A SCANNING ELECTRON AND LIGHT MICROSCOPY STUDY OF THE RED TIDE DINOFLAGELLATE *GYMNODINIUM* SP. FROM FALSE BAY, SOUTH-AFRICA

BOTANY HONOURS PROJECT

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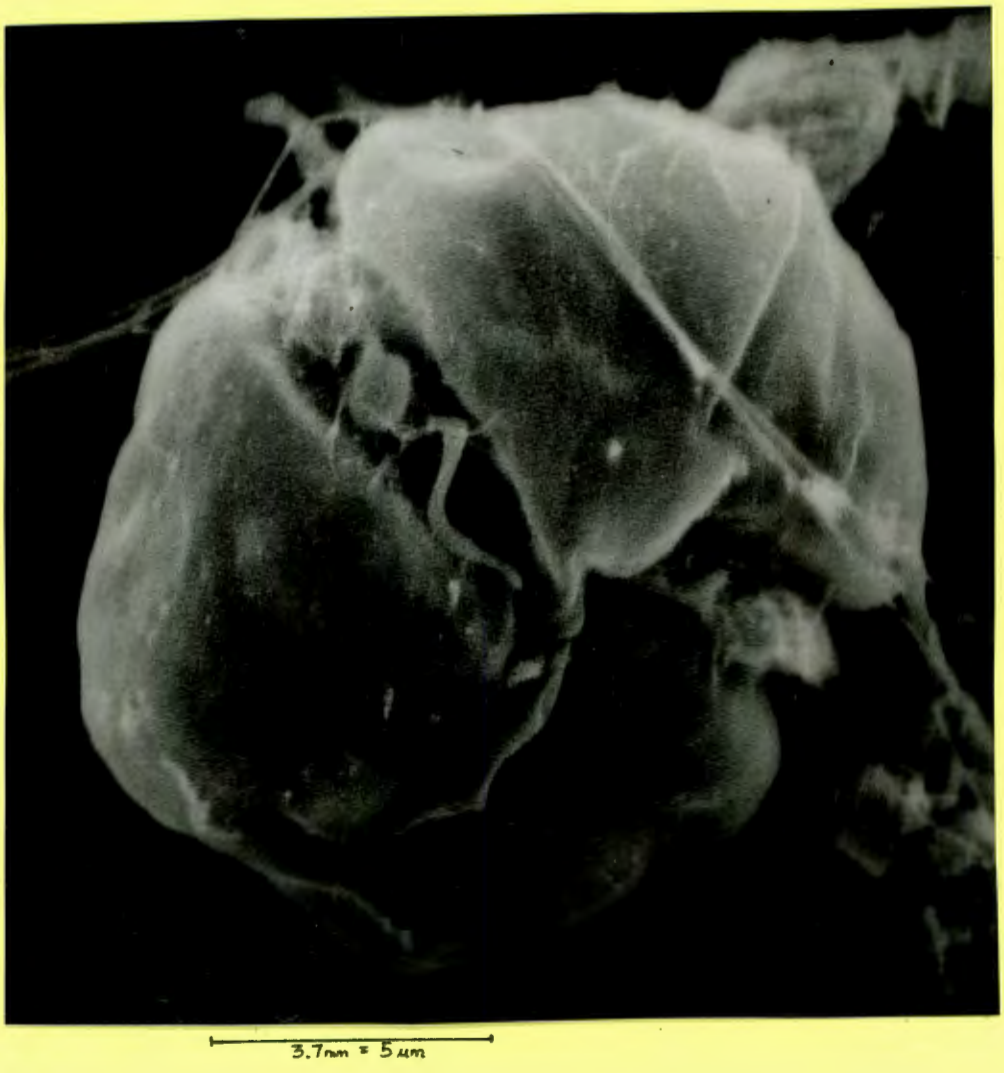


CONTENTS:

The figure on the cover is a scanning electron micrograph of Gymnodinium sp. Mag : x 4700

ABSTRACT:

A previously unrecorded red tide dinoflagellate bloomed in False Bay during 1988 and 1989, causing faunal mortalities as well as eye, nose and lung irritations to bathers and fishermen. Light microscopy and scanning electron microscopy was used in an attempt to identify this species which shared certain features with the following species:
(a) *Gymnodinium nagasakiense* Takayama et Adachi, the western Pacific species, with which it has the external morphology in common,
(b) *Gyrodinium aureolum* Hulburt, the North Atlantic species which is common in northern European and eastern USA waters, with which it shares the cell shape and the nucleus position,
(c) *Ptychodiscus brevis* (Davis) Steidinger, the north American species, which has a similar toxic effect.
Ptychodiscus brevis differed from the False Bay organism in several aspects, including cell contour, the possession of an apical protrusion, and the toxins of *P.brevis* are lipid soluble whereas the toxins of *Gymnodinium sp.* are water soluble. The local species was morphologically similar to *Gymnodinium nagasakiense* but had the nucleus in a different position. *G.nagasakiense* has the nucleus in the left side of the cell, whereas in the local species the nucleus was situated sub-centrally to centrally. It is concluded that *Gymnodinium sp.* was most similar to *Gyrodinium aureolum* but differed in their toxic effects. The epithet *Gyrodinium cf. aureolum* is suggested although the local species may be a new species. Further research using DNA contents and transmission electron microscopy are needed to clarify this problem.

INTRODUCTION:

Dinoflagellates are a diverse group of unicellular, biflagellated organisms which form an important component of the marine, brackish and fresh water phytoplankton. They produce blooms or red water (which are commonly called "red tides") when the concentration of cells becomes so dense that the ocean is colored locally red, red-brown or yellow (Bold and Wynne 1985), also orange and purple (Horstman 1981) and yellow-green (Davis 1948). Some bloom dinoflagellates produce toxins which are associated with fish kills and the mortality of other marine organisms. Most blooms off the southern and western coast of South Africa are non-toxic and due to dinoflagellates and ciliates (Horstman 1981).

There are three categories of toxic blooms of dinoflagellates (Steidinger 1973 cited in Bold and Wynne 1985). Those which kill fish but few invertebrates, e.g. *Ptychodiscus brevis* (Davis) Steidinger, the Florida red tide; those which kill invertebrates mainly, eg. *Gonyaulax*; and those which kill few marine organisms but toxins are concentrated in filter-feeding bivalves, causing paralytic shellfish poisoning (PSP), eg. *Gonyaulax catanella* which produces a neurotoxin, called saxitoxin, that is 100 000 times more toxic than cocaine (Bold and Wynne 1985).

Toxins affect organisms in 3 ways: they cause paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), and neurotoxic shellfish poisoning (NSP). In DSP and PSP, the toxic effects in humans usually result from the ingestion of affected shellfish whilst in NSP the toxic effect is due to contact with the toxins, which have been released into the water by cell lysis or physiological processes, and not from the ingestion of affected shellfish (Horstman *et al.* 1990). During a PSP event, the poison is

accumulated in the digestive glands of the bivalve which causes paralysis. Death of the bivalves is usually due to exposure when the organism is washed up on the beach (Horstman 1981). In humans the symptoms of PSP are numbness of lips, tongue and finger tips within a few minutes of eating the contaminated shellfish. Numbness of the arms, legs and neck and a general muscular incoordination associated with respiratory distress follows. Death from respiratory paralysis can occur within 2 - 24 hours, depending on the quantity of the toxin ingested (Horstman 1981). In South Africa PSP is the usual type of toxic event (Horstman 1981).

There is a globally significant increase in the frequency of the occurrence of red tides accompanied by the regional spreading and the involvement of more species (Smayda 1989). This phenomenon has been linked to the increase in pollution and industrialisation. Since South Africa is becoming an increasingly industrialised country, the frequency of red tides may increase and this could become a major problem for marine industries, such as fishing and related industries. Horstman (1981) has noted an increase in the annual number of reported red water outbreaks but cautions that this may be due to increased public awareness and not a reflection of a real increase in these events. See Horstman (1981) for a review of red water occurrences and shellfish poisoning in South Africa.

Several factors have been suggested to trigger blooms of phytoplankton. These are all basically nutrient and grazing hypotheses. Changes in the environment, for example upwelling or tidal turbulence, brings nutrients to the surface, allowing increased growth. Red tide dinoflagellates may have an advantage over coastal diatoms during upwelling periods because they can take up and assimilate nitrate in the dark, thus allowing them to

outcompete the diatoms (Harrison 1976, in Bold and Wynne 1985). Species introduced to areas may be filling empty niches. Anthropogenic factors are increasing in importance too, especially in the area of nutrient enrichment of water bodies. A fourth factor is the changes in the effect of grazing when marine cultures are started (Smayda 1989). These factors all contribute to bloom formation. In South Africa, active upwelling usually results in diatom blooms (contradictory to the idea of Harrison (1976 as cited by Bold and Wynne 1985) that dinoflagellates bloom under upwelling conditions. Dinoflagellates bloom when wind-induced turbulence is weak, which allows stratification of the surface layers. During conditions intermediate to stratification and active upwelling coexistence of diatoms and dinoflagellates in blooms can occur (Horstman 1981).

During 1988 and 1989 red waters associated with the mortality of shellfish occurred in the False Bay area (Horstman *et al.* 1990). Blooms were recorded for the late summer/autumn period, when most outbreaks of red water in South Africa occur (Horstman 1981). This is due to warm conditions prevailing because of reduced upwelling. The bloom lengths were two to three weeks. After the 1989 bloom the species was still present in False Bay, in low concentrations. A bloom was predicted for the March/May period of 1990 (G.Pitcher, Sea Fisheries, Roggebaai, pers. comm.) but did not occur as water temperatures remained remarkable low during the latter part of summer (1990). When investigated, it was found that this organism had never previously been recorded for False Bay. It was named *Gymnodinium sp.* in this preliminary investigation (Horstman *et al.* 1990).

The studied organism has caused considerable damage in the above area. During 1989 alone, the bloom area extended from Fish Hoek to Pearly Beach (fig. 1).

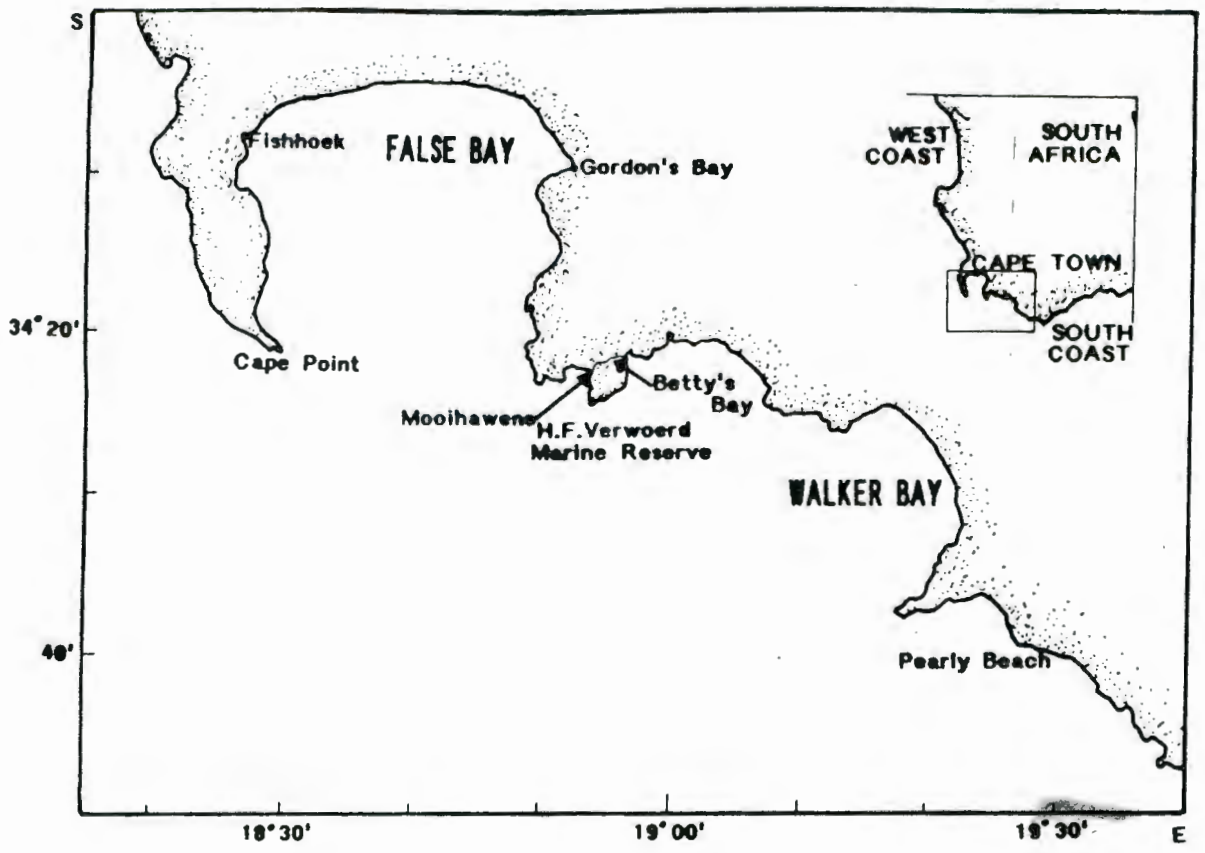


FIGURE 1. Localities of the blooms of Gymnodinium sp.
In 1988 it bloomed from Fishhoek to Pearly Beach.
In 1989 it bloomed in Gordon's Bay.

Faunal mortalities caused by *Gymnodinium .sp.*, amounted to 40 tons (6.25% of the annual harvest) of *Haliotis midae* (abalone), mainly from the H.F.Verwoerd marine reserve in Betty's Bay (Horstman *et al.* 1990). The abalone was affected only to a depth of three metres. Other marine organisms affected were *Jasus lalandii* (rock lobster), which moved to deeper waters during the bloom, *Diplodus sargus* (Blacktail) and octopi were killed. In addition to the effect on the local fauna, fishermen suffered from eye and respiratory problems. During the previous year (1988) blooms occurred at Gordons Bay, and the fauna killed were *Hepsetia breviceps* (vlei sardine), *Turbo sarmaticus* (ollycrock), *Octopus granulatus*, chitons and starfish. The toxin(s) from the local organism killed filter feeders, deposit feeders, grazers and predators. This differs from the usual PSP occurrence, which affects only filter-feeders (Horstman *et al.*, 1990, In press). The toxic effect is similar to NSP. If it persists or increases with time, *Gymnodinium sp.* could have a severe impact on the local abalone population.

Prior to *Gymnodinium sp.* there have been 3 dinoflagellate species which were reported to cause fish mortality in False Bay. There was *Gonyaulax polygramma* in 1962 which was a major problem by causing fish kills due to hypoxic conditions. In 1967 *Noctiluca scintilans* caused fish kills locally in Simonstown, by ammonia production, and in 1976 *Gymnodinium sp.* caused fish kills due to gill clogging in the Gordon's Bay area (Brown *et al.* 1979 in Horstman *et al.* 1990). The 1988/1989 *Gymnodinium sp.* had the NSP effect and not gill clogging. Only two organsims, *Gonyaulax catanella* and *G.grindleyi* were found to be toxic. Non-toxic organisms affected the fauna indirectly through gill-clogging and anoxic conditions. See Horstman (1981) for a list of all recorded phytoplankton blooms in False Bay from 1959 to 1980, and their effects on the local fauna. Severe

mortalities among the mussel population, caused changes to the population structure which took several years to recover (Horstman (1981).

A brief outline of the morphology of naked dinoflagellates, as pertaining to the species under observation, is essential for facilitating an understanding of the concepts used in this project report. The definitions used are according to Takayama and Adachi (1984). These definitions are listed in a glossary attached at the end of the report.

THE UNARMOURED DINOFLAGELLATE: TERMINOLOGY

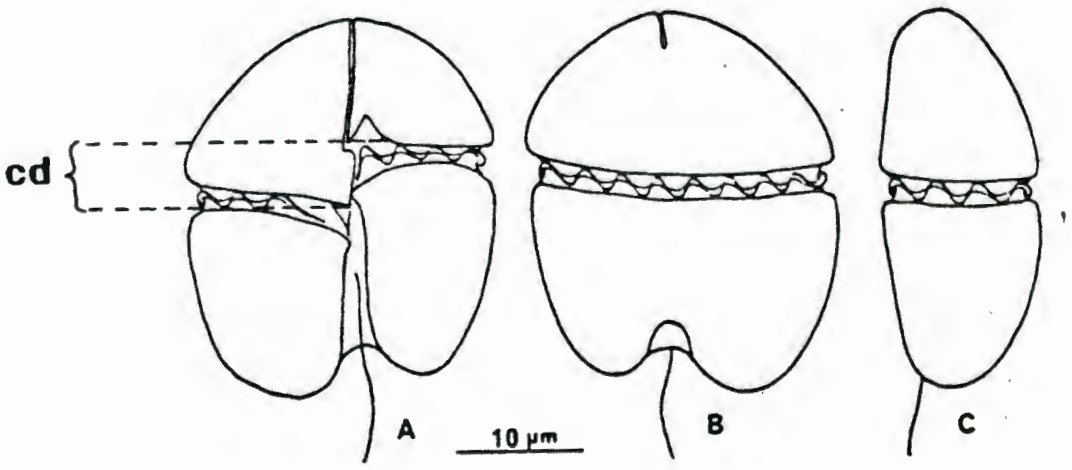


Fig. 2. The external features of *Gymnodinium nagasakiense* Takayama et Adachi.
a: ventral view, B: dorsal view, C:lateral view showing the dorsi-ventral flattening (ex Takayama and Adachi 1984). **cd = cingulum displacement**

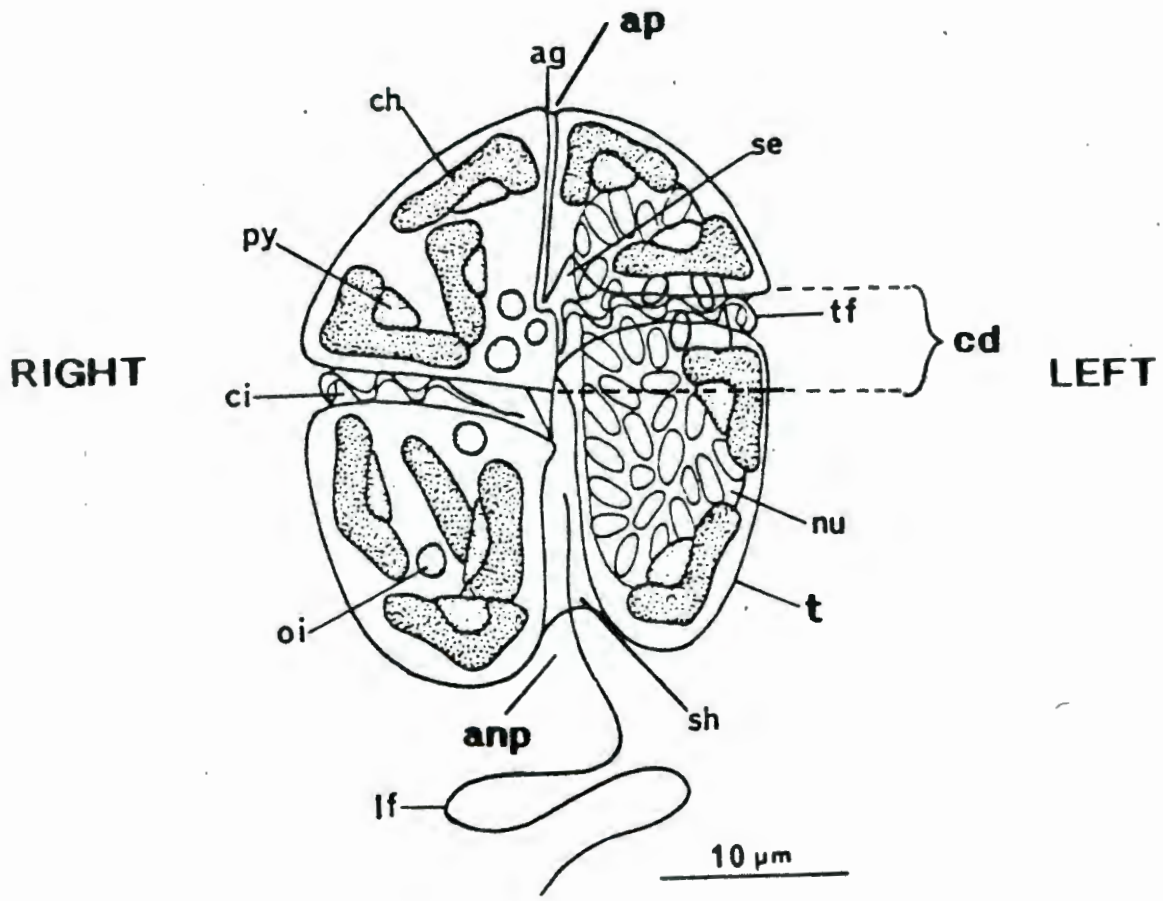


Fig. 3. A schematic representation of *Gymnodinium nagasakiense* Takayama et Adachi.
Depicted on the diagram is the following: epicone, hypocone, the left and right sides of the cell, ag - apical groove, ci - cingulum, tf - transverse flagellum, lf - longitudinal flagellum, nu - nucleus, se - sulcus on the epicone, sh - sulcus on the hypocone, ch - chloroplast, cd - cingulum displacement, ap - apex, anp - antapex, t - theca (extracted from Takayama and Adachi 1984).

TAXONOMY

Gymnodinium sp. was first observed in False Bay (South Western Cape, South Africa) in 1988 when it caused an olive-green discolouration of the water (Horstman *et al.* 1990). It is an unarmoured (naked) dinoflagellates.

Dinoflagellates are divided into two morphological groups at the level of the light microscope. This division is based on the theca structure and form. Armoured forms (thecate) have a theca consisting of membranes and thecate vesicles with structural polysaccharides which appear as plates, whilst unarmoured (naked) whilst having a theca consisting of several membranes, the thecal vesicles do not form plates. With the use of the electron microscope, this feature has been studied in more detail, and intermediate forms have been found that are unarmoured but with thin plates (Dodge and Crawford 1970).

The taxonomic status of *Gymnodinium sp.*, following the system of Bold and Wynne (1985), is shown below:

DIVISION: Pyrrophyta

Unicellular; biflagellated organism with starch food reserves; nucleus with permanently condensed chromosomes, lacks centromeres and spindle; the cell covering (amphiesma) consists of layers of membranes; cells are unarmoured (naked) or armoured (thecate).

CLASS: Dinophyceae

Motile cells are biflagellate. One ribbon-like flagellum with a vibrating beat, located in a transversely aligned groove, called the cingulum, which encircles the cell in the equatorial region; the other extending in a posterior direction from the cell in a longitudinally orientated groove, called the sulcus.

ORDER: Gymnodiniales

Cells motile, solitary and free-living, cell covering without thecal plates.

GENUS: *Gymnodinium* STEIN

(Gr. gymnos, naked and Gr. dinein, to whorl)
Cingulum in the median position; cingulum may form a complete circle by the ends meeting on the ventral surface, or may be offset; if the cingulum displacement is greater than 1/5 of the cell length, the generic name is *Gyrodinium*, and if it is less than 1/5, it is known as *Gymnodinium*. The genus *Ptychodiscus* is differentiated from *Gymnodinium* by the presence of one ventral flagellar pore in *Gymnodinium* , whilst *Ptychodiscus* has two ventral flagellar pores.

Ptychodiscus was first described by Stein (1883 cited in Boalch 1969) as a monospecific genus. It is described as being shaped like a pair of bellows, consisting of anterior and posterior parts connected by a girdle region. The smaller epicone bore a lamella-like structure. The epicone diameter was 60-93um and the hypocone diameter 66-120um (Boalch 1969). The genus is very little flattened dorso-ventrally and has thecal ridges in the cingulum.

The local species designation has been based on morphological features from a preliminary light microscope

study by researchers at the Sea Fisheries Research Institute, Roggebaai, Cape Town.

The aim of this project is the identification of the organism from False Bay referred to as *Gymnodinium sp.*. Identification is of importance, not only to those interested in the field of taxonomy; but economically too, since the red waters affects fisheries products; socially, as red water has caused public health problems; and identification facilitates the prediction of potential impacts to public health and marine communities. The last of these reasons is linked to the second and third ones, for, when monitoring programmes are established, improved forecasts of possible effects can be made and control measures can be applied, if feasible. When the species is known it can be compared, from the published literature, to the effects it has in other areas in the world. An accurate identification prevents ambiguity during any communication of the problem.

POSSIBLE SPECIES

From a preliminary light microscope investigation, the False Bay organism has been tentatively assigned to the Japanese dinoflagellate species, *Gymnodinium nagasakiense* Takayama et Adachi, which has caused considerable damage to fisheries products in Japanese and Korean waters (Takayama and Adachi 1984) and along the coast on New Zealand (Partensky *et al.* 1988). The local species is morphologically similar (in cell shape) to *G.nagasakiense* Takayama et Adachi. However, it also shares characteristics of two other species. It resembles *Gyrodinium aureolum* Hulburt in cell shape and the position and shape of the nucleus (a prime taxonomic character for dinoflagellates), and it resembles *Ptychodiscus brevis* (Davis) Steidinger by the presence of aerosol toxins.

GYRODINIUM AUREOLUM:

Gyrodinium aureolum (Hulburt) was described by Hulburt (1957) on the east coast of the USA and is known to be present in the north west and the north east Atlantic ocean, where it is common in north European waters. It causes damage to marine life and the shellfish industry. Although it is known to be toxic, the toxin(s) have not been identified (Partensky and Sournia 1986). The toxic effect is not by PSP, but probably due to oxygen depletion of the water. It has an internal pyrenoid in the chloroplasts. Chloroplasts are lobed and globular (Tangen and Bjornland 1981). It was described as having a central nucleus by Hulburt (1957) and by Tangen (1977) (Fig. 4a) but the nucleus was found to occur on the left by Tangen and Bjornland (1981) and Partensky *et al.* (1988). Either *G.aureolum* has a variable nucleus position or several different taxa may have been attributed to this species.

PTYCHODISCUS BREVIS

Ptychodiscus brevis (Davis) Steidinger is an unarmoured dinoflagellate first described by Davis (1948) as *Gymnodinium breve* when its aerosolized toxin caused respiratory irritation and fish mortality (Pierce 1986). This designation was based on light microscopy. The transfer of the species to the genus *Ptychodiscus* by Steidinger (1979) has been criticized because this species differs from the typical *Ptychodiscus* and has a carinal groove which is considered equivalent to the apical groove in *Gymnodinium* (Takayama 1985). It is a notorious red tide organism in the Gulf of Mexico (Abbot *et al.* 1975). The species has a north Atlantic distribution and blooms take 2 to 4 weeks to develop. The species was characterised by the dorso-ventral compression, the dorsal convexity and ventral concavity, the prominent apical process (fig. 4b) which is ventrally directed, the sulcus on the epicone, a large posterior nucleus and the lateral contraction of the cells.

In 1978 and 1979 this species was redescribed by Steidinger, at the LM and TEM levels. It was discovered to have a highly vesiculated cytoplasm, lobed peripheral chloroplasts with multistalked pyrenoids, thecal ridges in the cingulum, a distinct apical process, it lacked the xanthophyll pigment peridinin, the median apical groove extends the length of the carina (dorsally and ventrally) and the groove has a thick rolled edge (Steidinger *et al.* 1978, Steidinger 1983). This species also has two ventral flagella pores and one large dorsal pore in the cingulum. The ventral pores are not clearly visible at the LM level.

Ptychodiscus brevis is notorious along the coast of Florida for its effects on marine life. Its toxic effect is NSP. The toxins are aerosolized ie. the airborne irritant was caused by wave action lysing the cells, releasing the toxin(s) and producing airborne toxic particles in sea spray carried ashore by the wind (Pierce 1986). It causes fish kills by respiratory inhibition, at concentrations of 0.25 x 10^4 cells.l^{-1} and kills some invertebrates. The cells are very fragile and rupture as they pass through the gill passages of fish, releasing the toxins with the cell contents. Mortalities are extensive and direct, and this organism is a problem. The toxins also affect humans more directly and contact with it may cause skin irritations (Abbot *et al* 1975). Symptoms are numbness, tingling, cramps and may lead to coma in severe cases. The toxins also cause respiratory irritation in humans and animals due to the aerosolization of the toxins. The toxins are 3 polyether (brevetoxins) neurotoxins and 2 phosphorus (ichthyotoxins) (Yasumoto 1985), and by 1989, 8 toxins had been discovered (Smayda 1989). They are soluble in an organic solvent (lipid soluble neurotoxin is one of them). The toxins are not destroyed by cooking the shellfish (Steidinger 1983).

GYMNODINIUM NAGASAKIENSE

Gymnodinium nagasakiense Takayama et Adachi was described by Iizuka (1975) and Takayama (1981) and was named *Gymnodinium sp* (type-'65) and *G.nagasaki*. The type-'65 refers to it having been observed blooming in 1965. This species is known in Japan, Korea (Matsuoka *et al.* 1989) and New Zealand (Partensky *et al.* 1988). Cell dimensions vary for the different studies completed and these are listed separately in Table 2. From the original description, cells are dorso-ventrally flattened, with flattening 33% to 75% of the width and up to 50% of the width in the middle section, no surface striations, the apical groove is sculptured on the epicone and starts immediately above or to the right of the apical end of the sulcus, the nucleus is ellipsoidal or reniform (longer than wide) and always on the left of the cell (Matsuoka *et al.* 1989, Partensky *et al.* 1988, Takayama and Adachi 1984). The chloroplasts are shapeless and brown with an external pyrenoid attatched to each chloroplast. The cingulum displacement is 11% to 25% of the cell length, but average 14% to 20%. It is thus mainly below 20%, which is why it is placed in the genus *Gymnodinium*. Cells are rounded in ventral outline, with the epicone sub-hemispherical in ventral view, and dorso-ventrally flattened, the hypocone is notched by the sulcus at the antapex, which reaches to the dorsal side and chromosomes are granular (Takayama and Adachi 1984). It has a reduced apical process when compared to *P.brevis*, has a distally upturned cingulum that joins the broad anterior end of the sulcus, the sulcus on the hypocone is deeply excavated, the apical groove is at a distinct angle but is shorter than in *P.brevis*, which has thecal ridges in the cingulum and a dorsal pore which the latter has too, but differs mostly in the degree of sulcal intrusion on the epitheca and the cingulum displacement (Steidinger 1983) (fig. 4c). This species exhibits a high degree of morphological variability. The major difference being the presence of a carinal groove

in *P.brevis* and an apical groove in *G.nagasakiense* (Takayama 1985).

A SPECIES COMPLEX?

The identification becomes more problematic because of the confused taxonomic status of the genera *Gymnodinium* and *Gyrodinium*. In fact the distinguishing character between the two genera *Gymnodinium* and *Gyrodinium*, as mentioned before, is the girdle displacement (Bold and Wynne 1985, Taylor 1985, Takayama 1981). If the displacement is greater than 20% of the cell length it is placed in *Gyrodinium* and if less, in *Gymnodinium*. The species *Gymnodinium nagasakiense* Takayama et Adachi and *Gyrodinium aureolum* Hulburt are on the boundary between these two genera. *G.nagasakiense* Takayama et Adachi from the western Pacific and *G. aureolum* Hulburt from the Atlantic are considered to be conspecific by Taylor (1985) who based the conclusion on morphology and biochemistry of the pigments. He found the above two species to be indistinguishable from a third species, *Gymnodinium mikimotoi* and closely related to *Ptychodiscus brevis*. Taylor (1985) is supported by Matsuoka *et al.* (1989), Steidinger and Tangen (1985) and Steidinger *et al.* (1989), although Partensky *et al.* (1988) have shown that *G.nagasakiense* and *G. aureolum*, although morphologically identical, can be differentiated by their DNA content. *G.nagasakiense* had 41% more DNA than *G. aureolum* (Table 2).

Another problematic feature concerning *Gyrodinium aureolum* Hulburt is that the original description of the genus was made in 1957 (Hulburt 1957) using light microscopy and may be inaccurate concerning certain morphological details, since important taxonomic features such as sulcus extension and the apical groove are not easily viewed on a light microscope. Recent observations of these species with SEM (scanning electron microscopy) and TEM (transmission

electron microscopy) have been used in comparison with the original descriptions. *Ptychodiscus brevis* (Davis) Steidinger too has had its designation changed and had originally been described as *Gymnodinium breve* (Davis 1948). The criterion for distinguishing between the genera seems not to work for these species.

The typical confusion about the species mentioned emphasises the challenge that the identification of the False Bay red tide organism poses. By means of the scanning electron microscope a more detailed study is possible and this technique should in the future be used to reinvestigate all species, and consequently a reevaluation of the currently accepted taxonomy of these toxic unarmoured dinoflagellates will be necessary.

The taxonomic characters currently used for the identification of the species are morphological (and considered conservative). These characters have been selected from the literature and follow the suggestions of Partensky and Sournia (1986), for identifying members of the Gymnodiniaceae, in the format followed by Takayama and Adachi (1984). Both scanning electron microscope and light microscope techniques have been employed. The specific characters observed with each technique are listed in the materials and methods section.

Once the morphology of *Gymnodinium sp.* has been established it will be compared to the species descriptions of three other species with which it shares characteristics.

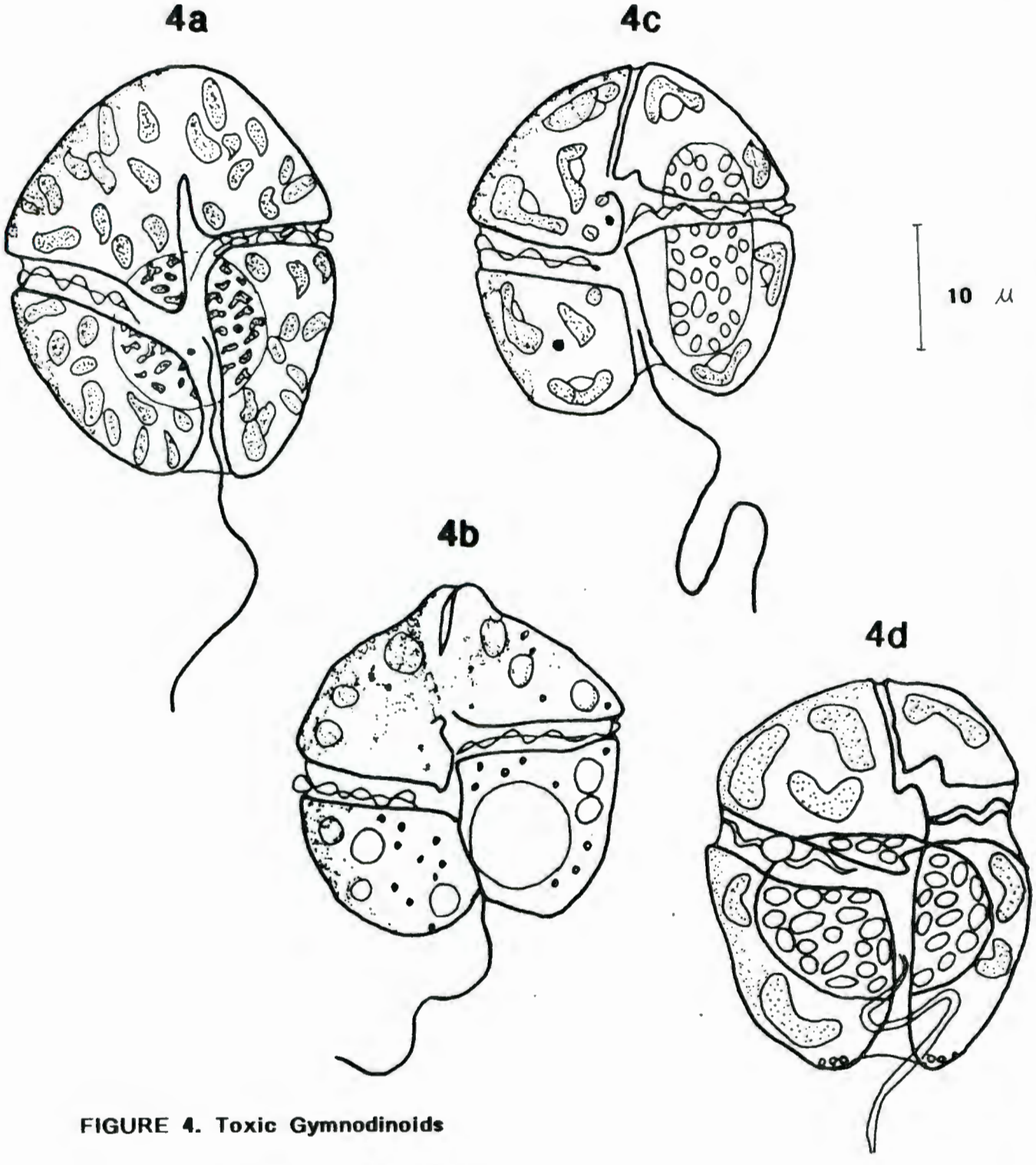


FIGURE 4. Toxic Gymnodinoids

a. Gyrodinium aureolum

b. Ptychodiscus brevis

c. Gymnodinium nagasakiense

d. Gymndinium sp.

Figures 4 a, b, c after Taylor 1985.

METHODS AND MATERIALS:

Source of material:

Material for the study was obtained from G.Pitcher, Sea Fisheries Research Institute, Roggebaai, Cape Town. These samples were collected from False Bay in 1988 and 1989 and fixed in 4% borate-buffered formalin. The sample was multialgal with armoured species present as well.

Light microscopy:

For light microscopy, material were prepared as temporary slides, and stained with a nuclear stain (Aceto-carmine and Haematoxylin[x] were used) and a counter stain (Fast Green[O]) (Johansen D.A. 1940). The coverslip was ringed with transparent nail varnish to seal it temporarily. No permanent slides were prepared due to the species extreme sensitivity to chemicals used in fixation, which causes drastic distortion. These were viewed with a light microscope, under phase contrast and differential interference contrast. Zeiss photomicroscopes was used for differential interference contrast microscopy and phase contrast, and a Nikon-Japan light microscope for preliminary observations.

Characters studied with the light microscope included cell dimensions (length and width of the cells, length of the epicone and hypocone), and the length and direction of the apical groove on the epicone. The cell length was taken from the apex of the cell to the antapex. Width, was measured as the maximum length from right to left and was found to occur at the highest part of the hypocone, immediately below the cingulum. External morphology was not readily distinguishable by light microscopy, thus electron microscopy became an essential tool for determining the morphology.

x A 1% solution of Haematoxylin was made in absolute alcohol.
O A 5% solution of Fast Green FCF in water.

For cytology, position of the nucleus, shape of the nucleus, number and shape of the chloroplasts, chromosome number and morphology were observed. Staining was carried out on the microscope slide. Some fixed material was pipetted into the centre of a drop of aceto-carmine. The coverslip was then placed on the drop and surplus aceto-carmine was flushed out. A light pressure was applied to the coverslip to spread the cells (Dodge 1963, 1966). The Dinophyceaen nucleus shares characteristics with Eukaryotes and Prokaryotes, and is called a Mesokaryotic nucleus, which means intermediate nuclear organisation type (Dodge 1966). The chromosomes are unique in that they are continually condensed.

Scanning electron microscopy:

To obtain a clear micrograph, biological specimens must be prepared for the scanning electron microscope. Preparation of samples, includes fixation, post-fixation, dehydration, critical point drying and coating.

FIXATION

Fixation preserves dead material in a life-like form. Fixation was by chemical media, in 4% borate-buffered formalin where cells were kept in suspension.

POST-FIXATION

Approximately 1 to 2ml of the sample were filtered using a 5.0um transparent 13mm Millipore filter (Millipore Corporation, Bedford MA). The filter is placed in a filter-holder. The filter-holder used was the Swinnex filter unit (Watson *et al.* 1980). The sample was pipetted into the filter holder and allowed to filter by normal gravity filtration. Syringing the sample into the filter unit and applying pressure during this process must be avoided as this causes the cells to collapse. These were washed with a 0.1M Na cacodylate buffer for 1 hour. Thereafter they were

post-fixed in 2% osmium tetroxide made up in a 0.1M Na cacodylate buffer (in a 1:1 ratio) for 2hrs and then rinsed with distilled water for 10 minutes to wash the specimens of any unwanted debris.

DEHYDRATION

Scanning electron microscopy requires dry specimens. Air drying causes distortion of the cells due to the action of the surface tension forces, therefore critical point drying is used. As water is immiscible with the transitional fluid used during critical point drying it has to be removed from the specimens. This is done by using a dehydration fluid, which is called the intermediate fluid (alcohol was used in the present study, but acetone is an alternative). Dehydration was completed using an ethanol series of increasing concentration, 30%, 50%, 70%, 90%, 95% and 100%, with the sample being washed with each concentration for 10 minutes (although this period can range from 5 to 20 minutes). The graduated alcohol concentrations are necessary to maintain osmotic equilibrium. Once again, whilst filtering it is essential not to pressurise the cells. Cells were concentrated by normal gravity filtration.

CRITICAL POINT DRYING

This process achieves the transition from the liquid to the vapour phase without the passage of the phase boundary and the accompanying forces due to surface tension. The specimens (still in the filter holders), were enclosed in the specimen chamber of the critical point drying apparatus. The transitional fluid (liquid CO_2) was admitted into the chamber. The dehydrating or intermediate fluid is flushed out. The chamber is heated to above the critical temperature and pressure. Above the critical temperature, the chamber is slowly vented. The samples were thus critical point dried.

COATING

Coating refers to the deposition of a heavy metal onto the surface of the specimen to make it electrically and thermally conductive. This avoids charging of the sample, which affects the image obtained from the microscope, and secondly it achieves a high yield of secondary electrons which makes the resolution of the image good (Watson *et al.* 1980).

The filters (with specimens on them) were mounted on 13mm aluminium stubs with a liquid graphite/glue mixture. Coating was with gold/paladium (Au/Pd). Micrographs were taken with the Cambridge S200 in the Electron Microscope Unit at the University of Cape Town.

Characters observed from electron microscopy include the body contour (cell length, width and dorsiventral flattening), the shape of the epicone, the sulcus extension on the epicone and hypocone, the apical groove, the cingulum displacement, the shape and length of the flagella and the prescence of pores or markings on the amphiesma (cell covering).

Possible sources of error were mainly due to the preparation of the samples for electron microscopy. This procedure has distorted the cells drastically. The use of preserved material also aggravates the aim of species identification of naked dinoflagellates since it effects changes in morphological features of the organisms as noticed by Kimball and Ferguson Wood (1965) when studying an organism which at first appeared to be *Gymnodinium mirabile* and in later cultures it took on characteristics similar to those of *Gyrodinium mirabile*. The degree of cingulum displacement changed with culturing, causing the generic shift. Thus the source of the material is a factor which affects the

conclusions made, and should be considered when comparing to published results.

RESULTS:

SPECIES DESCRIPTION

MORPHOLOGY:

All morphological details are shown in plates I and II.

Body contour: Cell shape and size

The species *Gymnodinium sp.* is on average 25um (±4) long, and ranges between 17 and 39um. The width is 22um (±4), with a range of 13 to 33um. Cells are longer than wide (plate I). Cells are dorsiventrally flattened (plate I), on average 61% of the width (this is the ratio of the lateral width to the ventral width), and the range of flattening is from 42% to 72%. The length and width, were determined from LM and the flattening from SEM. The SEM results for the length and width were excluded from the calculation of the length and width due to excessive shrinkage during cell preparation for SEM but were included in calculation of the ratios of cell length:cell width and cell breadth:cell width. These results are listed in Table 1.

Epicone: Apical groove and sulcus on the epicone

In ventral view, the epicone is sub-hemispherical and has an **apical groove (ag)** which extends from the right of the sulcus (on the epicone) to the apex and continues onto the dorsal side of the epicone (plate I). In ventral view (plate I) the apical groove is 4.9um (±1.5) long and ranges between 2.7 and 7.1um in length. On the dorsal side of the epicone it is 5.6um (±0.7) long with a range of 4.8 to 6.5um. Measurements listed above are from SEM micrographs of the shrunken samples. Since the SEM samples had shrunk from the preparation, it is contradictary that the LM

measurements for apical groove on the ventral surface were lower than the SEM measurements. This result may be due to inaccurate measuring of the apical groove with the LM, due to the groove not being fully distinguishable using this technique. The right ridge of the apical groove is thickened in ventral view (plate I). In dorsal view the epicone is sub-hemispherical with the apical groove at the apex, which continues from the ventral side to about half-way down the dorsal side of the epicone (plate I). The sulcus on the epicone (se) starts immediately above the cingulum (explained below), where it forms a small notch, pointed towards the apex in the epicone. From there it extends down to the antapex in the hypocone (plate I).

Hypocone: Sulcus on the hypocone, and the longitudinal flagellum

The hypocone, in ventral view is notched by a sulcus (sh). The sulcus is a wider, deeper groove than the apical groove and occurs in the middle of the hypocone in a vertical direction. It extends from the epicone to the antapex on the hypocone and to the antapex of the dorsal side where it is visible as a small indentation. The hypocone is longer than the epicone. See plate I for these characteristics. The longitudinal flagellum extends vertically down the sulcus on the hypocone (fig. 5).

Cingulum: Transverse flagellum

The cingulum is the 'groove' which divides the cell into epicone (from the cingulum to the apex) and the hypocone (from the cingulum to the antapex). It is supra-centrally positioned, causing the epicone to be smaller in length than the hypocone. It also houses the transverse flagellum, which encircles the entire cell (plate I and fig 5). The cingular displacement is 21% (±3) of the cell length and this ratio varied between 18% and 26%. This ratio was obtained from SEM micrographs.

Surface ornamentation

No striations or pores were visible on the amphiesma.

CYTOLOGY:

Nucleus (nu)

There are two main taxonomic characters associated with the nucleus. These are shape and position.

Shape: The nucleus was found to be ellipsoidal to reniform, ie. wider than long (plate II). It was 11.7um (±2.6) wide, with a range of width from 7.5 to 20.1um. The length was 8.1um (±2.2) and ranged from 4.5 to 12.6um.

Position: The nucleus was located sub-centrally and sometimes centrally (Plate II).

There was no nucleolus visible.

Chromosomes

These were condensed and granular. The chromosome number was found to be 53 (±5) and this varied from 45 to 60. Variation in the chromosome numbers of dinoflagellate species have been found to occur (Dodge 1963) (Plate II) or may be due to error during counting.

Chloroplasts (cl)

They appear to be circular at first glance, but are lobed and L-shaped. The lobes are circular in cross-section and appear to be two chloroplasts (Plate II). There were on average 16 (±3) chloroplasts per cell, but their numbers per cell varied greatly from 10 to 22.

The chloroplasts in the hypocone are aligned around the nucleus, along the periphery of the cell, with a few on the nucleus. In the epicone they were densely and irregularly packed.

No pyrenoid was observed associated with the chloroplast.

OTHER CELL INCLUSIONS:

There was at the antapex, an accumulation of an unknown substance. This formed a thin layer of globules which was clearly visible with the light microscope.

The results for the False Bay organism are summarized in Table 2, which also contains statistics for the species *Gyrodinium aureolum* and *Gymnodinium nagasakiense* for general comparison. Generally, the morphology of *Gymnodinium sp.* conformed to the description of *G. nagasakiense*, but differed in aspects of the cytology of this species.

During the preparation for SEM, a significant amount of shrinkage of the cells occurred. When compared to the LM results, shrinkage amounted to approximately 40% of the cell length and width as measured by LM (Table 1).

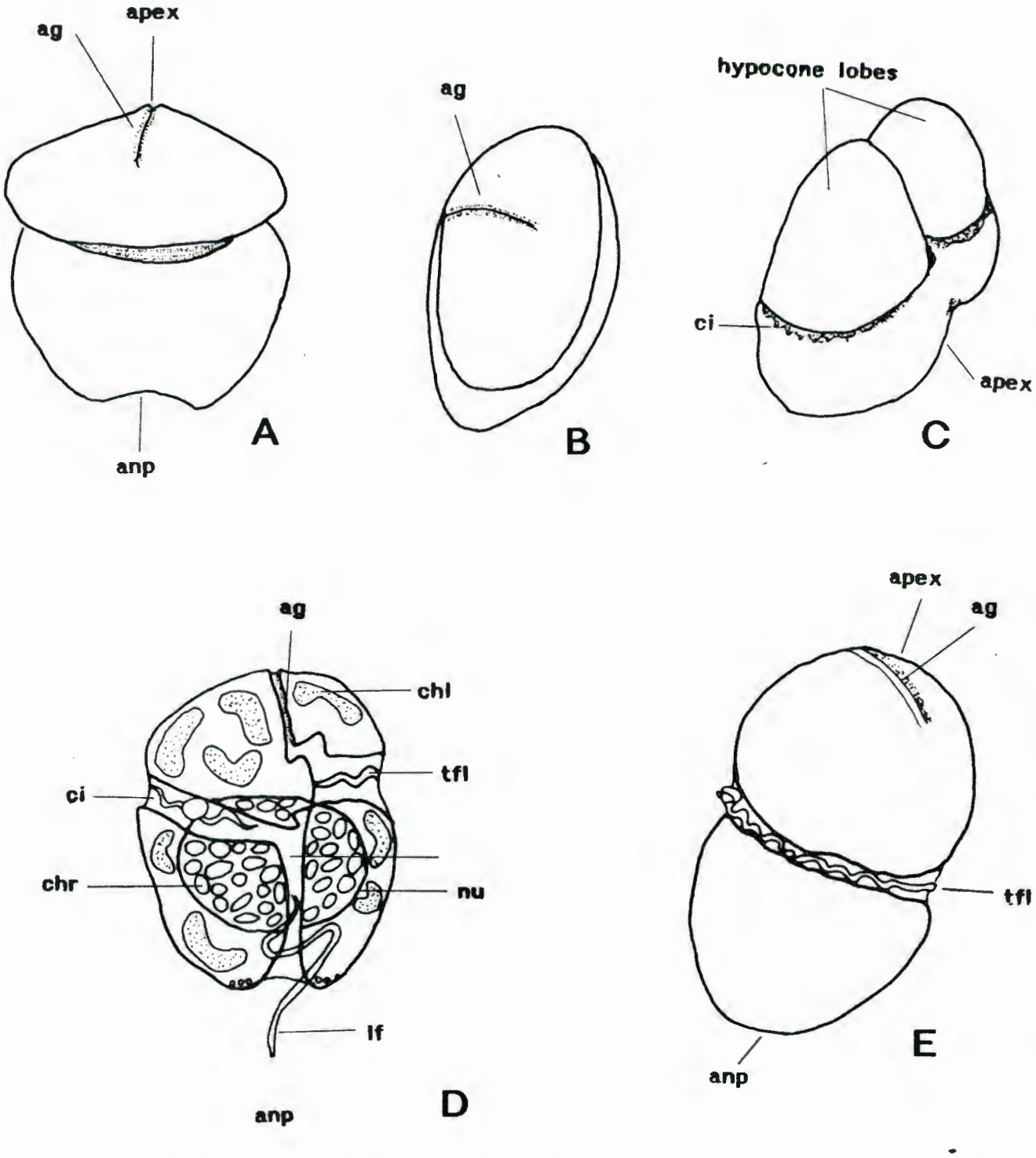
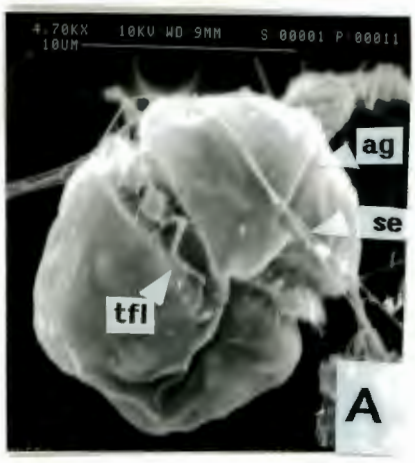
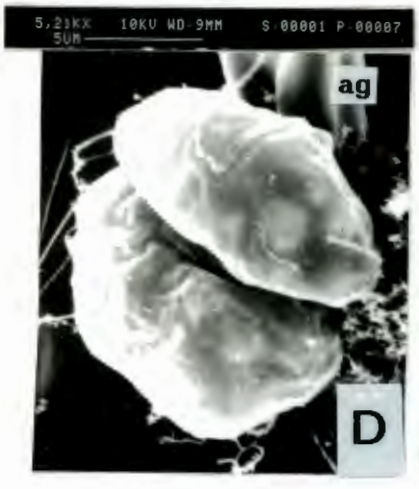
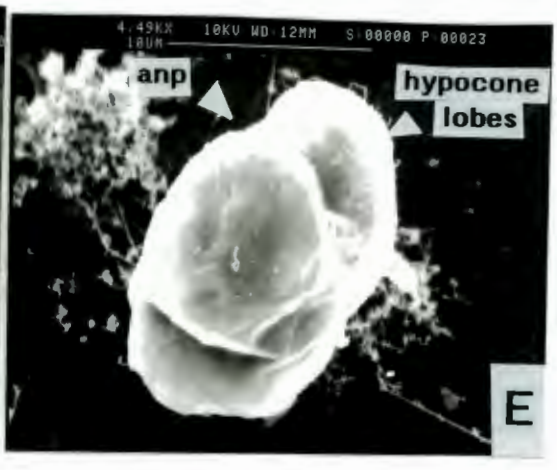
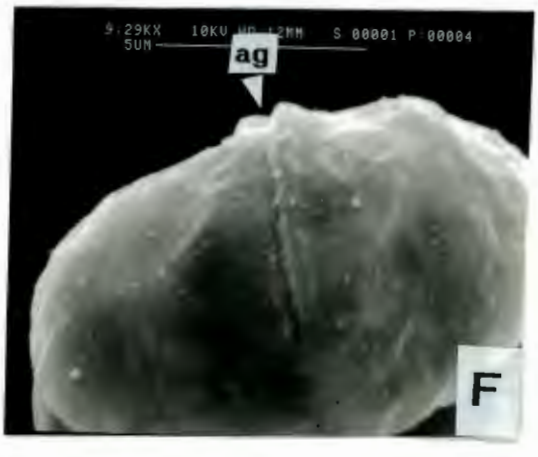


FIGURE 5. Composite line drawings of *Gymnodinium sp.*, from SEM and LM. Depicts the external morphology determined from SEM and the cytology determined from LM. (a) dorsal view (b)apical view (c) antapical view showing hypocone lobes (d) composite of cell in ventral view (e) oblique view showing transverse flagellum. anp-antapex, chr-chromosome, nu-nucleus, ag-apical groove, ci-cingulum, chl-chloroplast, cd-cingulum displacement, lf-longitudinal flagellum, tfl-transverse flagellum









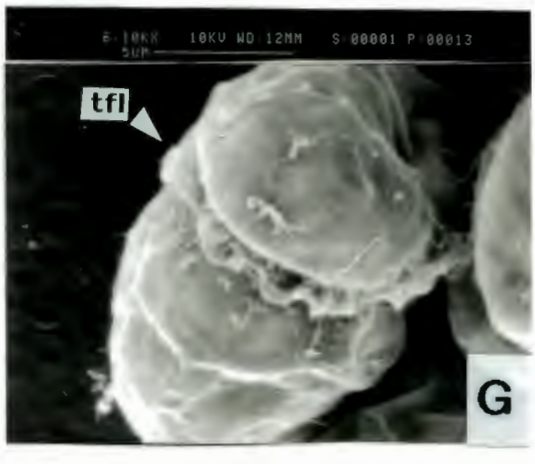


PLATE I: SEM micrographs of *Gymnodinium sp.* (A)ventral view showing sulcus extension, cingulum, apical groove and transverse flagellum, (B) Close-up of A, (C) Apical view showing apical groove and the degree of cell flattening, (D) dorsal view (E)antapical view showing hypocone lobes, (F) close-up of apical groove, (G) lateral view showing the ribbon-like transverse flagellum. anp-antapex, chr-chromosome, nu-nucleus, ag-apical groove, ci-cingulum, chl-chloroplast, cd-cingulum displacement, lf-longitudinal flagellum, tfl-transverse flagellum

se?

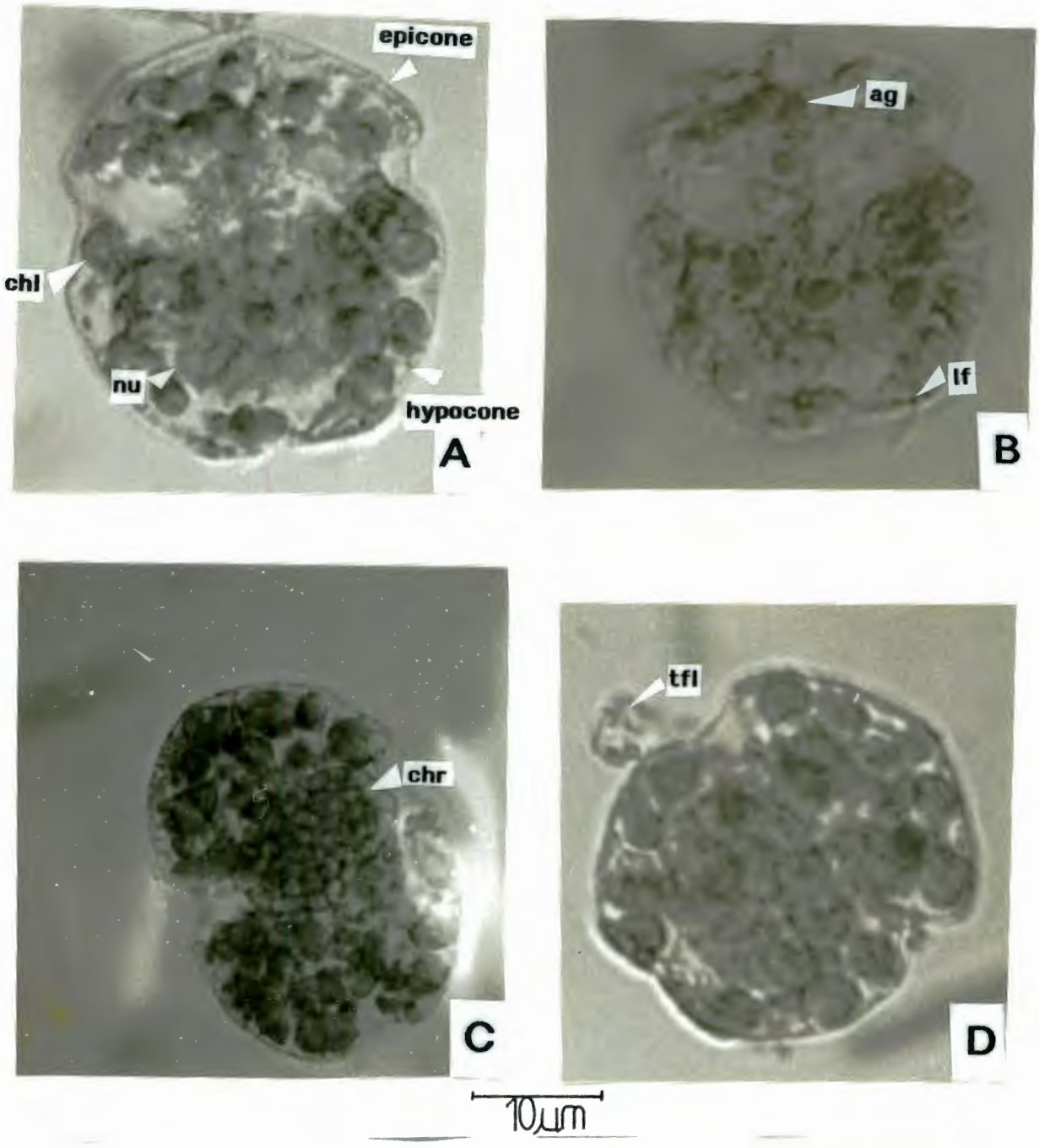


PLATE II: LM micrographs of the cytology of *Gymnodinium sp.* (A) General outline showing the shape of the epicone and the hypocone, the sub-central nucleus and the lobed chloroplasts, (B) the cell outline is more clearly defined, the cingulum displacement is visible , the longitudinal flagellum and the apical groove (C) nucleus with granular chromosomes (D) transverse flagellum anp-antapex, chr-chromosome, nu-nucleus, ag-apical groove, ci-cingulum, chl-chloroplast, cd-cingulum displacement, lf-longitudinal flagellum, tfl-transverse flagellum

Mag **X2000**

	LIGHT MICROSCOPY	SCANNING ELECTRON MICROSC	REDUCTION (%)
CELL LENGTH	24.9 ± 3.8 (n=50)	15.2 ± 2.7 (n=17)	39
CELL WIDTH	22.1 ± 3.7 (n=50)	13.4 ± 2.8 (n=22)	39.5
CELL BREADTH	-	8.1 ± 2.3 (n=6)	-
LENGTH:WIDTH	1.14 ± 0.15 (n=50)	1.10 ± 0.07 (n=15)	
BREADTH:WIDTH	-	0.61 ± 0.11 (n=5)	
NUCLEUS LENGTH	8.1 ± 2.2 (n=48)	-	
NUCLEUS WIDTH	11.7 ± 2.6 (n=48)	-	
CHROMOSOME NUMBER	51 ± 6.5 (n=7)	-	
NUCLEUS POSITION	SUB-CENTRAL	-	
CHLOROPLAST NUMBER	5 ± 3 (n=26)	-	
CHLOROPLAST LENGTH	3.1 ± 0.5 (n=5)	-	
CHLOROPLAST WIDTH	7.0 ± 1.5 (n=5)	-	
CHLOROPLAST SHAPE	LOBED	-	
APICAL GROOVE (VENTRAL)	3.4 ± 0.6 (n=3)	4.9 ± 1.5 (n=11)	46.3
APICAL GROOVE (DORSAL)	-	5.6 ± 0.7 (n=3)	
CINGULUM DISPLACEMENT(CD	-	3.0 ± 0.7 (n=9)	
CD:LENGTH OF CELL		0.21 ± 0.03 (n=8)	
FLAGELLUM WIDTH	-	0.27 ± 0.02 (n=2)	

TABLE 1. Dimensions of *Gymnodinium sp.* Includes average, population standard deviation and the sample number. Measurements were from light microscopy (LM) and scanning electron microscopy (SEM).

	Gymnodinium sp.	Gymnodinium nagasakiense (Partensky et al 1988)	Gymnodinium nagasakiense (Takayama et Adachi 1984)
CELL LENGTH (um) $\bar{X} \pm SE$:	24.9 ± 3.8 (n = 50), LM	28.3 ± 2.8 (n = 58)	25 - 35
RANGE:	10.5 - 39.3	21 - 34	18 - 37
CELL WIDTH (um) $\bar{X} \pm SE$:	22.1 ± 3.7 (n = 50), LM	23.5 ± 2.7 (n = 58)	23 - 33
RANGE:	12.6 - 33.3	16.1 - 29.2	14 - 35
CELL BREADTH (um)	8.1 ± 2.3 (n = 6), SEM		
RANGE:	3.8 - 10.3		
LENGTH : WIDTH	1.1 ± 0.1 (n = 65), LM+SEM	1.2 ± 0.1 (n = 58)	
RANGE:	0.93 - 2.0		
BREADTH : WIDTH	0.6 ± 0.1 (n = 5), SEM		
RANGE:	0.42 - 0.74		33 - 75 %
CINGULUM DISPLACEMENT (um)	3 ± 0.7 (n = 9), SEM		
RANGE:	1.9 - 3.9		
CING.DISPL :LENGTH (%)	21 ± 3 (n = 8), SEM	22 ± 3 (n = 58)	14.3 - 20
RANGE:	18 - 26	17 - 27	11 - 25%
NUCLEUS POSITION	SUB-CENTRAL/ CENTRAL, LM	LEFT	LEFT
NUCLEUS SHAPE	ELLIPTICAL LM	RENIFORM	ELLIPTICAL/ RENIFORM
NUCLEUS LENGTH (um)	8.1 ± 2.2 (n = 48), LM		
RANGE:	4.5 - 12.6		
NUCLEUS WIDTH (um)	11.7 ± 2.6 (n = 48), LM		
RANGE:	7.5 - 20.1		
CHLOROPLAST NO.	15.2 ± 2.7 (n = 26), LM	18 ± 6 (n = 58)	
RANGE:	10 -22		
CHROMOSOME NO.	53 ± 5 (n = 5), LM	117 ± 3 (n = 5)	
RANGE:	45 - 60		
DNA CONTENT(pg)		62	
TOXINS	WATER SOLUBLE HEAT SENSITIVE RESPIRATORY IRRITATIONS		WATER SOLUBLE
FAUNAL MORTALITY	FISH/INVERTEBRATES		FISH KILLS

TABLE 2. Comparison of *Gymnodinium nagasakiense* *Ptychodiscus brevis*. deviation and sample

DISCUSSION:

The approach taken in this discussion is comparative. The *Gymnodinium sp.* is compared to each of the species that it closely resembles. Thereafter, the implications of recent developments in the taxonomy of this group are assessed, including the idea that the Japanese and European species are conspecific. See table 2 for the summary of the comparison.

COMPARISON WITH *PTYCHODISCUS BREVIS* (Davis) Steidinger

P.brevis is distinguished by several features. Foremost is the shape of the apex, which forms a protrusion that is ventrally directed. This structure is called the apical carina (fig 4b). The samples of *Gymnodinium sp.* do not possess an apical carina (fig. 4d and fig.5).

The cell contour of the two species differs as well. *P.brevis* has a square outline and *Gymnodinium sp.* a more rounded appearance. The cells of the former are longer than the latter.

In *P.brevis*, the extension of the sulcus into the epicone is much deeper than in the *Gymnodinium sp.* or *Gymnodinium nagasakiense* (fig. 4).

The cingulum displacement, a prominent feature of *Gymnodinium sp.* (21% of the cell length), appears much less in *P.brevis* (fig 4).

Both organisms produce aerosol toxins. *P.brevis* produces neurotoxins which kill fish and causes respiratory irritation in people and animals. The toxic effect is caused by the lysing of cells, which releases the toxins, and produces airborne particles in the seaspray, which are

carried ashore by the wind (Pierce 1986). A similar effect occurred with the *Gymnodinium sp.* *Gymnodinium sp.* has toxins which were found to be water-soluble and heat labile, whilst *P.brevis* has lipid soluble toxins.

The species in False Bay does not correspond to the description of *P. brevis*.

COMPARISON WITH *GYMNODINIUM NAGASAKIENSE* Takayama et Adachi

Morphologically, *Gymnodinium sp.* is almost indistinguishable from the *G.nagasakiense*, the Japanese species, as described by Takayama and Adachi (1984). It has similar dimensions of body length and width, though slightly shorter on average, than the Japanese and it is slightly less flattened and the cingulum displacement (when taken as a ratio of body length) is slightly more. The only other difference was found in the lower region of the left epicone, at the junction with the sulcus on the epicone. *G.nagasakiense* has a lesser sulcus extension into the epicone than *Gymnodinium sp.*(fig. 4). Even though the morphology pointed to synonymy between the species, their cytology did not corroborate this.

The nucleus position, a major tool for identifying unarmoured dinoflagellates, differed between the two species. In *G.nagasakiense*, the nucleus has always been located on the left side of the cell, whereas in *Gymnodinium sp.* it occurred subcentrally (fig. 4). The shape of the nucleus was found to be similar, although the latter species had nuclei that were more elliptical than reniform. Chromosomes were granular in both. The Japanese species has slightly more than twice the number of chromosomes as *Gymnodinium sp.* The chloroplasts of the two species were similar. The chloroplasts of the Japanese species are described as shapeless (Takayama and Adachi 1984), are

described in Table I and II as L-shaped. The False Bay organism has chloroplasts which are usually lobed or L-shaped as in the Japanese species (fig. 5). The chloroplast number of the local organism is lower, on average 16 as compared with 18 in the Japanese (Partensky et al 1988). The Japanese taxon has external pyrenoids. No such pyrenoids were observed in *Gymnodinium sp.* It may occur internally.

The statistical tests (T-test, Zar 1984) for comparing cell dimensions, showed that the means of cell length, cell width and length:width ratio were significantly different for the two species, even though their standard errors overlapped. Cingulum displacement:length ratio was equal. There was no clear deviation from any of the characters Applying statistical tests for similarity does not help in elucidating affinities. Although cell dimensions are different according to the T-test, the overlapping of the standard errors points to these differences being non-significant. Differences observed could be attributed to the preservation technique employed. The nucleus shape and position and the number of chromomosomes were different. Although morphologically similar to *G.nagasakiense*, *Gymnodinium sp.* is not this species when cytology is considered.

Gymnodinium sp., bloomed in sea temperatures of 12.5°C to 20°C in False Bay. *G.nagasakiense* occurs widely in west Japan and south Korea, mainly in temperatures of between 24° and 29°C, although red tide has been reported for this species at lower temperature conditions (13° to 21 °C). Thus *G.nagasakiense* blooms in tropical as well as temperate waters. The most favourable temperature for reproduction of this species is approximately 26°C in tropical waters. When considering temperature tolerances of these two species, they cannot be distinguished.

COMPARISON WITH *GYRODINIUM AUREOLUM* Hulburt

There were a few differences observed, when compared to the original description by Hulburt (1957). The cells of *G. aureolum* are longer than *Gymnodinium sp.*, but are of very similar widths. The cingulum displacement was 20% of the cell length, slightly less (but not significantly different) than that of *Gymnodinium sp.* (21%). The sulcus on the epicone, was said to extend to the cell apex, when in fact it is the apical groove which extends to the apex. The apical groove is commonly mistaken for the sulcus on the epicone when examination is by light microscopy (Partensky *et al* 1988).

material was fixed!

The chloroplasts were more yellow-green than yellow-brown as described by Hulburt and were lobed and L-shaped, and seldom elliptical as *G. aureolum*. The chloroplasts radiated around the nucleus, in the same manner as *G. aureolum*. The nucleus, was similarly positioned and shaped. Both have nuclei that are wider than long (spherical). Chromosomes were more granular in *Gymnodinium sp.* (ie. less elongate than in Hulburts original description of *G. aureolum*).

Subsequent to Hulburt (1957), this organism has been frequently recorded in European waters and has been the subject of several studies. Tangen (1977) found, as Hulburt described, that the nucleus of *G. aureolum* was normally situated sub-centrally, but it was also situated in the left hypocone during early division stages. In addition, chloroplasts were disc or kidney-shaped and occurred peripherally in the cells. This conforms to the description of the nucleus and chloroplasts of the False Bay organism.

Recently, Partensky *et al.* (1988) investigated the morphology of several strains of *G. aureolum* and compared

this to *G. nagasakiense*. Although the two species were morphologically indistinguishable, they could be differentiated on the basis of DNA content. *G.nagasakiense* has approximately 50% more DNA than *G.aureolum*. Thus, the two species could not be discriminated , using morphological characters for identifying the Gymnodiniaceae (proposed by Partensky and Sournia 1986). Both species were found to have nuclei positioned in the left hypocone, and nuclei were longer than wide. When compared to Hulburts original description of the nucleus, which had a sub-central to central nucleus, this revelation could be explained by several similar species having been included in the species *G. aureolum* as described by Hulburt (1957). Another peculiar characteristic was that the strains investigated were of two distinct size classes. The one was as large as *G. nagasakiense* and the other was distinctly smaller. The mean length and width of *Gymnodinium sp.* was intermediate between the smaller and larger strains of *G. aureolum* investigated by Partensky *et al.* (1988).

If there were several different species which have all been attributed to *G. aureolum*, it would make any comparison and subsequent identification difficult to analyze. The major problem is the nucleus position. From the above studies, *G.aureolum* has been seen to have a central to sub-central nucleus (Hulburt 1957), a sub-central nucleus with the nucleus in the left hypocone during early division stages (Tangen 1977) and nucleus always on the left (Partensky et al 1988). Thus the nucleus position is a controversial taxonomic character for this species.

The statistical analysis, using the T-test (Zar 1984), showed the cell length, width and length:width ratio to be significantly different for *G. aureolum* (Partensky *et al.* 1988) and *Gymnodinium sp.* Once again, the standard errors for cell length, cell width, length:width ratio, cingulum

displacement:length ratio and the number of chloroplasts indicated that these were not significantly different. I conclude that there is no significant difference in cell characteristics measured above. It is curious though, that one study found the nucleus of *G.aureolum* on the left side of the cell (Partensky *et al.* 1988), whilst other studies found the nucleus position in mature cells always to occur centrally or sub-centrally. The False Bay species differs from *G.aureolum* in its toxic effects. *G.aureolum* causes mortality of fish and invertebrates by oxygen depletion of the water. Its toxin(s) are still unidentified. *G.cf. nagasakiense* causes mortality of fish and invertebrates with an unknown toxin(s) and also has an aerosol (airborne) toxin which causes respiratory irritations in humans and animals. .*G.aureolum* has no aerosol toxin.

The False Bay species is very similar to *G.aureolum* in morphology and cytology, but differs in the effects of its toxins.

CONCLUSIONS:

Although *Gymnodinium sp.* in False Bay produced a toxic effect similar to NSP, which is commonly associated with *P. brevis*, it differs from *P. brevis* in several morphological features. It can be concluded that these are two distinct species.

Gymnodinium sp. is morphologically similar to *G.nagasakiense*, but differs in the shape and position of the nucleus, the organisms which it affects and its temperature tolerance. *Gymnodinium sp.* is a distinct species from *G.nagasakiense*.

Gymnodinium sp. is most similar to *G. aureolum*, with respect to morphology and cytology but differs in toxic effects.

I conclude that *Gymnodinium sp.*, the species found in False Bay showed the most affinity to *G. aureolum* and would therefore name it *G.cf. aureolum* since it has the most features in common with the former species. However, *Gymnodinium sp.* may be a new species since it differed from all three species.

The taxonomy of unarmoured dinoflagellates, especially those that produce toxic red tides needs urgent revision. More reliable techniques such as molecular and genetic approaches are needed for the identification of these nuisance species, since conservative (morphological) characters have been found to be less useful at species level. Since South Africa is becoming an increasingly industrialised country, the impact of toxic red tides may increase, which means that a sound taxonomy is essential for the identification, monitoring, and control of these blooms.

GLOSSARY

acronematic: a flagellum that is smooth and terminated with a fine fibril, thus tapered at its distal end.

antapex: the "bottom" or furthest area vertically from the cingulum, when viewed ventrally or dorsally.

apex: the "top" or highest part of the cell, when viewed ventrally or dorsally. Occurs at the section where the apical groove is.

apical groove (ag): syn. acrobase. This is a narrow groove which is present in the epicone (apical region). It extends from the dorsal side of the epicone to the ventral side, as far as the sulcus.

cingulum (ci): a constriction with a transverse orientation which occurs equatorially in the cell. It encircles the entire cell and houses the transverse flagellum. The two ends of the cingulum meet at the ventral side and may be offset. The cingulum divides the cell into hypocone and epicone.

cingulum displacement (cd): syn. girdle displacement. This refers to the offset between the ends of the cingulum, which meet on the ventral side of the cell.

dorsivental flattening: This refers to the width of the cell in lateral view. It is measured as a ratio of the cell width in ventral view.

FLATTENING(%) = LATERAL WIDTH/VENTRAL WIDTH x 100

epicone: Is the half of the cell which extends from the cingulum to the apex, anterior to the cingulum.

hypocone: Is the half of the cell which extends from the cingulum to the antapex, posterior to the cingulum. In ventral view, with the apex at the top, the hypocone is the lower half of the cell.

longitudinal flagellum: This flagellum is housed in the sulcus on the hypocone. It extends towards the antapex.

sulcus: a longitudinal groove on the ventral side of the dinoflagellate cell, which is wider and deeper than the apical groove. Extends from the apical groove in the epicone, where it is known as the sulcus in the epicone (se), to the antapex, where it is called the sulcus in the hypocone (sh). A flagellum is located in this region.

transverse flagellum: This flagellum is housed in the cingulum and has a ribbon-like structure. It encircles the entire cell. It is responsible for propulsion of the cell in an anterior direction.

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