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Gymnodinium natalense sp. nov. (Dinophyceae), a new tide pool dinoflagellate from South Africa

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A new dinoflagellate, *Gymnodinium natalense* Horiguchi et Pienaar (Gymnodiniales, Dinophyceae) is described from a tide pool at Amanzimtoti, Natal south coast, South Africa. The organism possesses typical dinoflagellate organization. The epicone is conical, while the hypocone is almost trapezoidal with a moderate depression at the antapex. The cingulum is left-handed, well excavated and is displaced 1/2–1/1 of its own width. The sulcus is short, deep and reaches antapex. The chloroplast is single, green to yellowish-brown and is connected to the pyrenoid which is located in the epicone. The dinokaryotic nucleus is ovoidal and is located in the upper part of the hypocone or in the center of the cell. The eyespot is rectangular in ventral view, C-shaped in apical view and bright red in color. It is extraplastidial and possesses unique ultrastructural features. It is composed of several layers which contain many regularly arranged rectangular crystalline structures. No trichocyst has been observed, although the bottle shaped mucocysts have been observed in the peripheral region of the cell. Only the asexual reproduction has been observed. Two motile cells are formed within the parental amphiesma by binary fission.

Key Index Words: Dinophyceae—Gymnodinium natalense *sp. nov.*—*Gymnodiniales*—*South Africa*—*taxonomy*—*tide pool.*

Some dinoflagellates are known to inhabit tide pools and produce dense blooms under favorite conditions (Hirano 1967; Horiguchi and Chihara, 1983; Horiguchi and Chihara, 1987; Lombard and Capon, 1971; Taylor, 1983). During the course of our studies on tide pool and sand-dwelling dinoflagellates of the Natal coast, South Africa, we often encountered such dinoflagellates. The dinoflagellate we describe in this paper is one of them. It is a small unarmored dinoflagellate and produces dense bloom in tide pools in summer. It is also characterized by an eyespot with unusual ultrastructure. Since the eyespot possesses many unusual features, it deserves detailed description and therefore, the ultrastructure and ontogeny of the eyespot will be published elsewhere (Horiguchi and Pienaar, 1994).

Materials and Methods

The organism used in this study was collected from a tide pool at Amanzimtoti, Natal south coast, South Africa on 18 August 1986. At the time of collection, the dinoflagellate produced dense bloom in the tide pool.

For transmission electron microscopy, the cells were processed as described before (Pienaar and Aken, 1985) and for scanning electron microscopy, the same technique which was employed for *Scrippsiella arenicola* Horiguchi and Pienaar (1988) was used. When the samples were fixed for transmission electron microscopy, most of the cells were in the non-motile stage and therefore, the cells in sections were often observed to be enclosed by the pellicular layer of the parental amphiesma. Observations were made using a JEOL 100CX transmission electron microscope and a HITACHI S-570 scanning electron microscope.

Description

Class: Dinophyceae Fritch
Order: Gymnodiniales Lemmermann
Family: Gymnodiniaceae Lankester

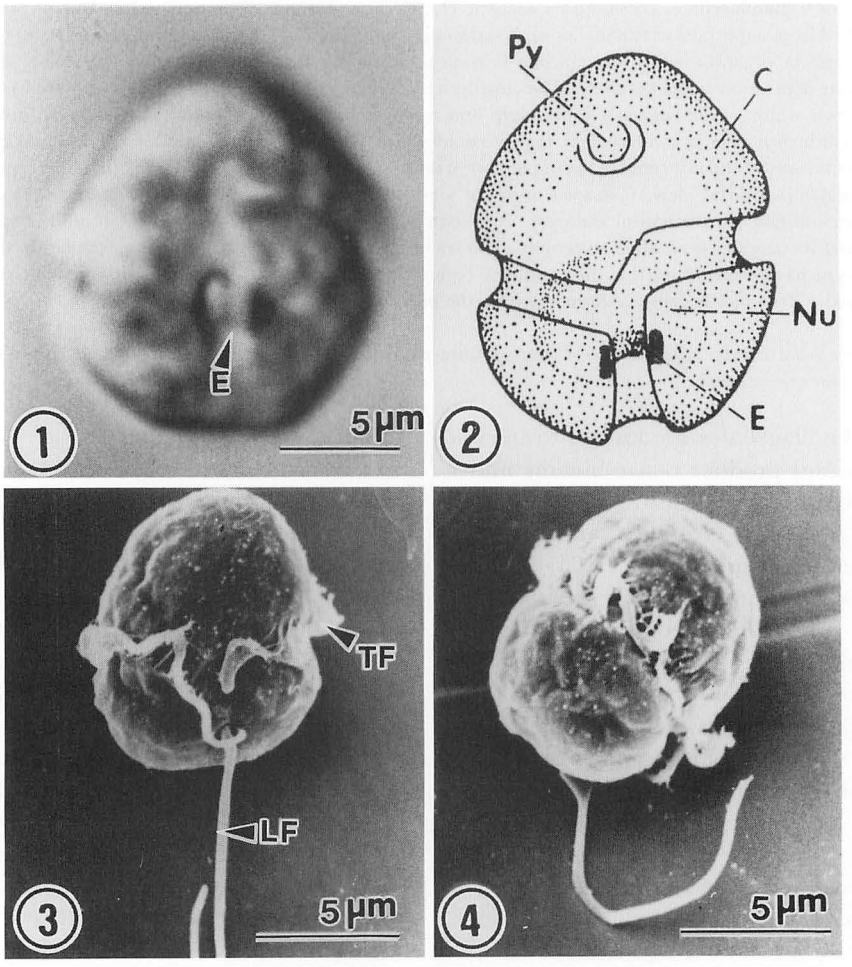
***Gymnodinium natalense* Horiguchi et Pienaar, sp. nov.**

Cellula ex epicono et hypocono constans, 14.4–18.0 μm longa, 9.9–12.6 μm lata; epiconus conicus; hypoconus fere trapezoideus cum moderate depresso antapice; cingulum bene excavatus, 1/2–1/1-plo latitudine cinguli descendens; sulcus brevis, profundus, ad antapicem attingens; nucleus dinokaryotics, ovoideus, in supero parte hypoconi vel medio cellula situs; chloroplastus viridis vel luteibrunneus, in peripheria situs; pyrenoides sphaerica, per vaginam amylorum obtecta, saepe partim in chloroplasto inclusa; stigma rectangulare a ventre visus, C-forme a apice visus, in sulco situm; planta marina.

Holotype: Figure 2.

Type locality: Amanzimtoti, Natal south coast, Republic of South Africa.

Cell consisting of epicone and hypocone,



Figs. 1–4. *Gymnodinium natalense* Horiguchi et Pienaar, sp. nov. Fig. 1. Light micrograph showing live cell. Fig. 2. Line drawing, showing ventral side of the cell (Holotype). Fig. 3. SEM. Ventral view. Fig. 4. SEM. Dorsal view.

Abbreviations used in the figures (1~13). Ac: accumulation body; C: chloroplast, CM: cytoplasmic membrane; E: eyespot, LF: longitudinal flagellum, M: mucocyst; Nu: nucleus, OM: outer membrane; OPM: outer plate membrane; P: pellicle; Py: pyrenoid; S: starch grain; TF: transverse flagellum

14.0-18.0 μm in length, 9.9-12.6 μm in width; epicone conical; hypocone trapezoidal with moderately depressed antapex; cingulum well excavated, displaced 1/2-1/1 of its own width; sulcus short, deep, reaching antapex; nucleus dinokaryotic, ovoidal, located in upper part of hypocone or middle of cell; chloroplast green or yellowish brown, peripherally situated; pyrenoid spherical, surrounded by starch sheath, partially embedded in chloroplast; eyespot rectangular in ventral view, C-form in apical view, situated at sulcus; organism marine.

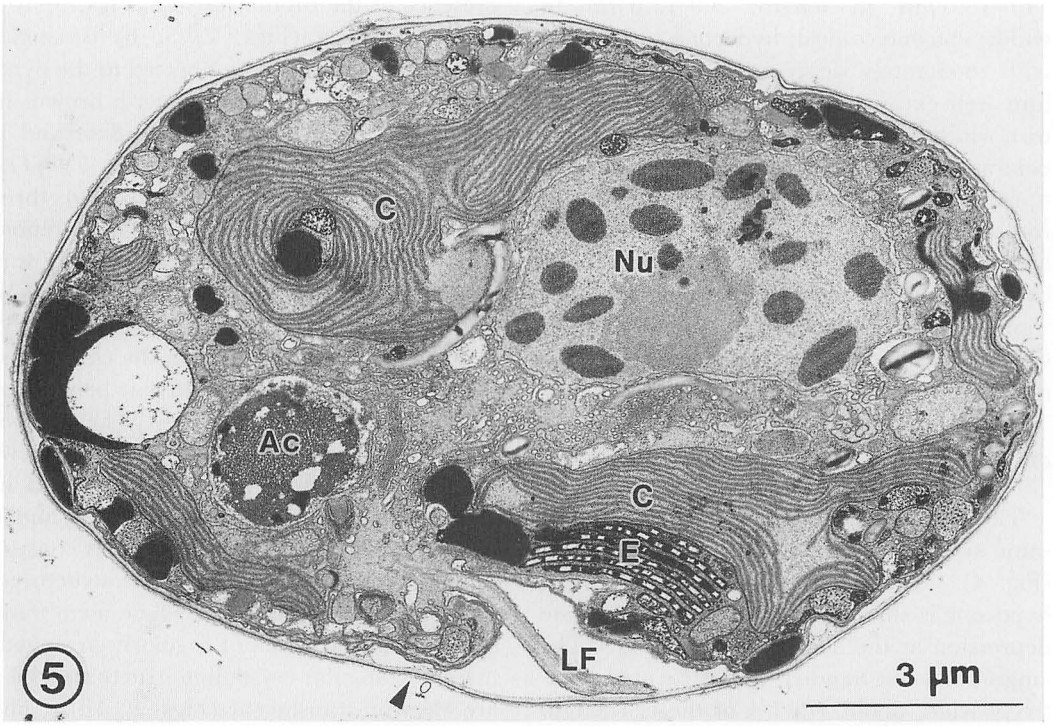
Observations

The epicone and the hypocone are almost same size when viewed from the dorsal side (Fig. 4). The epicone is conical, while the hypocone is almost trapezoidal with moderate depression at the antapex (Figs. 1, 2). The cingulum is left-handed, well excavated, relatively wide, about 1/5-1/4 of the cell length and is displaced about 1/2-1/1 of its own width. The sulcus is short, deep and reaches to the antapex, but does not invade into the epicone (Fig. 2). The SEM photograph (Fig. 3) reveals that the transverse and the longitudinal flagella emerge from the different pores. The distance between these two pores is about 1.3 μm. The transverse flagellum shows typical semihelical nature (Figs. 3, 4). No peduncle has been observed.

The nucleus is typical of dinokaryotic (Fig. 5). It is ovoidal and is located upper part of the hypocone or in the center of the cell (Fig. 2). The mitochondrial profiles reveal presence of the tubular cristae (Figs. 5, 10). The chloroplast (Figs. 2, 5, 6) is single, peripherally arranged, connected to the pyrenoid and is green to yellowish-brown in color. It is typical of dinoflagellate and is bounded by a triple membrane (Fig. 7). The lamella typically consists of two to three appressed thylakoids (Fig. 7). The pyrenoid (Figs. 2, 6) is single, spherical, partially surrounded by starch sheaths and is located in the epicone. The pyrenoid matrix is partly, or often mostly, embedded in the chloroplast (Fig. 6). The pyrenoid matrix is penetrated by single thylakoids (Fig. 6). The eyespot (Figs. 1, 2) is rectangular in ventral view, C-shaped in apical view and bright red in color. It is extraplastidial (Figs. 9. 10) and is situated at the sulcus (Figs. 1, 2). It consists of several layers of the crystalline structures. Each layer is enclosed by a single membrane (Fig. 10) and consists of regularly arranged, many rectangular crystalline structures which are electron-translucent (Figs. 9, 10). The dictyosomes are arranged in the circular manner in the center of the cytoplasm (Fig. 13). The fine fibers which are thought to be the flagellar hairs have been observed in the endoplasmic reticulum (Fig. 12). The amphiesma consists of, from outside to the cytoplasm, the outer membrane, the outer plate membrane, the pellicle and the cytoplasmic membrane (Fig. 11). No thecal plates have been observed. Although the trichocysts are absent, the trichocyst-like structures have been observed in the peripheral region of the cell (Fig. 11). They are thought to be the mucocyst. The mucocysts

Figs. 5-8. *Gymnodinium natalense* Horiguchi et Pienaar, sp. nov. Fig. 5. Longitudinal section of a cell, showing general arrangement of the organelles. Note that the cell is fully developed motile cell prior to release and is still enclosed by the parental amphiesma (arrowhead). Fig. 6. Detail of the chloroplast and the pyrenoid. Fig. 7. Close up of the chloroplast, showing triple membrane chloroplast envelope and lamellae consisting of three appressed thylakoids. Fig. 8. Nonmotile cell containing two daughter cells. Note that cell division takes place within the parental amphiesma.

Figs. 9-13. *Gymnodinium natalense* Horiguchi et Pienaar, sp. nov. Fig. 9. Longitudinal section through the eyespot. The eyespot consists of six layers of crystalline structures. Fig. 10. Tangential section through the eyespot, showing many rectangular crystalline structures. Note that the crystalline layer is enclosed by a single membrane (arrowhead). The mitochondrial profile (upper left corner) reveals tubular cristae. Fig. 11. Bottle-shaped mucocysts and detail of the amphiesma. The amphiesma consists of outer membrane, outer plate membrane, pellicle and cytoplasmic membrane. Arrowhead indicates pellicular layer of the parental amphiesma. Fig. 12. Flagellar hair produced in ER. Fig. 13. Dictyosomes which are arranged in a circular manner in almost center of cell (arrowheads).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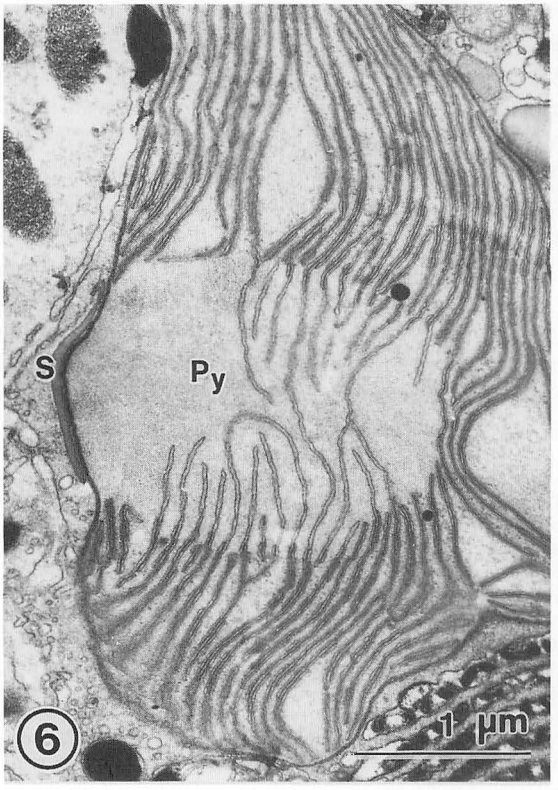
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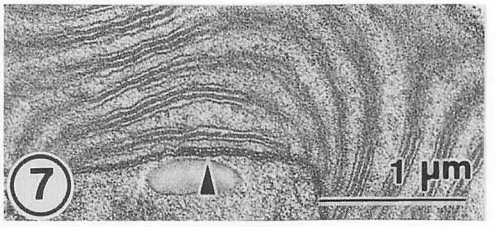
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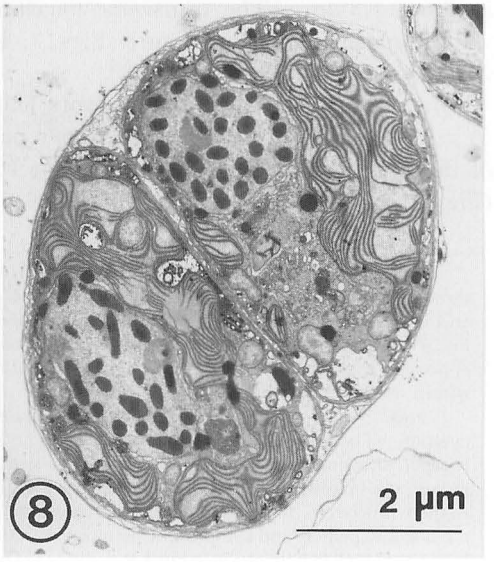
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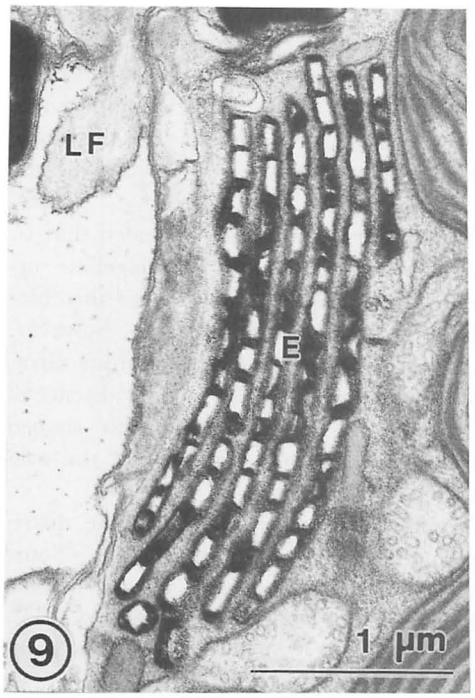
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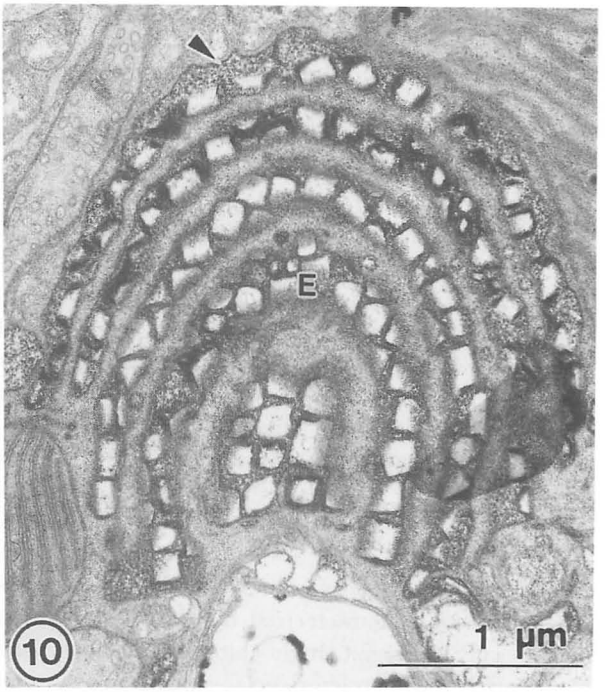
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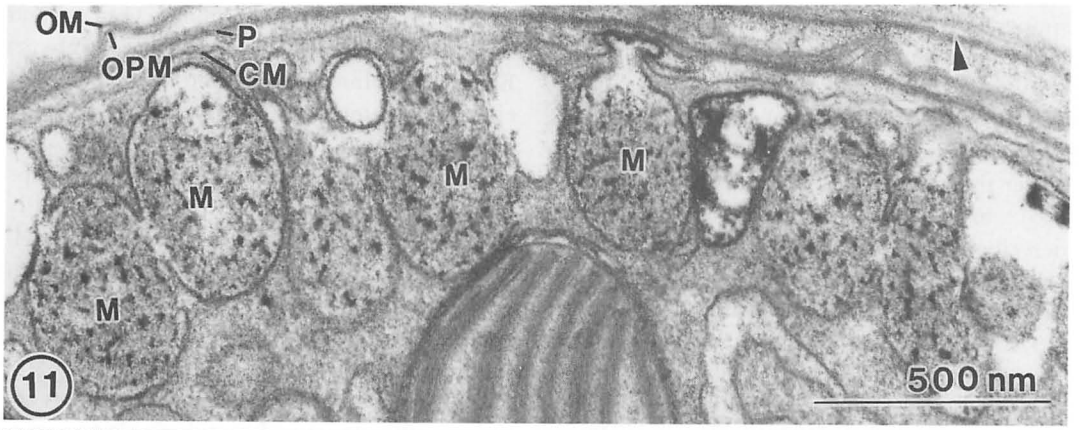
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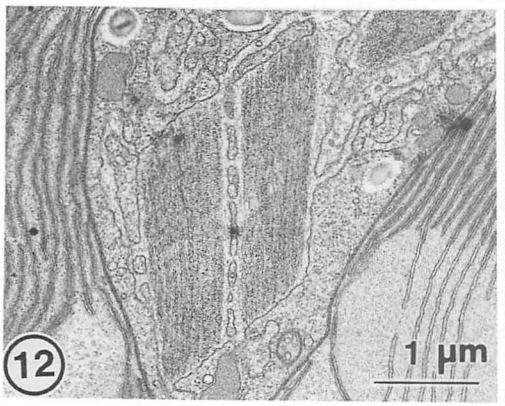
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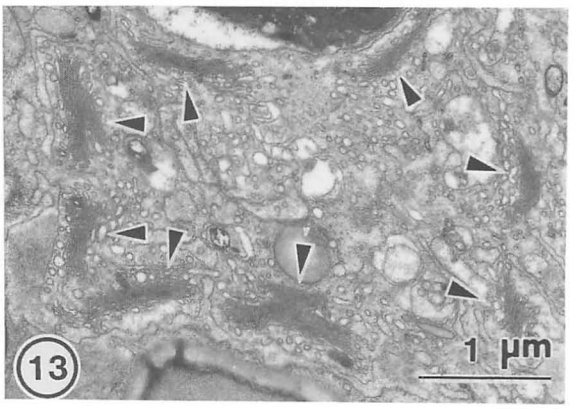
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are single membrane-bounded, bottle-like in shape and contain moderately electron-dense fibrous material with scattered dark dot-like materials (Fig. 11). The accumulation body has often been observed somewhere in the cytoplasm (Fig. 5).

Only asexual reproduction has been observed. *G. natalense* alternates motile stage with nonmotile stage in its cell cycle. The nonmotile cell is spherical to ovoidal and neither the cingulum or the sulcus is visible. Cell division is confined to the nonmotile phase and is accomplished by the binary fission of the protoplast within the parental amphiesma (Fig. 8), resulting in the formation of two motile cells.

Discussion

G. natalense is characterized by small size, peripherally arranged single chloroplast with a pyrenoid and characteristic C-shaped eyespot. Because of the cell shape and its unarmored nature, it is evident that the species belongs to the genus *Gymnodinium* of the order Gymnodiniales. Many of the species in the genus *Gymnodinium* are relatively large, viz. larger than 50 μm and small forms (20 μm or less) are relatively rare. Among the described species, the following species have somewhat similar cell shape and cell size to the present species: *G. albulum* Lindemann (1928), *G. arcticum* Wulff (1916), *G. cassiei* Norris (1961), *G. halophilum* Biecheler (1952), *G. incoloratum* Conrad et Kufferath (1954), *G. lacustre* Schiller (1933), *G. pyrenoidosum* Horiguchi et Chihara (1987) and *G. simplex* (Lohmann) Koffoid et Swezy (1921). However, *G. natalense* can be distinguished from these species for the following reasons: *G. albulum* possesses no eyespot, *G. arcticum* has many chloroplasts whose shape is distinctly elliptical, *G. cassiei* has nucleus located in the epicone and has chloroplasts with numerous pointed arms that radiate from a center located in the hypocone, *G. halophilum* also possesses a nucleus in the epicone and apical groove called an "acrobase" (Biecheler, 1952), *G. incoloratum* has no chloroplast, *G. lacustre* possesses a narrow sulcus which does not reach antapex, *G. pyrenoidosum* possesses different type of eyespot and *G. simplex* has four chloroplasts. Based on the differences described above, it is obvious that this dinoflagellate belongs to a new species of the genus *Gymnodinium*.

The ultrastructural study revealed that *G. natalense* possesses typical dinoflagellate organelles, including the nucleus and the chloroplast. It should be pointed out, however, that *G. natalense* possesses a few unique ultrastructural features. These include absence of the trichocysts, possession of bottle shaped mucocysts and unique structure of the eyespot.

The trichocyst is ejectile organelle distributed in the majority of dinoflagellates. Some dinoflagellates are, however, known to lack trichocysts. These include *Prorocentrum cassbicum* (Woloszynska) Dodge (unpublished observation), *Dinophysis acuminata* Claparede et Lachmann, *D. fortii* Pavillard (Lucas and Vesk 1990), *Actiniscus pentasterias* (Ehrenberg) Ehrenberg (Hansen 1993) and the present species. It is interesting to note that all of these five species which lack trichocysts possess mucocysts, although the structure and shape of the mucocysts of each species are slightly different from each other. These dinoflagellates belong to the taxonomically distant groups and therefore, the absence of trichocysts and the gain of mucocysts seem to be the result of parallel evolution.

The eyespot of *G. natalense* possesses very unique ultrastructural features. It is extraplastidial and is composed of several layers of crystalline structures. This type of eyespot has never been reported for any other groups of algae, including dinoflagellates (see Horiguchi and Pienaar 1994). It is of great interest to investigate the eyespot of various species of the dinoflagellates in order to elucidate whether *G. natalense* is truly an only dinoflagellate which possesses this type of unique eyespot or not.

The mode of cell division of *G. natalese* is similar to those of other tide pool bloom-forming dinoflagellates, such as *Gymnodinium*

pyrenoidosum (Horiguchi and Chihara 1987) and *Scrippsiella hexapraecingula* (Horiguchi and Chihara 1983).

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堀口健雄*・R. N. Pienaar**：南アフリカのタイドプールから採集された渦鞭毛藻の一新種 *Gymnodinium natalense*（渦鞭毛藻綱）について

南アフリカ，ナタール地方南部のアマンチムトーティーのタイドプールから新種の渦鞭毛藻 *Gymnodinium natalense*（渦鞭毛藻綱，ギムノディニウム目）を記載した。本種は典型的な渦鞭毛藻の外部形態を呈する。上殻は円錐形で，下殻はほぼ台形，後端にややくぼみをもつ。横溝は左巻きで深く，溝の幅のおよそ 1/2–1/1 の分のずれを生じる。縦溝は短いが深く，細胞後端に達する。葉緑体は 1 個で緑色または黄褐色を呈し，ピレノイドに結合している。ピレノイドは上殻中に位置する。渦鞭毛藻核は楕円形で，下殻上部または細胞中央に存在する。明るい赤色の眼点は正面観では長方形，頂面観では C-字型を呈する。眼点は葉緑体とは独立して存在し，特徴的な微細構造をもつ。すなわち，規則正しく並んだ多数の直方体の結晶様構造から成る，いくつかの層から出来ている。トリコシスト（毛胞）は見られないが，細胞周辺部にはびん型のミューコシスト（粘液胞）が観察される。本種では無性生殖のみが観察された。すなわち細胞分裂は，親の細胞の細胞外被中で細胞質が 2 分裂し 2 個の遊走細胞を形成するという様式を示す。(*060 札幌市北区北10条西 8 丁目　北海道大学理学研究科生物科学専攻，** Department of Botany, University of the Witwatersrand, Private Bag 3, P. O. Wits, 2050 Republic of South Africa)

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