Jpn. J. Phycol. (Sôrui) 39: 227–237, September 20, 1991

Dinoflagellates collected from aquaculture ponds in southern Taiwan

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Su, H.-M. and Chiang, Y.-M. 1991. Dinoflagellates collected from aquaculture ponds in southern Taiwan. Jpn. J. Phycol. 39: 227–237.

Five species of photosynthetic dinoflagellates, *Prorocentrum minimum, Gyrodinium instriatum, Pyrophacus horologium, Gonyaulax verior*, and *Alexandrium tamarense*, collected from aquaculture ponds in southern Taiwan where a PSP incident due to ingestion of cultured purple clams occurred and caused the death of two men, are described with some taxonomical considerations. *A. tamarense*, which previously has been reported to be the first toxic strain isolated from tropical Pacific waters, differs from those reported from temperate waters in having the ability to grow at very low salinity and high temperature, and small size (11–34 μm).

Key Index Words: Alexandrium tamarense—*dinoflagellate*—Gonyaulax verior—Gyrodinium instriatum—*phytoplankton*—Prorocentrum minimum—Pyrophacus horologium—*Taiwan*.

Dinoflagellates are widely recognized to produce "blooms" or "red tides", and some of them are found associated with the production of toxins, resulting in fish kills and mortality of other marine organisms (Baden 1983, Carmichael 1986) Those toxins also can be accumulated by filter-feeding shellfish to cause paralytic shellfish poisoning (PSP) to the mankind (Prakash 1963, Prakash and Taylor 1966, Wood 1968). As reported from various parts of the world (Taylor 1984), a PSP incident due to ingestion of cultured purple clams collected from Tungkang, Pingtung Hsien, Taiwan (Fig. 1) occurred on Jan. 1, 1986 and caused the death of two men (Hwang *et al* 1987).

The incident brought about our interest to study if there were any toxic dinoflagellates growing in the ponds of that area. Between 1986 and 1987, we made extensive investigations on the occurrences of dinoflagellates in aquaculture ponds at Tungkang area, and isolated five species of photosynthetic dinoflagellates. This paper presents the morphological and ecological characteristics of these algae with taxonomical considerations.

Unialgal cultures of these dinoflagellates are kept both in the Institute of Oceanography, National Taiwan University, Taipei and in Tungkang Marine Laboratory, Tungkang.

Materials and Methods

Dinoflagellates were collected from shrimp ponds and/or crab ponds in Tungkang area, but special attention was paid to those ponds surrounding the one from where toxicated clams were harvested (that pond was closed after the PSP event) (Fig. 1). The algae were isolated with micropipette method (Hoshaw and Rosowski 1973). Their clonal cultures were grown in "K" medium (Keller and Guillard 1985) with salinity 15 ppt, and were maintained at 25±1°C, under a 12 : 12 LD cycle at 80 $\mu E/m^2/s$ provided by cool-white fluorescent lamps. The growth rates (K) of *A. tamarense* grown in 3-45 ppt salinity and at 16-35°C were measured to find the optimum conditions.

Both living and Lugol's solution fixed cells were observed with the optical LM system filled with phase contrast and Nomarski interference contrast optics. Thecal plates were

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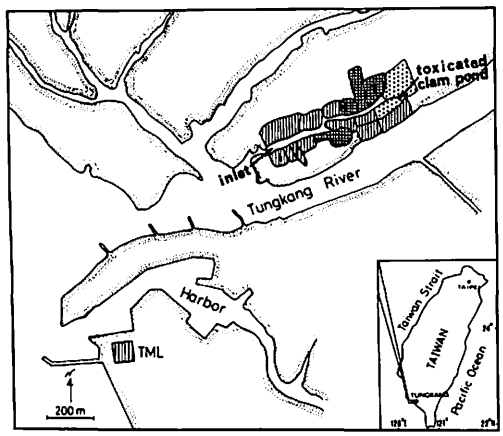

Fig. 1. Map of sampling place. ▦ Purple clam pond, ▦ crab pond, ▨ shrimp pond. TML: Tungkang Marine Laboratory.

dissociated with a slight pressure on the cover glass. For scanning electron microscopy (SEM), cells were either fixed in 1% OsO_4, or fixed in 0.5% GTA and 0.2% OsO_4 simultaneously, or double fixed in 2.5% GTA and 1% OsO_4, or fixed in Lugol's solution with the culture medium as buffer solution. The fixed cells were transferred to a capsule with a Nuclepore filters (8 μm), and then washed and dehydrated in a graded acetone series. After critical point dry, the material was shadowed with gold and viewed with a scanning electron microscope (Hitachi model S-520).

Results and Discussion

***Prorocentrum minimum* (Pavillard) Schiller** (Figs. 2-9)

Toriumi 1980, p. 107, Figs. 4, 10. Dodge 1982, p. 35, pl. I f, g.

Cells are about 14 to 20 μm long and 14 to 18 μm wide (mostly 15 to 16 μm) and ovate, triangular and sometimes heart-shaped in valve view (Figs. 2, 3). Young cells are flattened, while older cells are rounded. Cells develop, with age, a widely developed intercalary bands (Figs. 4, 5). The posterior end of the cell is usually round and the anterior one truncates with a very slight depression. The surface of the valve is covered evenly with many small, pointed spines (Figs. 2, 3, 4, 5). However, on the intercalary bands, the spines occur in rows lying perpendicular to the anterior-posterior axis (Figs. 4, 5) except in the apical area where the cell surface appears smooth (Figs. 5, 6). Trichocyst pores are mainly located around margin of the valves (Figs. 2, 3, 4). There are two unequally sized pores located at the cell's anterior end (Figs. 3, 5, 6, 7). The larger one has a oblong shape confined by a collar-like ridge. The smaller one has a circular shape. A forked structure (as) arises between the two pores (Figs. 5, 6) and is seen as a single spine (as) at the anterior end of the cell from the valve view (Fig. 2). The "apical tooth" (at) is a curved (from the valve view, Fig. 6) or a straight (from the side view, Fig. 2) bilaminar structure, which arises from a part of the edge of the smaller pore (Fig. 6). Generally, two kinds of flagella arise from the apical area of the cell (Fig. 8), one is thread-like and long (1f), the other is hemi-helical and undulate (tf). The latter rounds transversely and in an anti-clockwise direction to the anterior-posterior axis of the cell. Although it is not shown in Fig. 8 that there two flagella emerge from the larger or the smaller pore, only one

Figs. 2–9. *Prorocentrum minimum*. Fig. 2. Valve view. Showing surface spines, apical spine (as), apical tooth (at) and trichocyst pores (tp) on the border (SEM). Fig. 3. Valve view. Showing the large (l) and small (s) anterior pores. Fig. 4. Side view. Showing the straight and striate intercalary bands, and surface spines distributed evenly in the valve surface (SEM). Fig. 5. Apical view. Fig. 6. Enlargement of Fig. 5. Showing the shape and structure of large (l) and small (s) anterior pores, forked spine (as) and apical tooth (at) (SEM). Fig. 7. Valve view. Showing one longitudinal flagellum extending from the flagellar pore. Fig. 8. Apical view. Showing longitudinal (lf) and transverse flagella (tf) (SEM). Fig. 9. Apical view. Showing two transverse flagella (tf) emerging from a flagellar pore (SEM).
(Figs. 2–34, All scales=5 μm except where indicated)

Figs. 10–13. *Gyrodinium instriatum*. Fig. 10. Ventral view. Showing the contour of the cell and a large nucleus (N) in the anterior center of epicone, and many chromatophores. Fig. 11. Ventral view. Showing the girdle, sulcus and one longitudinal flagellum (SEM). Fig. 12. Dorsal view. Showing the transverse flagellum (tf) and the excavated antapex sulcus (SEM). Fig. 13. Ventral view. Showing the apical groove (ag) (SEM).

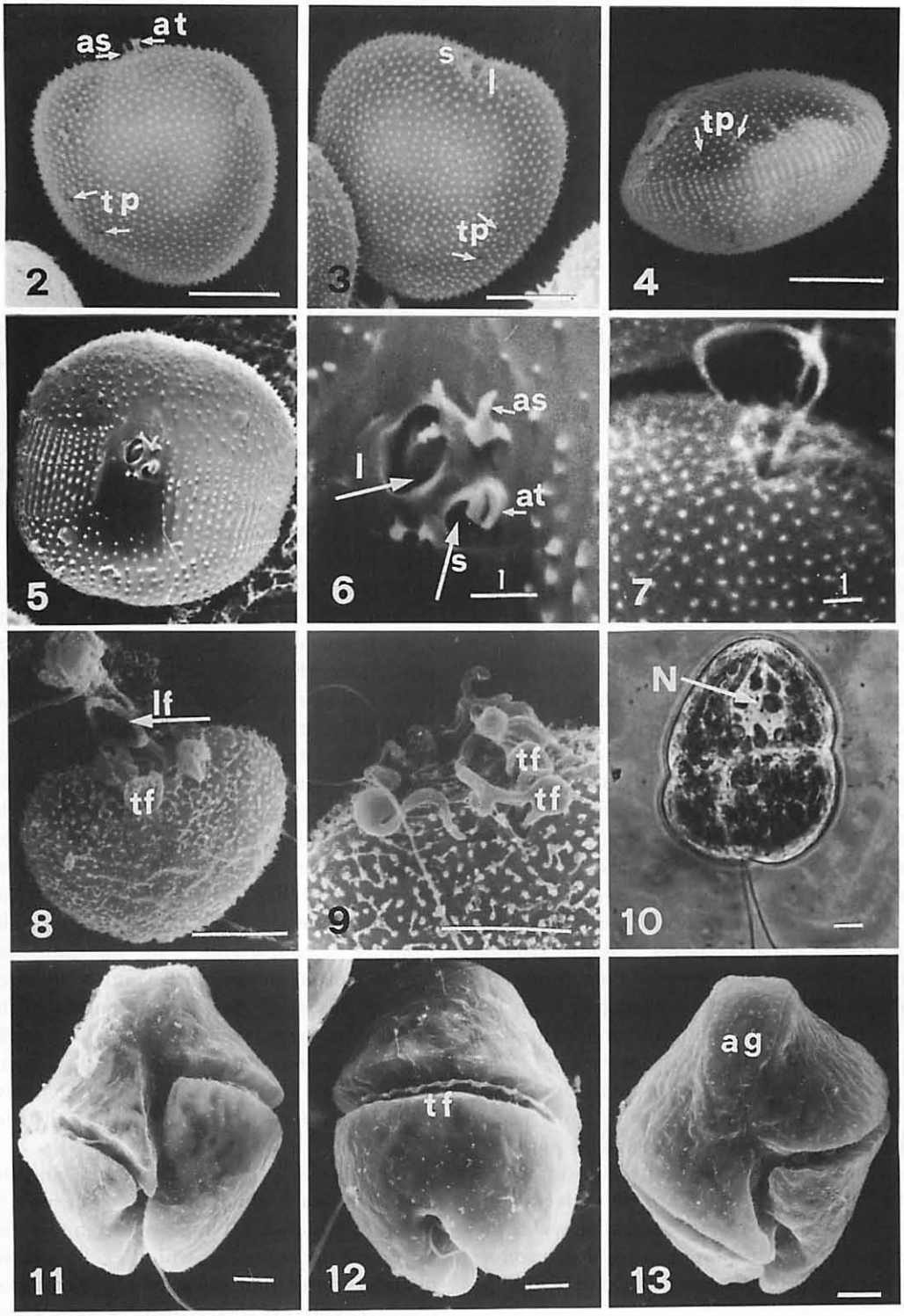
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Table 1. Occurrence of dinoflagellates and pond water parameters during 1986–1987.

Species	Month of occurrence	Maximum densities (cells/ml)	Water temperature (°C)	Salinity (ppt)	pH	D.O. (ppm)	Transparency (cm)
Prorocentrum minimum	Nov.–June	47,000	21.6–30.0	10–34	7.50–7.92	7.2–16.7	29–50
Gyrodinium instriatum	Oct.–June	1,700	17.9–30.9	10–25	7.50–8.70	3.8–16.7	20–70
Pyrophacus horologium	Oct.–Dec. Mar.–July	60	21.6–30.9	10–19	7.51–8.70	3.8–16.7	30–70
Gonyaulax verior	Nov.–July	320,000	17.9–30.9	10–25	7.51–8.70	4.1–16.7	20–68
Alexandrium tamarense	Nov.–Apr.	1,600	17.9–30.0	10–19	7.51–8.70	4.1–16.7	25–68

longitudinal flagellum (Fig. 7) or two transverse flagella (Fig. 9) emerge from a pore, which is the larger or the smaller cannot be confirmed in figures.

This alga appeared from November to June of next year (Table 1) in the area. Blooms with cell densities of more than 10,000 cells/m*l* were found in ponds with salinity 12 ppt and 32 ppt in January and April respectively. This species has been reported in Japan, the west coast of U.S.A., Gulf of Mexico, Caspian Sea, Mediterranean sea and Sargasso Sea and it often forms blooms.

Our specimens resemble *P. minimum* described by Honsell and Talarico (1985), Toriumi (1980) and Loeblich *et al* (1979) and *P. mariaeebouriae* (Faust 1974), which is the synonym of *P. minimum* (Toriumi 1980), in structure of cell surface and anterior pores, cell shape and size. Loeblich *et al.* (1979) and Croome and Tyler (1987) showed two longitudinal flagella of *P. minimum, P. rhathymum, P. triestinum, P. playfairi* and *P. foveolata* emerging from a flagellar pores. While Honsell and Talarico (1985) reported both transverse and longitudinal flagella emerging from the same pore. We found only one longitudinal flagellum emerging from a pore (Fig. 7), or two transverse flagella emerging from a pore (Fig. 9). Therefore, the pore from which both flagella emerge is the flagellar pore and the other one is auxiliary pore as indicated by Loeblich *et al.* (1979). The cells with two longitudinal or transverse flagella may be the results of sexual fusion or asexual division as that reported in *P. micans* (Soyer *et al.* 1982).

***Gyrodinium instriatum* Freudenthal and Lee** (Figs. 10-14)

Freudenthal and Lee 1963, p. 183, Figs. 8-17; Fukuyo 1980, p. 206, pl. 1 Figs. 1-14; Takayama 1985, p. 130, pl. I Fig. 9.

Cells are ovoid 34–56 μm (commonly 40–47 μm) in length and 22–40 μm (mostly 30–37 μm) in width. Epicone is tapering gently toward truncate apex and sometimes slightly dorsoventrally compressed (Figs. 10, 11). Hypocones is also tapering gradually to a moderately sulcus-grooved antapex. Girdle is narrow, deep, and its right end bends sharply and descends about 1/3 the body length posteriorly (Fig. 11). Sulcus projects anteriorly into epicone, joints the apical groove (Fig. 11), and extends posteriorly into a moderately excavated antapex, continuing across it to dorsal sulcal side (Fig. 12). The apical groove extends to the left side of the apex, crossing the dorsal surface to turn downward and then upward to the right side of its proximal end (Figs. 13, 14). The longitidual flagellum extends about a body length or less from the antapical end, while the transverse one is located in the girdle and terminated slightly short of the girdle length. The nucleus is large, spheroidal and centrally located in anterior epicone (Fig. 10). Chromatophores are many, ochre, elongate, radiating toward the center of the cell (Fig. 10).

This species is the most common and the most abundant dinoflagellates occurred in ponds of this area. It appeared from October to June of the next year, with the cell densities of always more than 100 cells/m*l*. But it may reached as high as 1000 cells/m*l* or more sometimes (Table 1). This alga was also found

blooming in Inland Sea of Japan (Fukuyo 1980) and New York waters of U.S.A. (Freudenthal and Lee 1963).

Our specimens of this alga fit very well with the features of *G. instriatum* described by Freudenthal and Lee (1963), Fukuyo (1980) and Takayama (1985) in the shape and structure of apical groove, girdle displacement of 1/3 body length and the extension of sulcus to epicone and hypocone. The differences among *G. instriatum, G. ovoidium, G. fissum, G. striatissimum, G. uncatenum* and *G. lebourae* have been discussed by Freudenthal and Lee (1963). They indicated that *G. instriatum* differs from *G. fissum* by the absence of pellicular striations in the former, and is dissimilar to *G. fissum* and *G. striatissimum* in absence of similar cytoplasmic inclusion. This species is also different from *G. lebourae* due to smaller size of the latter (15 μm). Although *G. instriatum* resembles *G. uncatenum* very closely in the ventral aspect and deeply excavated antapex (Coats *et al.* 1984, Figs. 8, 10), they differ from each other in that the former is less dorsoventrally compressed, while the latter flattens laterally.

***Pyrophacus horologium* Stein** (Figs. 15-22)

Steidinger and Davis 1967, Figs. 1-5; Wall and Dale 1971, p. 234, Figs. 31-37, Dodge 1982, p. 144, Figs. 17A, B.

Cells are 36-74 μm in transdiameter. Theca are oblate, discoidal, and almost circular in anterior and posterior views (Fig. 15), but biconvex in dorsoventral and lateral views (Fig. 16). Both epitheca and hypotheca are convex, usually the latter is slightly flattened. Girdle is equatorial and with lists (Figs. 16, 17). Thecal plates are smooth and with many trichocyst pores (Fig. 17). Plate formula is po (apical pore plate), 5-6′, 0-1a, 7-10″, 9c, 8-10‴, 0-1p, 3-5⁗, commonly is 5′, 0, 9″, 9c, 9‴, 1p, 3⁗ (Figs. 18-21). Two spores produced asexually in theca are observed in Fig. 22.

This cosmopolitan dinoflagellates occurred frequently but less abundantly than the other species of dinoflagellates in cell number in this area (Table 1).

This species can be recognized easily by its clam-like shape and many easily recognized plates. Our specimens of this alga fit clearly with the characteristics of *P. horologium* as described by Steidinger and Davis (1967), Wall and Dale (1971). The plate tabulation which may vary in some ranges in species of the genus *Pyrophacus*, as reported by Wall and Dale (1971), is also observed in our specimens (Fig. 19 with 5′, 9″, while Fig. 20 with 6′, 10″ plates in epitheca).

***Gonyaulax verior* Sournia** (Figs. 23-25)

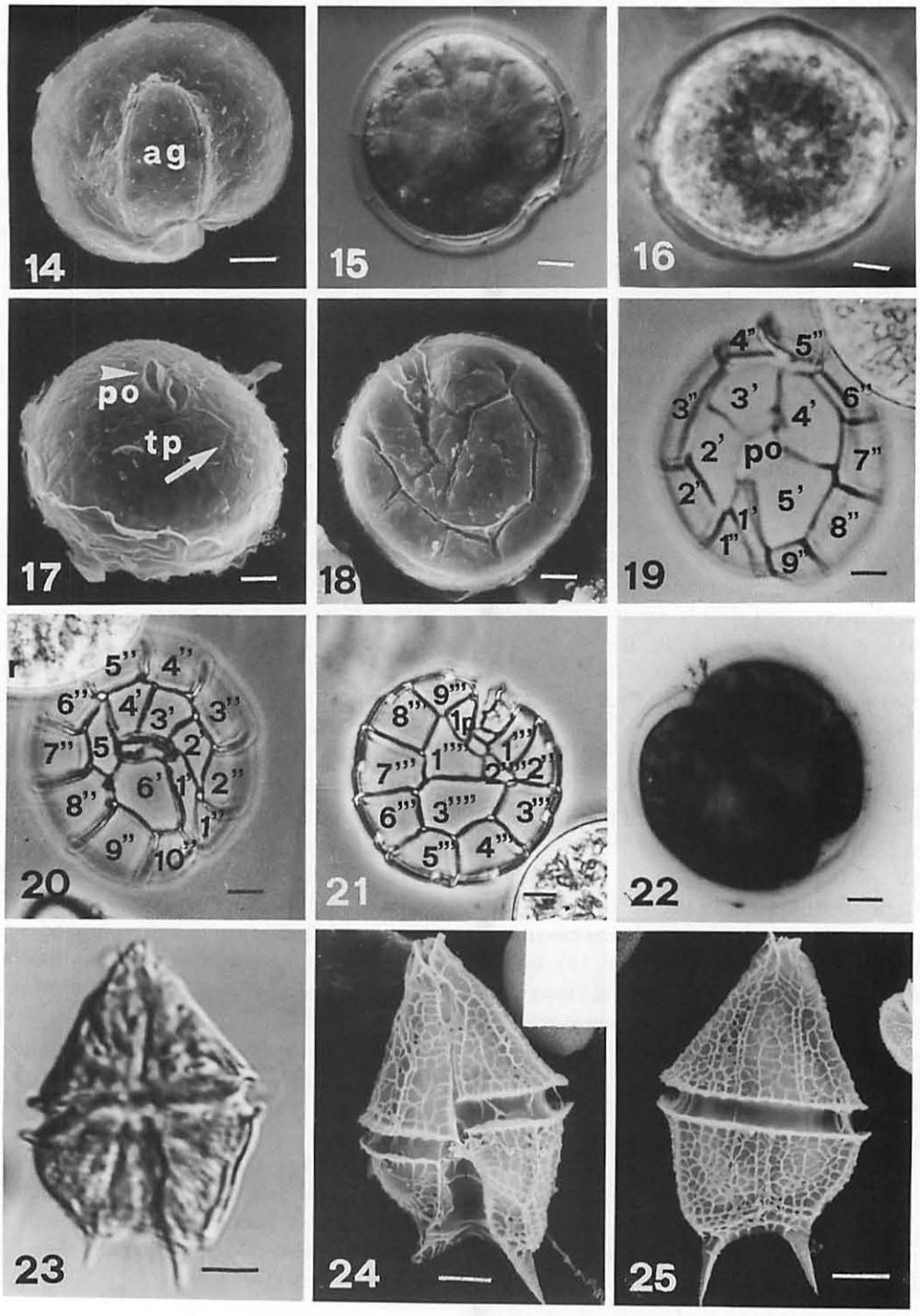
Dodge 1982, p. 217, Fig. 256; 1985, p. 81; Matsuoka *et al.* 1988, Figs. 8-14.

Cells are 30-42 μm (commonly 28-32 μm) in length, 20-34 μm (mostly 20-22 μm) in width. They are elongated, cordiform, and dorsoventrally flattened (Fig. 23). Epitheca is longer than hypotheca, and trianguloid with convex sides tapering into an apical horn. Hypotheca is trapezoid with two conspicuous spines, of which left one is longer than the right one (Figs. 24, 25). Girdle is situated below midpoint of the cell, and offset by one girdle's width. Thecal plate is reticulate. Plate formula is po, 3′, 2a, 6″, 6‴, 1p, 1⁗ and with the first apical plate narrow, smooth and unornamented. Cell is weakly pigmented, with light brown color.

The characteristic shape of the conical epitheca and two large antapical spines makes this species quite easy to be recognized. Our specimens fit quite well with the features of *G. verior* described by Dodge (1985) and Matsuoka *et al.* (1988). It occurred almost all year round with the exception during August to October (Table 1). The cell density was usually less than 1,000 cells/m*l*, but sometimes it was found blooming in some shrimp ponds with cell number as high as 300,000 cells/m*l* (Table 1). This alga has been reported from British Isles, North Sea, Belgian coast, Mediterranean Sea and Japanese coastal waters.

***Alexandrium tamarense* (Lebour) Balech** (Figs. 26-34)

Balech 1985, p. 37 Fig. 20; Fukuyo 1985,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p. 531 Figs. 2A-G.

Cells are single (Fig. 26), but form a two-celled chain shortly after cell division (Fig. 27), globular in shape and slightly longer than broad. They are 14–34 μm long, 13–31 μm wide and with length/width ratio of 1.00–1.22 (mostly 1.12), width/transdiameter ratio of 1.00–1.13. The epitheca is conical and the hypotheca has a very marked excavation in the sulcal region, both are nearly equal in altitude (Fig. 28). Apex is rounded with a slight hump at the apical pore plate (po) (Fig. 29); antapex is also rounded and slightly depressed at where the sulcus reaches (Figs. 28, 30). Both shoulders of the epitheca are convex. The hypotheca is asymmetric with the height of right half slightly shorter than the left half (Fig. 28). The girdle is equatorial, descending, without overhang, deeply excavated without lists, and its right end displaced posteriorly about its width (Figs. 26, 28, 30). Sulcus is weakly impressed, widened posteriorly, and slightly indented anteriorly (Fig. 30). The plate formula is: po, 4′, 6″, 8s, 5‴, 2⁗ (Figs. 30, 31, 32). The apical pore plate (po) (Fig. 29) is triangular and has a fishhook-shaped cleft (Fig. 33). The 1st apical plate (1′) is slightly broad and rhomboidal. A conspicuous ventral pore is present at middle of suture between apical plates 1′ and 4′ (Figs. 28, 29, 31). The sulcal plates are composed of eight platelets. They include one anterior (s.a.), transitional (t.), left (s.l.), sulcal left anterior (s.l.a.), right (s.r.), posterior (s.p.) and two medians (Figs. 30, 32). Thecal plates are thin and smooth, with trichocyst pores, and covered with a delicate outer thecal membrane. Trichocyst pores are single or in pair, and distributed evenly (Fig. 33). A large U-shaped nucleus is located beneath the cingulum. Temporary cyst is round, while hypnocyst is cylindrical and with rounded ends (Fig. 34). This strain is toxic (Su *et al.* 1989) and nonbioluminescent.

This species was found from an inlet near the mouth of Tungkang river (Fig. 1) and from ponds whose waters were introduced from that inlet. This alga appeared during winter and spring with water temperatures around 10.9-30.0°C and the salinity about 10-19 ppt (Table 1). Cell densities were about 10-100 cells/m*l*, but on Nov. 5, 1986 we found it was blooming in a crab pond with the cell densities of about 1600 cells/m*l*. It was also blooming in a shrimp pond in Tainan Shien with the cell density as high as 10,000 cells/m*l* in June 1989 and cause death of shrimp (Su, unpublished data).

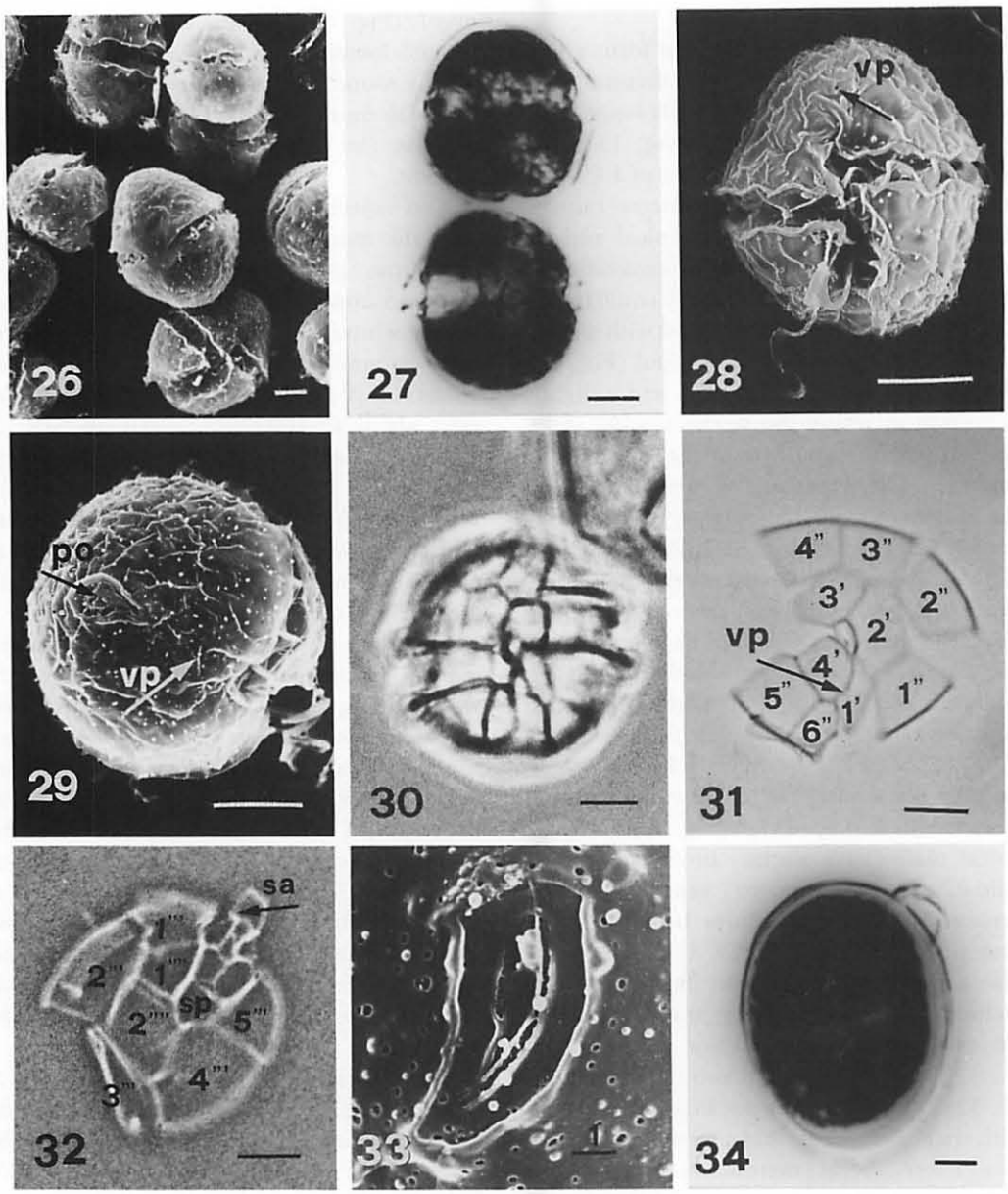
A. tamarense is a cosmopolitan alga occurred mainly in temperate coastal and estuary waters along the north part of the Atlantic and the Pacific Oceans (Taylor 1984) during summer time (Prakash 1963, Toriumi and Takano 1979). It also appeared in the tropical waters of Brazil and Venezuela along the west coast of the Atlantic Ocean (Balech 1971, Reyes-Vasquez *et al.* 1979). Recently, a nontoxic strain of this species was reported from Thailand coastal water (Fukuyo *et al.* 1989). This is the first record of toxic *A. tamarense* occurred in the tropical Pacific waters.

The intricate systematic problem of "Tamarensis" or "Catenella" group has been discussed (Loeblich and Loeblich 1975, 1979, Taylor 1975, 1979, 1984, Steidinger 1983, Fukuyo 1985). *Gonyaulax tamarensis, G.*

Fig. 14. *Gyrodinium instriatum*. Apical view. Showing the apical groove (ag) (SEM).

Figs. 15–22. *Pyrophacus horologium*. Fig. 15. Antapical view. Showing cytoplasm with numerous chloroplasts, and the thecae. Fig. 16. Dorsoventral view. Showing oblate shape. Fig. 17. Apical view. Showing apical pore plate (po) with its two slits and numerous trichocyst pores (tp) on the surface of the valve (SEM). Fig. 18. Antapical view. Showing the plate pattern and plain surface of the valve (SEM). Fig. 19. Phase contrast micrograph of the epitheca showing plates po, 5′, 9″. Fig. 20. The po, 6′, 10″ epitheca plates which different from those showing in Fig. 19. Fig. 21. Phase contrast micrograph of hypotheca. Showing plates 9‴, 1p, 3⁗ and sulcus platelets. Fig. 22. Antapical view. Showing two daughter cells inside the parental theca.

Figs. 23–25. *Gonyaluax verior*. Fig. 23. Ventral view. Showing the contour of the cell. Fig. 24. Ventral view. Showing the girdle, reticulate plate and two conspicuous spines (SEM). Fig. 25. Dorsal view. Showing the contour of the cell (SEM).



Figs. 26–34. *Alexandrium tamarense*. Fig. 26. The contour of the cell (SEM). Fig. 27. A two-cell chain. Fig. 28. Ventral view. Showing the displaced girdle, two flagella and a ventral pore (vp) (SEM). Fig. 29. Apical view. Showing the valve surface, po plate and ventral pore (vp) (SEM). Fig. 30. Ventral view. Showing the plate pattern. Fig. 31. Apical view. Showing the plates po, 4′, 6″ and ventral pore (vp). Fig. 32. Antapical view. Showing the plates 5‴, 2⁗, sulcal platelets. Fig. 33. Apical pore plate po. Showing trichocyst pores in single or in pair. Fig. 34. Hypnocyst.

tamarensis var. excavata, G. tamarensis var. tamarensis, G. excavata, Protogonyaulax tamarensis and *Alexandrium tamarense* are used to mention the same species. The morphological characteristics which have been used for identification of species are plate tabulation, cell shape, cell numbers of chain (Lebour 1925, Whedon and Kofoid 1936, Braarud 1945); lack or presence of ventral pore, toxicity and bioluminescence (Loeblich and Loeblich

1975); shape of cyst and sulcal platelets, contact of 1′ and Po plates (Taylor 1979, Fukuyo 1979, Balech 1985); the shape of apical pore plate, apical plate 1′ and sulcal posterior plate, and position of a posterior and an anterior attachment pore (Fukuyo 1985). But recently, the polymorphism of isozymes, DNA content and toxin spectrum have been used to help the identification of this group (Cembella and Taylor 1985). From the appearance of intermediate forms, and change of cell shape and cell numbers of chain in culture, Taylor (1984) suggested it may be more reasonable to put the "Tamarensis" group into intraspecific level. However, Balech (1985) thought some characteristics such as shape of apical pore, 1st apical plate 1′, sulcal anterior and posterior plate are conservative and can be used to distinguish different species.

Our specimens closely resemble *Alexandrium excavatum* (named as *G. tamarensis* var. *excavata* by Braarud) from Norway waters (Braarud 1945, Balech and Tangen 1985), *G. tamarensis* from Tamar estuary in England (173 strain in Loeblich and Loeblich 1975, Balech 1977) and *Protogonyaulax tamarensis* from Japanese coastal waters (Fukuyo 1985) in cell shape and plate tabulation. It also conforms to *G. tamarensis* var. *tamarensis* of Schmidt *et al.* (1978) in possession of a ventral pore and being toxic and nonbioluminescent. However, our alga differs from those reported from temperate waters in having ability to grow at very low salinity (3 ppt) and high temperatures (up to 33°C) in culture (Fig. 35A & B), and with smaller size (14–34 μm) (7 ppt and 25°C in Prakash 1967; 28–50 μm in Loeblich and Loeblich 1975). Although *Alexandrium tropicale* (Balech 1985) is a small sized and tropical species, and very similar to Tungkang strain of *Alexandrium tamarense* in cell shape, they still differ from each other in the position of nucleus (Balech 1971 Figs. 119, 120) and contact pattern of plate 1′ and po (Balech 1985 Fig. 7b). The contact pattern of plate 1′ and po was considered as a criterion for species (Loeblich and Loeblich 1979, Balech 1985), or for genus

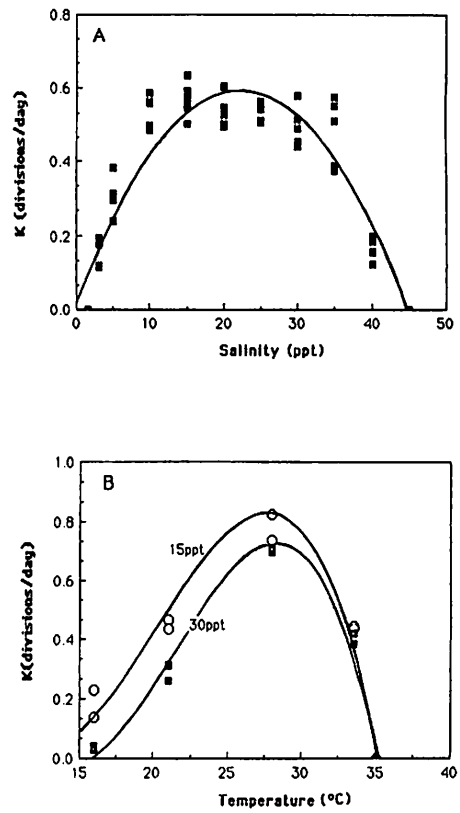


Fig. 35. The growth rates (K) of *Alexandrium tamarense* in various salinity (A) and temperature (B) under a 12 : 12LD cycle at 80 μE/m²/s light intensity.

(Taylor 1979). Because the contact is variable as that found in *A. excavatum* and *A. minimum* (Balech and Tangen 1985, Hallegraeff *et al.* 1988). We agree Balech's opinion that genus *Alexandrium* should include those species which have and have not the contact.

Acknowledgements

We thank Mr. C. S. Hsieh and Mr. L. Y. Yeh for the collection of water sample and measurement of water parameters, Ms. C. C. Chou for supplying SEM micrograph of Fig. 5, Dr. C. W. Li, Ms. C. Y. Lin and Ms. S. S. Rem for SEM expertise, other members of Tungkang Marine Laboratory for offering various invaluable assistance. This work was supported by National Science Council,

R.O.C. grant NSC77-0414-B002A-012.

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蘇　恵美*・江　永棉：台湾南部の養殖池から採集した渦鞭毛藻

養殖した貝 (purple clam) の摂取による麻痺性貝中毒事故が発生し2人の死者がでた台湾南部の養殖池から採集した光合成を行なう渦鞭毛藻5種 (*Prorocentrum minimum, Gyrodinium instriatum, Pyrophacus horologium, Gonyaulax verior, Alexandrium tamarense*) について記載し，若干の分類学的検討を行なった。*A. tamarense* は，熱帯太平洋水域で単離された最初の有毒種であると報告されているが，著しい低塩分ならびに高温で成育し，サイズが小さい (11–34 μm) 点で温帯水域で報告されているものとは異なる。(台湾台北市 国立台湾大学海洋研究所；*現所属：台湾屏東県 台湾水産試験所東港分所)