

Thecal Analysis of the Tropical Benthic Dinoflagellate *Gambierdiscus toxicus*

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Introduction

The dinoflagellate, *Gambierdiscus toxicus*, a causative agent in ciguatera poisoning, was originally described from material collected from the Gambier Islands in the Pacific Ocean (Adachi and Fukuyo, 1979). The earliest detection of this species in the Atlantic Ocean was by Sousa e Silva (1956) from plankton collected from the island of Boavista, Cape Verde Islands, in October and November 1948. She referred to this species as an unidentified *Goniodoma*. Along the coastal United States, *G. toxicus* has only been detected along the Florida Keys (Taylor, 1979; Bergmann and Alam, 1981; Besada et al., 1982). No detailed analyses of the morphology of the American isolates of *G. toxicus* have been made, and the only illustration of material collected in continental American waters is a single scanning electron microscopic view of

ABSTRACT—The theca of the benthic dinoflagellate, *Gambierdiscus toxicus*, has been determined, by the means of the chloral hydrate-hydriodic acid-iodine staining technique, to consist of the following plates: pp, 4', 6'', 6c, 8s, 5''', 2'''. The thecal plate overlap pattern, fission line, and position of the ventral pore were used to determine thecal plate homologies rather than relying strictly upon the relative position of a plate before assigning plates to a particular series. *Gambierdiscus toxicus* is considered to be closely related to members of the genera *Coolia* and *Ostreopsis* and the species of these three genera are placed in the family *Ostreopsidaceae*. These generic assignments are based on the possession of common morphological and biochemical characteristics.

an hypotheca (Bergmann and Alam, 1981).

The analysis of *G. toxicus* thecae by Adachi and Fukuyo (1979) of Gambier Island material and Taylor (1979) of Hawaiian material did not encompass a study of plates from disassociated thecae. This technique is necessary to reveal details of the sulcal series as well as spatial relationships of plates in the various series. We present an analysis of the thecae of a Florida isolate using the chloral hydrate-hydriodic acid-iodine staining technique (Stosch, 1969). Our assignment of plates to particular series is based on plate homologies determined by: Overlap patterns, path of the fission line, presence and position of a ventral pore, in addition to the common method of plate enumeration, size, and position. We have not relied strictly on plate position in the theca as the criteria for assigning them to a series, rather choosing to determine most probable homologies of plates when comparing two species. We have previously used these characteristics to reveal homologies in gonyaulacoid and peridinioid plate patterns (Loeblich, 1984; Loeblich and Loeblich, 1979), and have applied this methodology here in an analysis of *G. toxicus*.

Materials and Methods

Gambierdiscus toxicus used in this study was isolated from algal detritus in the intertidal region of the Straits of Florida, Windley Key, Fla., in August 1983. Cells of a clone (F-8) were grown

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in unialgal culture at 27°C in a 12:12 light dark photoregime under a light intensity of 300 ft.c. Culture medium used was a modified GPM medium (Loeblich, 1975), with salinity increased to 33‰, soil extract deleted, and nitrate and phosphate concentrations reduced by one-third. Chloral hydrate-hydriodic acid-iodine theca stain was used as described by Stosch (1969). Photomicrographs were taken on a Leitz Orthoplan¹ microscope equipped with an Orthomat camera.

Results and Discussion

Our findings suggest a thecal tabulation for *G. toxicus* of: Pore plate (pp), apical (4'), precingular (6''), cingular (6c), sulcal (8s), postcingular (5'''), and antapical (2'''). Illustrated in Figure 1 is the plate nomenclature for *G. toxicus*, the thecal plate overlap pattern where detected, and the fission line for this species. The path of the fission line for *G. toxicus* was determined earlier in our laboratory by Besada et al. (1982). It separates the theca into an anterosinistral moiety with the plates pp, apical, 1'', 2'', 1''', 2''', 3''', 1c, 2c, 3c, and a posterodextral moiety with the plates 4c, 5c, 6c, 3'', 4'', 5'', 4''', 5''', antapicals. The path of the fission line among the sulcal plates remains to be determined.

In the epitheca, plates of the posterodextral moiety overlap those of the anterosinistral moiety, while in the hypotheca, plates of the anterosinistral moiety overlie those of the posterodextral moiety. The overlap pattern was

¹Reference to trade names or companies does not imply endorsement by the National Marine Fisheries Service, NOAA

determined from the differential marginal growth in the sutures of two adjacent plates (Fig. 2). The overlap pattern could be deduced from observations of the differential intercalary growth at the margin of the dorsalmost

plates of the precingular and postcingular series. These two dorsal plates overlap the adjacent plates in their respective series as well as the apical and antapical plates that border them. The wide growth band on the plate edges of

thecae from older cells was used to determine that plates with these wide bands were overlying adjacent plates when viewed from the cell's exterior.

The apical pore has a curved slit in the plate that produces a tongue-like projection that is directed toward the third apical plate. An aberrant specimen was found with two apical pores (Fig. 3). An apical pore in the form of a slit is a feature this genus has in common with gonyaulacoids. A ventral pore is also present between plates 6'' and 1'. The above described thecal pattern with its associated plate assignments is gonyaulacoid in nature. See Loeblich and Loeblich (1979) for a discussion of gonyaulacoid nomenclature.

The epitheca (Fig. 4) has three large plates surrounding a pore plate; these are interpreted as three apical plates. The ventral side of the epitheca has two small plates in a precingular position. The smaller of the two plates we interpreted as an apical plate located in a position displaced from the apical pore. The other small plate we interpreted as the last member of the precingular series. The path of the fission line can be used to support the interpretation that there is an apical plate in a precingular position. Fission lines in other species (e.g., *Ostreopsis ovata*, Besada et al., 1982) separate the epithecae such that only the first two precingular plates are partitioned to the anterosinistral moiety. Thus in *G. toxicus*, the extra plate on the anterosinistral moiety is more properly interpreted as a member of the apical series. Figure 5 illustrates an aberrant specimen that has five apical plates due to an extra suture splitting the third apical plate.

The ventral pore in *G. toxicus* lies between the two plates in a precingular position that we interpret as the first apical and the last precingular. The ventral pore when present in gonyaulacoids and dinophysoids is most commonly associated with two apical plates, the first and last. Thus the position of the ventral pore in *G. toxicus* suggests that at least one of the two plates that borders the pore is a homolog of an apical plate despite its position in a precingular location. Illustrated in Figure 6 is a specimen with eight precingular plates; ex-

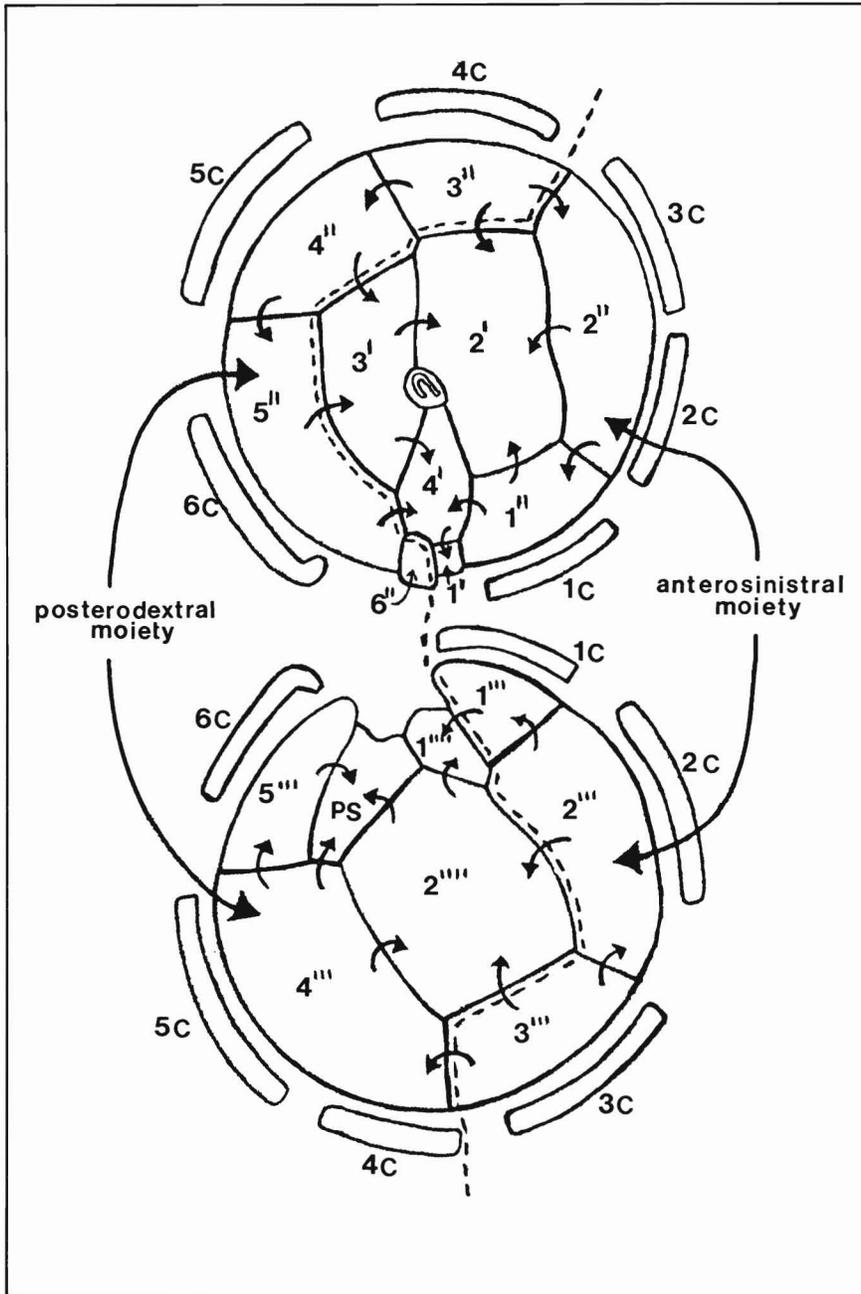
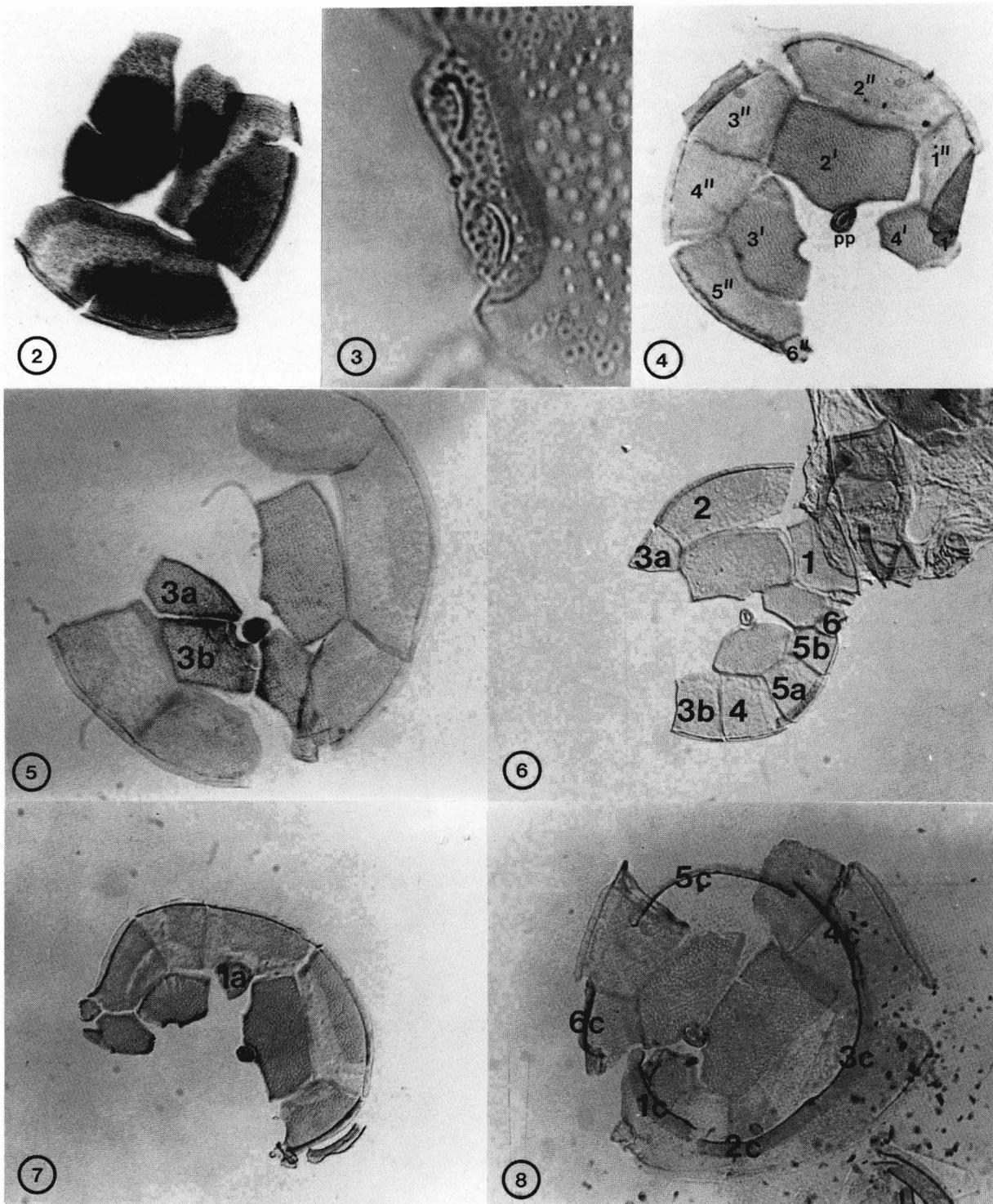
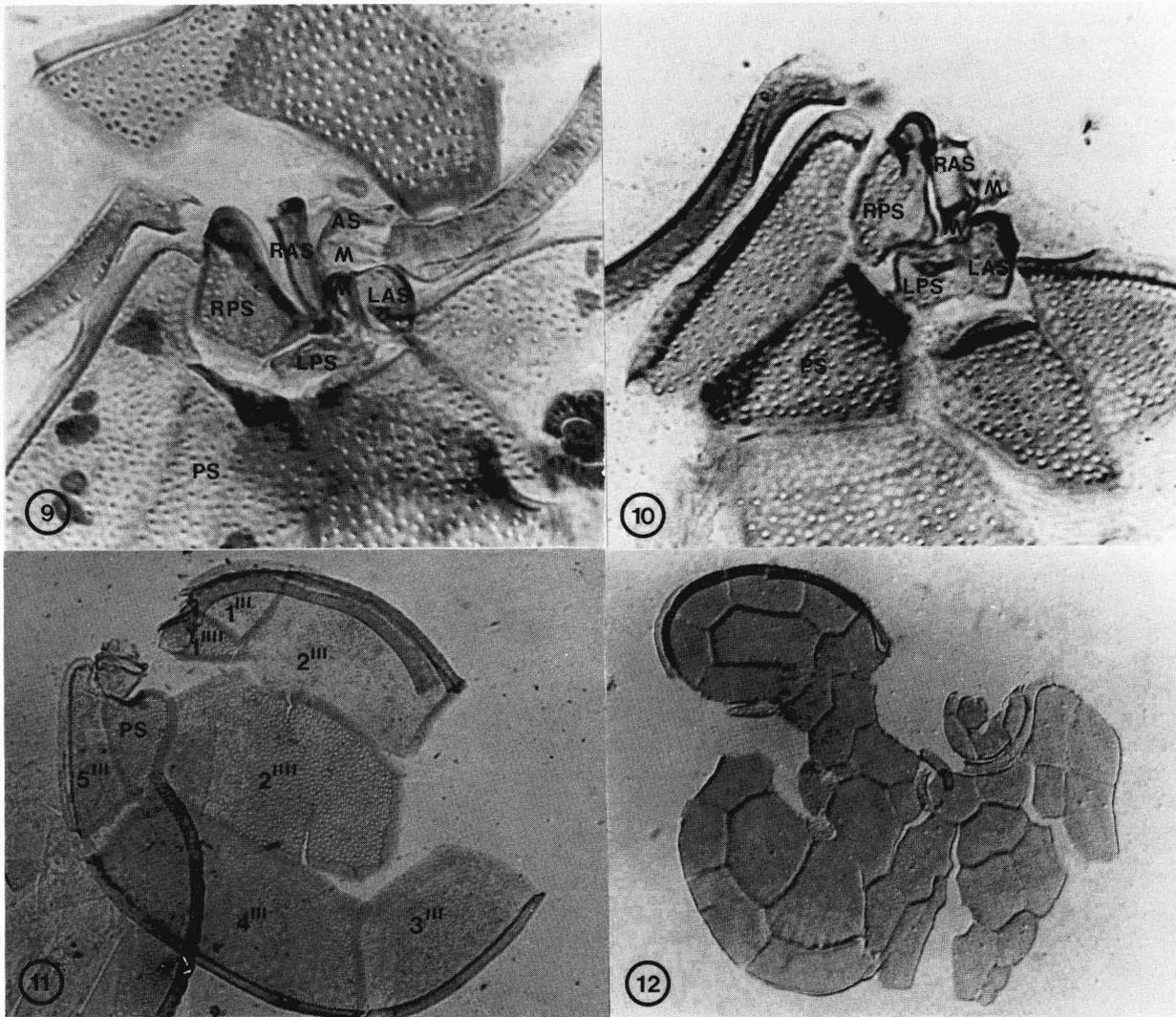


Figure 1.—Diagram of the theca of *Gambierdiscus toxicus* depicting the fission line (dashed), and the overlap pattern (small arrows indicate the direction of plate overlap), epitheca above, hypotheca below.



Figures 2-8.—Chloral hydrate-hydrionic acid-iodine stained theca of *Gambierdiscus toxicus*: Figure 2, marginal growth bands; Figure 3, aberrant theca with two apical pores; Figure 4, the epithecal plates; Figure 5, an aberrant epitheca with an extra suture splitting the third apical plate into two smaller plates (3a and 3b); Figure 6, an aberrant epitheca with eight precingular plates. Plates partitioned by the extra sutures are the third and fifth precingular plates; Figure 7, an aberrant epitheca with an anterior intercalary plate (1a); and Figure 8, an epitheca with the six cingular plates (c).



Figures 9-12.—Chloral hydrate-hydrionic acid-iodine stained theca of *Gambierdiscus toxicus*: Figures 9 and 10, the sulcal series with anterior (as), right anterior (ras), right posterior (rps), left anterior (las), left posterior (lps), posterior (ps) and two medial (m) plates; Figure 11, a hypotheca with a portion of the sulcal plates attached; and Figure 12, an aberrant theca considered to result from incomplete cytokinesis, note the two apical areas (p).

tra sutures occur between the third and fourth precingular plates and between the fifth and sixth precingular plates. The additional sutures can be deduced by comparing the plates that these precingular plates contact in adjacent plate series. Figure 7 illustrates an aberrant cell with an anterior intercalary plate.

The cingulum of *G. toxicus* is composed of six plates (Fig. 8), with the fifth cingular being the largest plate in this series. The last plate of the series

(6c) curves downward at its distal end into the sulcus. The sutures of the cingular series are collinear with those of the precingular and postcingular, except for the suture between 2c and 3c, which occurs in the middle of plate 2'' and the junction of 4c and 5c, which occurs in the middle of plate 4'''.

The pre- and postcingular plate series are each composed of five large plates separated by four sutures, while the six relatively equal cingular plates are sep-

arated by five sutures generating a non-collinear suture of the cingulum on the epitheca and hypotheca (Fig. 1).

The sulcus is composed of six large plates and at least two smaller internal plates (Figs. 9, 10). We were unable to find the two small plates (labelled Sar and Sal) that Adachi and Fukuyo (1979) found at the anterior of the sulcus. Perhaps these could be interpreted as thickening along the margin of the plate where the anterior sulcal contacts the

plates we designated as the 6'' and 1'. Taylor (1979) designated the last member of the postcingular series as a sulcal plate and the left anterior sulcal plate as the first postcingular plate, generating a postcingular series of seven plates. Taylor (1979) also designated the posterior sulcal plate as a third antapical plate. The overlap pattern suggests that this plate in question is the posterior sulcal as it underlies the 5''' and 2''' plates as would be expected of a posterior sulcal plate. Additionally, using Taylor's interpretation of this plate, no other plate in this genus could be interpreted as a posterior sulcal plate. We feel this designation does not reflect obvious thecal homologies when comparing *G. toxicus* to other dinoflagellates. We prefer to consider all plates lining the sulcal region as sulcal plates, especially those that occur within the sulcal depression.

We find the hypotheca (Fig. 11) to have five postcingular plates rather than the interpretation of six plates in this series as suggested by Adachi and Fukuyo (1979) and Taylor (1979). These investigators designate a very small plate located in the sulcal depression on the left side of the sulcus as the first postcingular plate. Such an interpretation is inconsistent when one compares the thecal plate assignments of peridinioids and gonyaulacoids. In peridinioids the plate in this position in the sulcus is a sulcal plate. In an effort to reveal the homologies between the theca of a variety of dinoflagellate genera, we consider those plates that are in the sulcal depression and form a complex encircling the flagellar pore region as sulcal plates. Thus, in our interpretation of the hypotheca of *G. toxicus* we find eight sulcal plates, five postcingular, and two antapical plates.

The hypotheca is interpreted to have two antapicals, the larger of the two located at the posterior of the cell (Fig. 11). We interpret this species to have two antapical plates rather than an antapical and a posterior intercalary plate. This interpretation better reveals homologies of the hypotheca, particularly when comparing peridinioids and gonyaulacoids. Support for interpreting the gonyaulacoid plate commonly referred to as

the posterior intercalary plate as an antapical is derived from the pattern of plate overlap on the hypotheca of both peridinioids and gonyaulacoids. In both these lineages the plate we have labelled as the second antapical overlaps the plate designated as the first antapical. The pattern of overlap of these two plates suggests to us that these two plates in both lineages are homologous plates. The difference in the hypotheca between the peridinioids and gonyaulacoids is in reality only one of the relatively greater size of the gonyaulacoid second antapical in relation to the first.

Our analyses support the hypotheca plate arrangement of Adachi and Fukuyo (1979) in finding that the plate we refer to as the second antapical does not contact the last (5''') postcingular plate. In contrast, Taylor (1979) illustrated a plate he considers the 3'''' that does not completely separate his 6'' and 2'' plates. As both Pacific (Adachi and Fukuyo, 1979) and Atlantic (described here) forms of *G. toxicus* have an identical hypothecal arrangement, Taylor's (1979) illustration of the hypotheca plates on the cell's right is most likely erroneous.

Depicted in Figure 12 is the theca of a cell that is difficult to interpret. It could represent a cell similar to the line drawing that Taylor (1979) presented as illustrating a zygote. An alternate conclusion that we prefer is that this specimen represents an aberrant cell that did not complete cytokinesis but formed a composite wall consisting of thecal plates that would have surrounded the two daughter cells. The presence of two apical pores on this specimen suggests that two thecae are involved, resulting from cell fusion or incomplete cytokinesis.

Assigning plates to particular series is only an aid in identifying and comparing species. We feel that the assignments should be made to reveal the greatest number of similarities between dinoflagellates from different genera and lineages.

What is important is to be consistent from species to species in choosing a number and series to denote the plate. As an aid in making these decisions we recommend that as many criteria as possible should be used in addition to

the widespread reliance on the position of the plate in the theca, e.g., overlap pattern, fission line, and thecal pores (see Loeblich (1984) for a discussion of the homologies of the dinoflagellate theca). An emphasis on detecting the homologies in the theca of one species when compared to another would have avoided the divergent thecal formulas proposed by Adachi and Fukuyo (1979), Taylor (1979), and Besada et al. (1982) for *G. toxicus*.

Gambierdiscus toxicus has a plate pattern that is very similar to the *Ostreopsis* and *Coolia* spp. These species differ in: 1) The relative size of the plates; 2) the shifting of the sutures in these genera, which has resulted in variations in the relative sizes of the plates; and 3) the position of the plates such that a particular plate may contact different plates in the different genera. Despite these thecal differences, a basic, common thecal pattern is apparent in these benthic dinoflagellates.

Interestingly, the sterol composition (Besada, 1982; Loeblich and Loeblich, 1983) and internal anatomy (Besada et al., 1982) also reveal that these species are closely related. The synthesis of dinosterol and cholesterol and their presence in a 1:2 ratio, respectively, in both *Ostreopsis ovata* and *Coolia monotis* suggests that these two genera are closely related and supports Lindemann (1928) in placing *Coolia* in the synonymy of *Ostreopsis*. *Gambierdiscus toxicus*, while possessing a similar ratio of these two sterols, differs in having the additional sterol, 24-methylcholesterol, suggesting that it is more distantly related to *Coolia* and *Ostreopsis* than they are related to each other.

All members of these three genera possess spirally coiled fibers that are vesicle bound (Besada et al., 1982). The absence of these structures in other dinoflagellates and the thecal plate similarities strongly suggest that these three genera are very closely related and perhaps should be considered congeneric. We consider them members of the family *Ostreopsidaceae* and place them in the gonyaulacoid lineage of families.

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