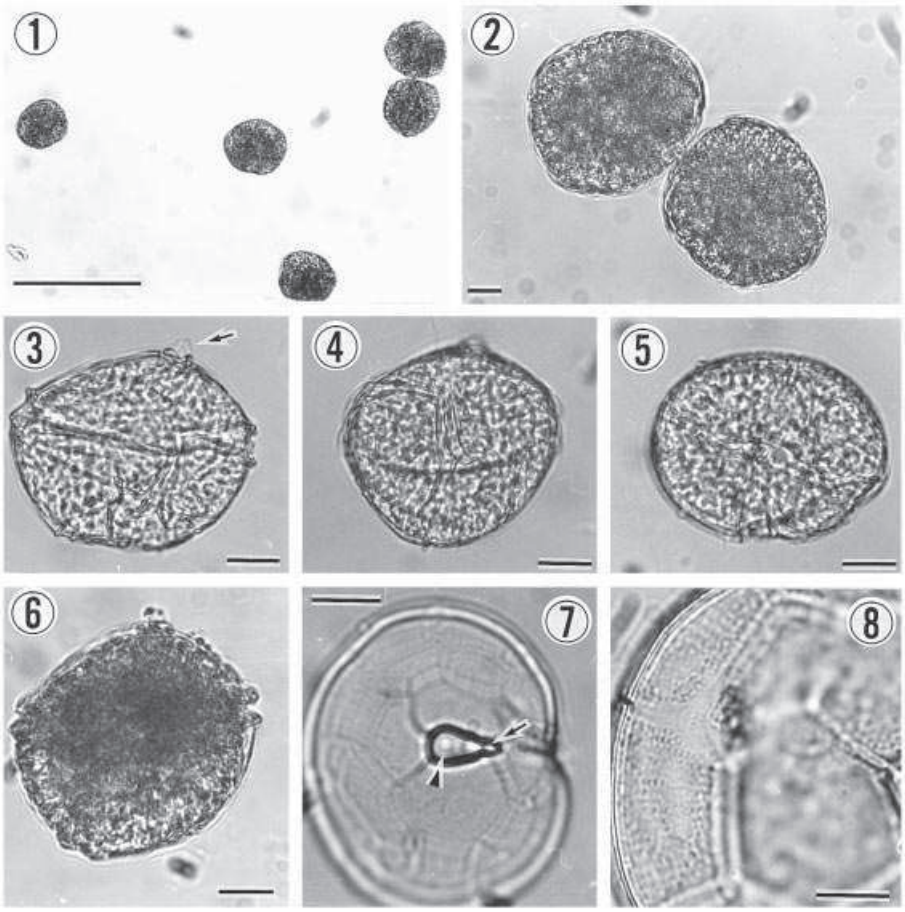
ISOTYPE: Fig. 10, see also Figs 11–14, 23–27.

TYPE LOCALITY: St Leu, La Réunion Island, French Indian Ocean (21°09′12′ S, 55°17′40″ E).

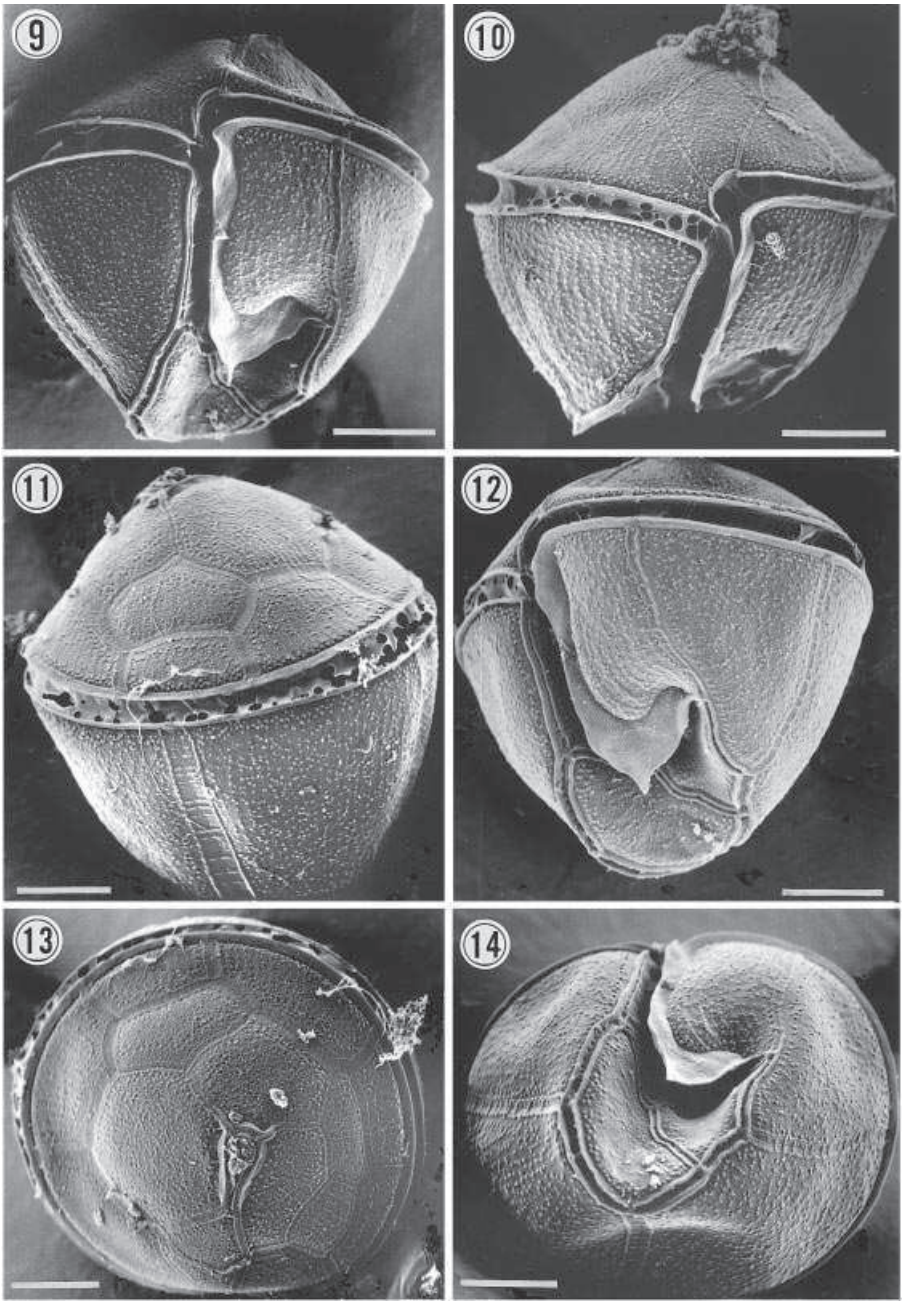
HABITAT: Marine, associated with sediment or attached to coral rubble and macroalgal turf surfaces.

ETYMOLOGY: Refers to the granular appearance of the thecal surface.

Bysmatrum granulosum sp. nov. is an armoured species (Figs 7, 9–14, 23). Cells measure 40–50 μm in length, 40–46 μm in width and are pentagonal in ventral view (Figs 3, 6, 10, 23) and subcircular in apical view (Figs 5, 7, 13, 25). The epitheca is conical



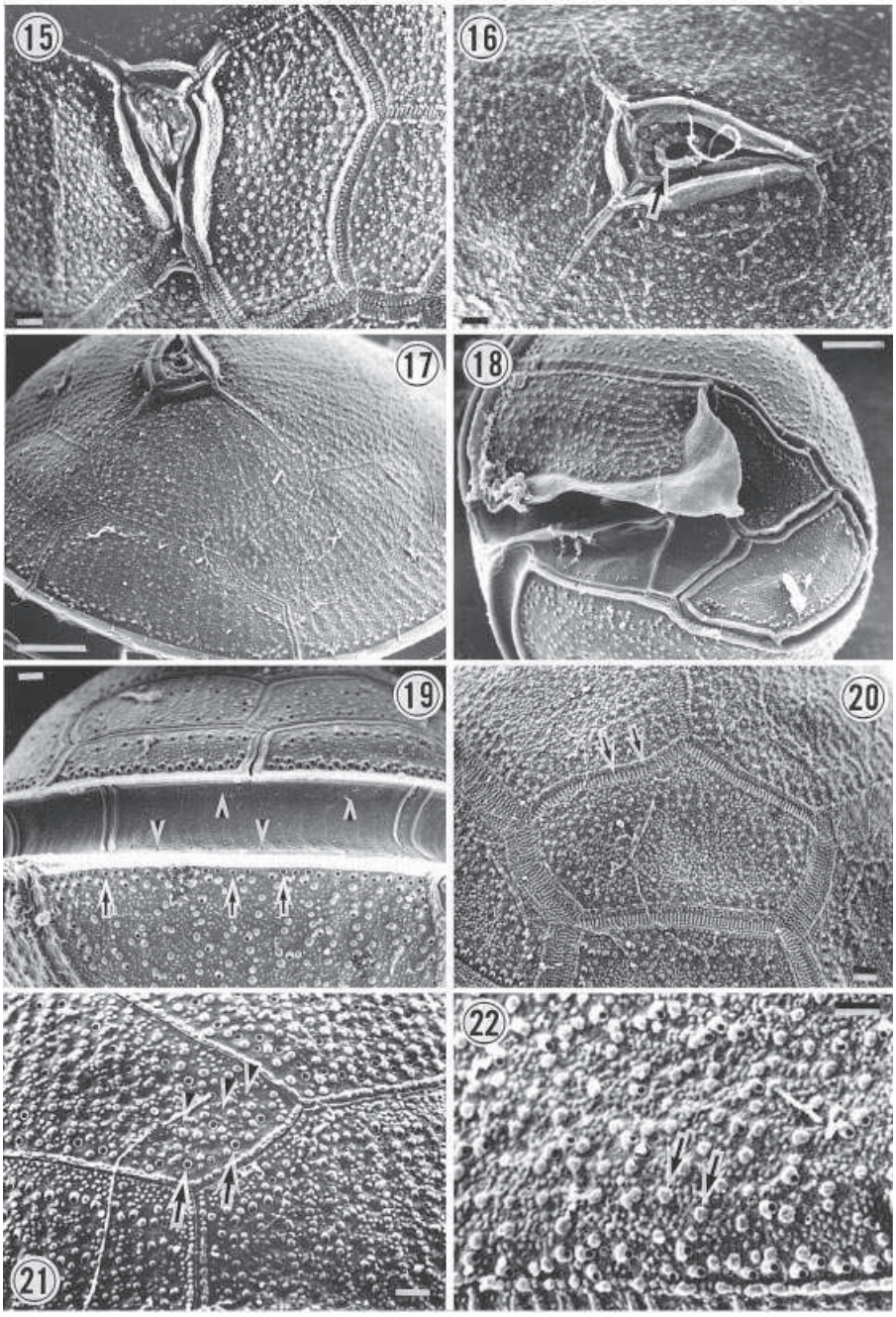
Figs 1–8. Light micrographs of *Bysmatrum granulosum* sp. nov. Fig. 1. Low magnification photomicrograph of five cells of *B. granulosum*. Fig. 2. Two cells in hypothecal view. Cellular contents are dense. Fig. 3. Ventral view. Arrow indicates the apical stalk. Fig. 4. Hypotheca viewed from the ventro-posterior side. Fig. 5. Hypotheca viewed from the antapical side. Fig. 6. Ventral view. Cellular content is very dense and prevents observation of the thecal structure. Fig. 7. Separated epitheca in apical view showing thecal arrangement of the epitheca and the apical pore complex. Arrowhead indicates plate P_0, and the arrow shows canal plate X. Fig. 8. Detail of precingular plates. The thecal surface appears longitudinally striated. Scale bars represent: Fig. 1, 100 μm; Figs 2–7, 10 μm; Fig. 8, 5 μm.



Figs 9–14. Scanning electron micrographs of the surface morphology of *Bysmatrum granulosum* sp. nov. Fig. 9. Cell viewed from the ventro-posterior side. Fig. 10. Ventral view. The epitheca is conical and smaller than the trapezoidal hypotheca. Fig. 11. Dorsal view. Fig. 12. Left lateral view. The sulcus is covered in part by a large left sulcal list. Fig. 13. Detail of the architecture of the epitheca including the position of the apical pore complex. Fig. 14. Antapical view of the hypotheca. Scale bars represent 10 μm.

and smaller than the hypotheca (Figs 10, 11). In ventral view, the hypotheca is trapezoidal with convex sides (Figs 9–11, 23). The cingulum is well excavated and descends 1–1·5 times its own width (Figs 10, 23). The sulcus is narrow, often hidden, and is covered partly by a large left sulcal list (Figs 9, 12, 23, 26, 27). The thecal surface is covered by small circular wart-like projections (Figs 21, 22). These projections are linearly arranged, radiating from the apical pore (Fig. 17), so that the thecal surface appears longitudinally striated under the light microscope (Fig. 8). Two sizes of wart-like projections are present. The smallest cover all the thecal surface. The biggest projections are less numerous and are localized at the surface of epithecal and hypothecal plates (not on the cingular and sulcal plates) (Figs 17, 18, 21, 22). In addition to the wart-like projections, thecal plates are perforated by round thecal pores (Figs 21, 22). We distinguish two sizes of pores. The smaller (0·07 μm) are rare and sparse. The larger (0·14–0·16 μm) are more numerous, principally situated at the bottom of the largest thecal projections and/or on the edges of each thecal plate. Marginal pores are present on both sides of the cingulum (Fig. 19). These marginal pores are similar in shape and in appearance to the largest thecal pores. Intercalary bands are finely striated (Fig. 20).

The epitheca of *Bysmatrum granulosum* is composed of the apical pore plate (P_0), the canal plate (X), four apical plates (4′), seven precingular plates (7″) and three intercalary plates (3a) (Figs 13, 25).

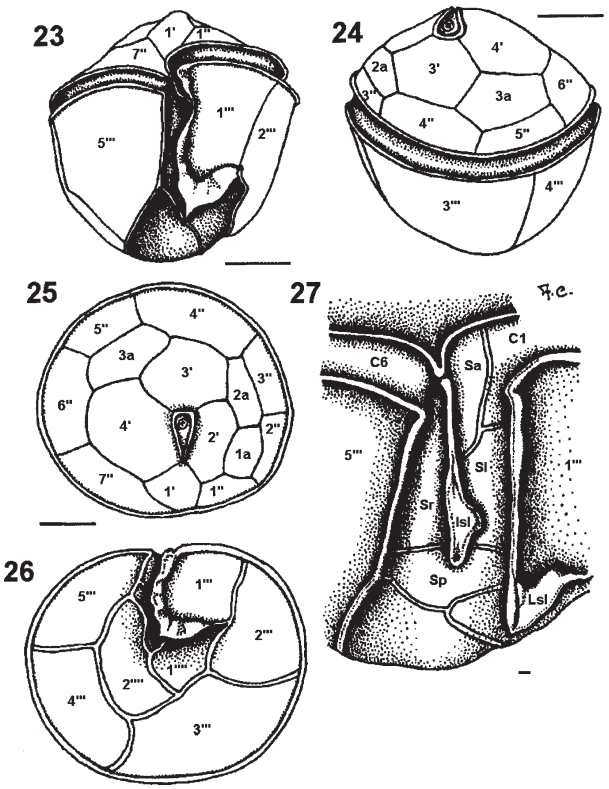


Figs 15–22. Scanning electron micrographs of details of the surface morphology of *Bysmatrum granulosum* sp. nov. Fig. 15. Apical pore complex. Fig. 16. The apical pore is circular with a raised margin (arrow). Fig. 17. Thecal plates showing the lines of small and minute wart-like projection, radiating from apical pore. Fig. 18. Sulcal view. Fig. 19. Cingular view. Cingular plates are striated, constituted by linearly arranged minute wart-like projections. Marginal pores are present on both sides of the cingulum (arrows). Small aligned pores are present along the inside of the cingulum lists (arrowheads). Fig. 20. Thecal plate 3a with transversely striated sutures (arrows). Fig. 21. Details of the thecal plate ornamentation. Arrows indicate large pores. Arrowheads indicate small pores. Fig. 22. Details of the thecal plate ornamentation showing small wart-like projections (arrows). Scale bars represent: Figs 15, 16, 19–22, 1 μm; Figs 17 and 18, 5 μm.

The apical pore complex (consisting of the apical pore P_0 and the canal plate X) is elongate (8–10 μm long) (Figs 7, 15, 16), and slightly polygonal, with margins formed by the raised and overlapping apical borders of the four apical plates (1′–4′) (Figs 13, 15, 16). The apical pore complex is directed toward the ventral centre of the cell, slightly to the cell's right (Figs 13, 25). The P_0 plate is situated between apical plates 2′, 3′ and 4′ (Figs 13, 15, 16, 25). The apical pore is round with a raised margin (Fig. 16). An apical stalk is often observed, formed by extrusion of mucilaginous material from the apical region (Fig. 3).

Arrangement of the epithecal plates is asymmetric. Apical plate 1′ is pentagonal and on the right of the hypotheca. The upper end of this plate touches the canal plate and the lower end touches the anterior margin of the cingulum (Figs 10, 23). Apical plates 2′, 3′ and 4′ are irregularly hexagonal. Apical plate 4′ is the largest, whereas plate 3′ is the smallest (Figs 13, 25). Plate 1a is four-sided, whereas plates 2a and 3a are respectively hexagonal and pentagonal (Figs 13, 24, 25). Plates 1a and 2a have one side in common, whereas plates 2a and 3a are separated from each other by a connection between plates 3′ and 4′ (Figs 13, 24, 25).

The cingulum is well excavated, is located in supraequatorial position, and descends 1–1·5 times its own width (Figs 9, 10). The cingulum is composed of six plates (6c). The right part of plate c1



Figs 23–27. Line drawings of *Bysmatrum granulosum* sp. nov. showing the thecal plate arrangement. Fig. 23. Ventral view. Fig. 24. Dorsal view. Fig. 25. Thecal arrangement of the epitheca. Fig. 26. Thecal arrangement of the hypotheca. Fig. 27. Detail of the sulcal plates (Sa, anterior sulcal plate; Sr, right sulcal plate; Sl left sulcal plate; Sp, posterior sulcal plate; Lsl, left sulcal list; Isl, internal sulcal list). Scale bars represent: Figs 23–26, 10 μm; Fig. 27, 1 μm.

forms part of the sulcus (Fig. 23). Pores are aligned along the inside cingular lists (Fig. 19). Cingular plates seem longitudinally striated because they are ornamented with a linear arrangement of minute wart-like projections (Fig. 19).

The sulcus consists of four plates (Sa, Sp, Sr, Sl) (Fig. 27). It is deep and narrow anteriorly and broad posteriorly.

The sulcus does not reach the antapex, and appears to extend to the two antapical plates 1′′′′ and 2′′′′ (Figs 14, 18). The left sulcal list (Lsl) is large, formed by the right and posterior margin of plate 1′′′, and pointed at its distal end. It covers sulcal plates and, partially, antapical plate 1′′′′. There is an internal sulcal list (Isl) on the right sulcal plate (Sr) (Fig. 27).

The hypotheca of *Bysmatrum granulosum* is composed of five postcingular plates (5′′′) and two antapical plates (2′′′′). Plate 1′′′ is rectangular, and is the smallest postcingular plate. Plates 2′′′ and 5′′′ are triangular and 3′′′ and 4′′′ are quadrangular (Fig. 26). The antapical plates 1′′′′ and 2′′′′ seem to form a part of the sulcus (Figs 14, 18, 26). The plates are ornamented with small wart-like projections, except in their marginal parts. Their margins are bordered by larger wart-like projections with large pores present at their lower margin. No antapical spine is observed in the hypotheca.

B. granulosum is a photosynthetic species containing many golden-brown chloroplasts. These numerous plastids obscure the inner cytoplasmic regions of the cells (Figs 1–6).

B. granulosum was associated with sediment or attached to coral rubble and macroalgal turf surfaces on the reef of St Leu (La Réunion Island) at 1–2 m depth. Dense populations of *B. granulosum* were also observed on the same substrata in the lagoonal waters of La Saline (La Réunion Island). This new species was collected with the potentially toxic dinoflagellates *Gambierdiscus toxicus* Adachi & Fukuyo (Nakajima *et al.*, 1981), *Gambierdiscus yasumotoi* Holmes (1998), *Ostreopsis mascarenensis* Quod (1994), *Ostreopsis siamensis* Schmidt (Usami *et al.*, 1995), *Prorocentrum arenarium* Faust (Ten-Hage *et al.*, 2000*a*), *P. belizeanum* Faust (Morton *et al.*, 1998), *P. borbonicum* Ten-Hage *et al.* (2000*b*), *P. concavum* Fukuyo (Hu *et al.*, 1993) and *P. lima* (Ehrenberg) Dodge (Murakami *et al.*, 1982). Other dinoflagellate species observed with *B. granulosum* in the samples were *Gambierdiscus belizeanus* Faust (1995*b*), *Prorocentrum emarginatum* Fukuyo (Fukuyo, 1981; Faust, 1990), *P. sculptile* Faust (1994), *Sinophysis canaliculata* Quod *et al.* (1999) and *S. microcephalus* Nie & Wang (Nie & Wang, 1944; Faust, 1993).

Discussion

Comparison of the morphology and cellular features of *Bysmatrum granulosum* sp. nov. and the three other *Bysmatrum* species – *B. arenicola*, *B. caponii* and *B. subsalsum* – is made in Table 1.

Cells of *B. granulosum* are larger than those of *B. caponii* (20–34 μm long, 20–34 μm wide) (Horiguchi & Pienaar, 1988*b*) and *B. subsalsum* (32–41 μm long, 31–51 μm wide) (Faust & Steidinger, 1998). *B. granulosum* differs also from other *Bysmatrum* species by the lack of prominent dorsiventral compression (Faust & Steidinger, 1998). Cells of *B. granulosum* are more or less pentagonal as are those of *B. caponii* and *B. subsalsum*; those of *B. arenicola* are ellipsoidal (Horiguchi & Pienaar, 1988*a*). The epitheca of *B. granulosum* is smaller than the hypotheca. Epithecas and hypothecas are equal in size in *B. arenicola* (Horiguchi & Pienaar, 1988*a*), *B. caponii* (Horiguchi & Pienaar, 1988*b*) and *B. subsalsum* (Steidinger & Balech 1977; Faust & Steidinger, 1998).

The apical pore complex of *B. granulosum* is similar in morphology to that of *B. arenicola* (Horiguchi & Pienaar, 1988*a*), *B. caponii* (Horiguchi & Pienaar, 1988*b*) and *B. subsalsum*

Table 1. Comparison of the morphology of the four *Bysmatrum* species

Features	*B. granulosum*	*B. arenicola*[a,c]	*B. caponii*[a,d]	*B. subsalsum*[a,b]
Length (μm)	40–50	40–58	20–34	32–41
Width (μm)	40–46	37–46	20–34	31–51
Shape	Pentagonal	Ellipsoidal	Pentagonal	Pentagonal
Cingulum	Sub-equatorial	Equatorial	Equatorial	Equatorial
Plate ornamentation	Wart-like	Wart-like	Reticulated	Striated
1a morphology	Quadra	Penta	Quadra	Quadra
2a morphology	Hexa	Penta	Hexa	Penta
3a morphology	Penta	Penta	Penta	Penta
Intercalary bands	Finely striated	Smooth	Striated	Striated
Antapical spine	–	–	Present	–
Colour	Golden-brown	Reddish-brown	–	Golden-brown
Pyrenoid	–	Present	–	–
Nucleus	Hypothecal	Hypothecal	Epithecal	Equatorial
Stigma	Present	–	Present	Present
Plastids	Many	Many	Many	Many
Ecology	Epibenthic	Tide-pools	Tide-pools	Epibenthic

[a] According to Faust & Steidinger (1998); [b] according to Steidinger & Balech (1977); [c] according to Horiguchi & Pienaar (1988*a*); [d] according to Horiguchi & Pienaar (1988*b*).
–, not observed.

(Faust, 1996*b*; Faust & Steidinger, 1998); the apical pore complex of *B. granulosum* is the longest (8–10 μm) among the *Bysmatrum* species. The presence of an apical stalk from the apical pore complex of *B. granulosum* may indicate that cells can attach to a substratum, as illustrated for *B. arenicola* (Horiguchi & Pienaar, 1988*b*; Faust & Steidinger, 1998).

The thecal surface of *B. granulosum* is covered by two distinct size classes of small wart-like projections. Wart-like projections are present on the thecal surface of *B. arenicola* (Horiguchi & Pienaar, 1988*a*); the surface morphology of *B. caponii* (Horiguchi & Pienaar, 1988*b*) and *B. subsalsum* (Faust, 1996*b*; Faust & Steidinger, 1998) has a reticulate surface pattern. In all four *Bysmatrum* species, thecal ornamentation is arranged in longitudinal rows radiating from the apical pore.

The thecal arrangement of *B. granulosum* is similar to that of other *Bysmatrum* species: intercalary plates 2a and 3a are separated by the apical plate 3′; this is an important morphological characteristic that distinguishes *Bysmatrum* species from other scripsielloid species (Faust, 1996*b*; Faust & Steidinger, 1998). Plate 1a is quadragonal in *B. granulosum*, *B. caponii* (Horiguchi & Pienaar, 1988*b*) and *B. subsalsum* (Steidinger & Balech, 1977; Faust, 1996*b*; Faust & Steidinger, 1998), whereas it is pentagonal in *B. arenicola* (Horiguchi & Pienaar, 1988*a*). Plate 2a is hexagonal in *B. granulosum* and *B. caponii* (Horiguchi & Pienaar, 1988*b*), but pentagonal in *B. arenicola* (Horiguchi & Pienaar, 1988*a*) and *B. subsalsum* (Steidinger & Balech, 1977; Faust 1996*b*; Faust & Steidinger, 1998).

Intercalary bands of *B. granulosum* are finely striated; those of *B. caponii* (Horiguchi & Pienaar, 1988*b*) and *B. subsalsum* (Faust, 1996*b*; Faust & Steidinger, 1998) are deeply striated whereas those of *B. arenicola* are smooth (Horiguchi & Pienaar, 1988*a*).

Bysmatrum granulosum sp. nov. is a tropical benthic dinoflagellate, as are the three other *Bysmatrum* species (Faust & Steidinger, 1998).

The description of this new species contributes, with our previous recent work (Quod *et al.*, 1999; Ten-Hage *et al.*, 2000*b*), to knowledge of the diversity of benthic dinoflagellates from the south-western Indian Ocean.

Acknowledgements

We thank Dr M.A. Faust, Smithsonian Institution, USA, and Dr B. de Reviers, Muséum National d'Histoire Naturelle, France, for their advice. We also wish to thank G. Mascarell and M. Dumont for their technical support. L.T.H. was supported by a fellowship from Ministère de l'Education Nationale, de la Recherche Scientifique et de la Technologie. This work was carried out in part with the support of Conseil Régional de La Réunion.

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