
Should *Gonyaulax hyalina* and *Gonyaulax fragilis* (Dinophyceae) remain two different taxa?

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Abstract :

Escalera *et al.* (2018. *Phycologia* 57: 453–464) concluded that *Gonyaulax hyalina* and *G. fragilis* were the same species. Here, the morphologies of *G. hyalina* and *G. fragilis* were restudied in field samples from different parts of the World Ocean. Results showed that Escalera *et al.* did not observe *G. fragilis*, as all strains used in their study belong to one species, *G. hyalina*. In consequence, it is hereby proposed that the two taxa remain as separate species until further genetic studies are carried out. Additionally, the interpretation of the anterior sulcal plate as unique within gonyaulacoids is considered here inaccurate, as the plate pattern of both species is similar to other species of *Gonyaulax*, e.g. *G. polygramma*. We also show that both species may co-occur in the same sample. Their observation of an anterior intercalary plate in cultures of *G. hyalina* was confirmed in field samples.

Keywords : Anterior sulcal plate, *Gonyaulax polygramma*, Misidentification

Gonyaulax Diesing (Dinophyceae) is a cosmopolitan thecate dinoflagellate genus found in a variety of marine, freshwater and brackish environments (Kofoid 1911, p. 188). It has been extensively studied by both paleontologists and neontologists as some of the extant *Gonyaulax* are related to the cyst-based genera *Spiniferites* Mantell and *Bitectatodinium* Wilson (Fensome *et al.* 1993, p. 93). Mertens & Carbonell-Moore (2018) reviewed the historical study of *Gonyaulax* of which Kofoid (1911) provides an excellent compilation of common species of *Gonyaulax*.

Gonyaulax fragilis (Schütt) Kofoid was first described by Schütt (1895, as *Steiniella fragilis*) from an unspecified location in the Atlantic Ocean. *Gonyaulax hyalina* Ostenfeld & J.Schmidt 1901 was first described by Ostenfeld & Schmidt (1901) from the Red Sea and the Gulf of Aden. Recently, Escalera *et al.* (2018) studied three strains which they considered as two *Gonyaulax* species, namely two strains of *G. hyalina*, one collected from the Tyrrhenian Sea (Mediterranean Sea) and the other from Tasman Bay (New Zealand), and one strain of purportedly *G. fragilis* from the Adriatic Sea (Mediterranean Sea). The isolates of the three strains were not identified initially by Escalera *et al.* (2018). Therefore, there was a possibility that the identifications had not been accurate. Escalera *et al.* (2018) concluded there were no significant morphological differences between the three studied strains. They also found these strains all clustered in the LSU-rDNA-based-phylogeny, and concluded that the two taxa were the same, adopting the name *G. fragilis* following the priority rule of the ICN (Escalera *et al.* used McNeill *et al.* 2012). Because their investigation was limited to only three strains and no field samples were included, we considered it important to investigate field samples. Through the examination of phytoplankton from different parts of the world (Table S1) using scanning electron microscopy (SEM), this note demonstrates that the external morphology of *G. fragilis* and *G. hyalina* is different enough to keep them as separate taxa. All three strains in Escalera *et al.* (2018) corresponded to only one species, *G. hyalina*, as these authors never worked with cells

of *G. fragilis*. Phytoplankton samples were prepared for SEM following Carbonell-Moore (2017). In addition, cells were treated with hexamethyldisilazane (HMDS) for one minute after dehydration with 5%, 20% 35% 60%, 80% and 100% ethanol to maintain thecal integrity. The plate nomenclature used here is an adaption of the nomenclature for Gonyaulacales suggested by Alvarez *et al.* (2016) and used by Mertens *et al.* (2018).

Figs 1 and 2 show scanning electron micrographs of *G. fragilis* and *G. hyalina* respectively. The agreement of external morphology with those of the original description and illustrations (or protologues) by Schütt and Ostenfeld & Schmidt, respectively, is remarkable (Figs 3 and 5). Although their plate pattern is the same (see Figs 9–12), the cell shape, plate ornamentation and size are not the same. *Gonyaulax fragilis* (Fig. 1) has a more tapered epitheca than *G. hyalina* (Fig. 2). In the case of Figs 1 and 2, *G. fragilis* is much longer (83 μm) than *G. hyalina* (53 μm), and relatively narrower (65 μm versus 51 μm , respectively). As only seven cells of *G. fragilis* versus 14 of *G. hyalina* were studied, a range of dimensions for *G. fragilis* cannot be established with certainty. However, *G. hyalina* presented much smaller sizes (24–53 μm long, $n = 14$) than *G. fragilis* (41–83 μm long, $n = 7$), which does not agree with Ostenfeld & J. Schmidt's length of 92 μm for the holotype. On the other hand, literature accounts have reported *G. hyalina* with a shorter cell than *G. fragilis* (see Escalera *et al.* 2018, their Table 1). However, given the difficulty identifying these taxa, the information in the literature should be treated with caution. For most authors, though, e.g. Kofoid (1911), Lebour (1925) or Balech (1962), *G. fragilis* is much longer than *G. hyalina*.

The plate pattern ornamentation is in all likelihood, the most striking difference. Though both species have long parallel ridges running antero-posteriorly, as well as short transversal ridges, it is the number and disposition of the short transversal ridges that makes them quite different. *Gonyaulax hyalina* shows many more of these short ridges, to the extent that

ornamentation appears as a rugged topographic map with many close contour lines (e.g. Figs S2, S3, S6, S9, S18, S20). This is not the case of *G. fragilis* (e.g. Figs S1, S4, S5, S7, S8, S11). In addition, the width and ornamentation of the growth bands is distinct in *G. hyalina* (e.g. Figs S2, S3, S9, S20, S22) while in *G. fragilis* they are simpler (e.g. Figs S7, S19, S23). The overlapping margins along precingular and postcingular plates are also different. For example, in the hypothecae of *G. fragilis* in Figs S19, S21 and S23, the overlapping margin of plate *2' over 1p is shown as a narrow ridge with mostly transverse lines. Figures S20, S22 and S24 of *G. hyalina* show this overlapping margin as mostly a series of long parallel lines.

It may be argued that in gonyaulacoids plate ornamentation changes with cell development as shown in *G. polygramma* (Taylor 1962) and *Pyrodinium bahamense* (Mertens *et al.* 2015). While Fig. 1 depicts a less-developed cell of *G. fragilis* (very similar to Schütt's protologue, Fig. 3 this study), Figs S1 and S5 display a well-developed cell of *G. fragilis*. The cell shows large flanges along not only the sutures but also on the plate surface, e.g. Fig. S1 on plate *6". These flanges were never observed in cells of *G. hyalina*. Despite of its large development, this cell of *G. fragilis* shows an ornamentation not much different from cells with more fragile thecae of the same species, i.e. less-developed cells (e.g. Figs S4, S7). None of the SEM images shown in Escalera *et al.* 2018 shows any resemblance to our *G. fragilis*, neither in shape or plate ornamentation, while all their SEM images agree with our images of *G. hyalina*. This indicates that the strain of *G. fragilis* they used was misidentified.

While the presence of *G. hyalina* in the Tasman Sea had been confirmed by MacKenzie *et al.* (2002), the cells of *G. fragilis* studied by Honsell *et al.* (1992) may suggest the presence of both species in the Adriatic Sea. Of note is that *G. hyalina's* typical ornamentation can be observed in their figs 4–6 at the same time that fig. 2 shows a light microscope image of a cell with the typical shape of *G. fragilis*.

The misidentification of the strain of the Adriatic Sea (GFA0203) as *G. fragilis* lead Escalera *et al.* to incorrectly conclude that all the strains belonged to a same species, which was confirmed by the sequences. The identifications were solely based upon cell shape (Escalera *et al.* 2018, p. 460).

In addition, the interpretation of Escalera *et al.* (2018) of the anterior sulcal plate (Sa) in *G. hyalina* is inaccurate. These authors labeled plate *4'p as the Sa, failing to see that this is the homologue of plate *4'p found in *G. polygramma* F.Stein (Figs 6–8). Plate Sa is always the plate that conforms the anterior part of the sulcus and is a conservative plate (Graham 1942, p. 7). It is safe to say that the anterior sulcal plate is never found above the sixth precingular plate because it is a sulcal plate associated with the flagellar pore. Figs 9–13 show the corrected plate pattern tabulation of both species in ventral and apical views including the sulcal region (Fig. 13). This was based on different images of cells in Figs S1–S18. Only those images identified as *G. hyalina* (e.g. Figs S14, S15, S18) agree with Escalera *et al.*'s images.

The close relationship of *G. fragilis* observed by Escalera *et al.* in the phylogenetic tree (their fig. 2) with *G. polygramma* is reflected in having the similar shape and size of the apical plates, especially plates *4'a and *4'p (Figs 6-8, S27-S30). Many *Gonyaulax* species follow *G. spinifera*'s pattern, where plates *4'a and *4'p are narrow (Figs 4, S25–S26).

The identification of *G. fragilis* and *G. hyalina* using light microscopy is not easy. Taylor (1976) in his monograph of dinoflagellates of the Indian Ocean, reported *G. fragilis* from only one station. However, the illustration he gave of this species (Fig. S36) is closer to his illustration of another species of *Gonyaulax* (Fig. S37) and far from our *G. fragilis* also from the Indian Ocean (Figs 1, S4, S8). On the other hand, some of his illustrations of *G. hyalina* may correspond to *G. fragilis* (Figs S33–S35 here). Balech (1962) illustrated a well-developed *G. fragilis* (Fig. S31) showing plate *4'p in the same position as our observations. With respect to

the sulcus, Balech just mentioned that it narrowed anteriorly, and widened posteriorly as shown in the present study (Fig. 13). Rampi (1943, his fig. 13) also identified *G. fragilis* from the Ligurian Sea (Mediterranean Sea), which also agrees with our observations of this species.

To conclude:

1. It is recommended here that *Gonyaulax fragilis* and *G. hyalina* should be kept as separate species. Escalera *et al.* 2018 studied only one of these species, namely *G. hyalina*. Further molecular studies would reconfirm the separation of both species.
2. The thecal plate pattern of *G. fragilis* and *G. hyalina* is corrected here (Figs. 9 and 10) reflecting our observations of cells from field material. It includes the accurate position of the anterior sulcal plate and the typical ventral pore (VP) of all *Gonyaulax* species, which was not observed by Escalera *et al.* (2018).
3. The SEM images of *G. fragilis* presented here are the first SEM images of this species in the literature. Those shown by Honsell *et al.* (1992, their figs 4–6) display the distinct ornamentation of *G. hyalina* not that of *G. fragilis*.
4. Escalera *et al.* (2018, fig. 12) found a small anterior intercalary plate surrounded by plates 2', *3' and 4'' which was also observed in our samples of *G. hyalina* (Fig. S18). At this point, we are unable to confirm its presence or absence in *G. fragilis*. This feature also needs to be investigated in other species similar to *G. polygramma* (Fig. S28), where plate *4'p is wide and short, e.g. *G. pacifica* Kofoid (Fig. S29), *G. aff. alaskensis* Kofoid (Fig. S30), or *G. turbynei* Murray & Whitting (Fig. S27), among others.
5. This study suggests that the two species can coexist, and can be found not only in the same ocean, but sometimes, in the same sample. For example, cells shown in Fig. 1 of *G. fragilis* and Fig. S20 of *G. hyalina* were observed in the same sample;

likewise, the cell shown in figure S7 of *G fragilis*, was found in the same sample of cells of *G. hyalina* (Figs 8, S3, S6) (see Table 1S). Figure 2 in Honsell *et al.* (1992) might be *G. fragilis*, while their figs 4–6 display the typical ornamentation of *G. hyalina*.

6. The identification of an isolated cell of either species for culturing, must be ratified with SEM. A light microscopy (LM) identification during isolation proceedings should not be sufficient unless the dinoflagellate species in question can be unequivocally identified under LM, which is neither the case of *G. fragilis* nor *G. hyalina*.

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FIGURE CAPTIONS

Figs. 1–8. Comparison of *Gonyaulax fragilis* and *G. hyalina* with other *Gonyaulax* species.

Fig. 1. Scanning electron photomicrograph (SEM) of *G. fragilis*. Ventral view. Cell from the Indian Ocean. Compare cell outline similarity with Schütt's drawing from 1896 (Fig. 3). White arrowhead points to plate *4' p, which was interpreted by Escalera *et al.* (2018) as the anterior sulcal (Sa).

Fig. 2. SEM of *G. hyalina*. Ventral view. Cell from the central equatorial Pacific Ocean.

Compare similarity of cell outline with original illustration by Ostenfeld & J. Schmidt (1901) (Fig. 5). Black arrowhead points to plate *4' p, which was interpreted by Escalera *et al.* (2018) as the anterior sulcal (Sa).

Fig. 3. Schütt's drawing of *Steniella fragilis* [= *G. fragilis* (Schütt) Kofoid] from 1896, ventral view.

Fig. 4. Close-up of ventral area of a cell of *G. spinifera* (SEM), Mediterranean Sea; same cell as Fig. S26. Notice narrowness of plates *4' a, p and 6" compared to homologues in *G. polygramma* (Fig. 7).

Fig. 5. Original drawing of ventral view of *G. hyalina* by Ostenfeld & J. Schmidt (1902).

Fig. 6. Close-up of ventral area of same cell of *G. fragilis* in Fig. 1. Note similarity of plates *4' a, p and 6" to those in *G. polygramma* (Fig. 7), and much wider than in *G. spinifera* (Fig. 4).

Fig. 7. Close-up of ventral area of *G. polygramma* (SEM). Note wider plates *4' a, p and 6" compared to homologues in *G. spinifera* (Fig. 4). Cell from the central equatorial Pacific Ocean.

Fig. 8. Close-up of ventral area of *G. hyalina* from Indian Ocean (SEM), same cell as Fig. S18. Note similarity of plates *4' a, p and 6'' to homologues in *G. polygramma* (Fig. 7), and much wider than in *G. spinifera* (Fig. 4).

Scale bars: Figs 1, 2: 20 μm ; Figs 6, 7: 10 μm ; Figs 4, 8: 5 μm .

Figs 9–12. Line drawings of plate pattern tabulation of *Gonyaulax fragilis* and *G. hyalina*.

Cingular plates not included. For sulcal area, refer to Fig. 13. A combination of Kofoidean and plate homology nomenclature is used (see text for details). 1': first apical plate. *3': homologue of the third apical plate; *4'a: homologue of the anterior fourth apical plate. *4'p: homologue of the posterior fourth apical plate; Sa: anterior sulcal plate; Ssa: left anterior sulcal plate; 1p: first posterior intercalary plate; *2''', *5''', *6''': homologues of the second, fifth and sixth postcingular plates, respectively.

Fig. 9. Ventral view of *G. fragilis*. Dash lines indicate sulcal plates which are usually hidden.

Fig. 10. Ventral view of *G. hyalina*. Dash lines indicate sulcal plates which are usually hidden.

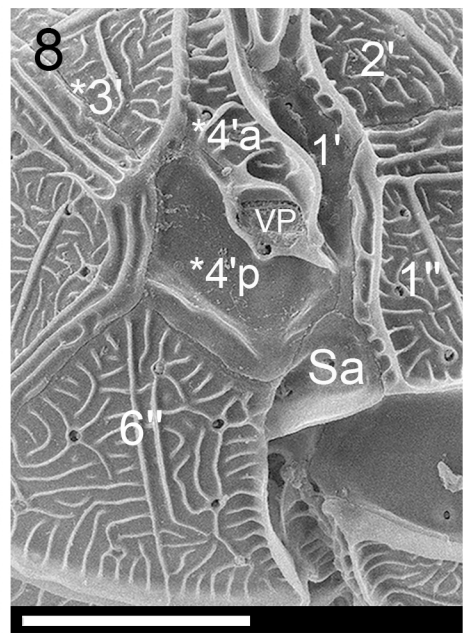
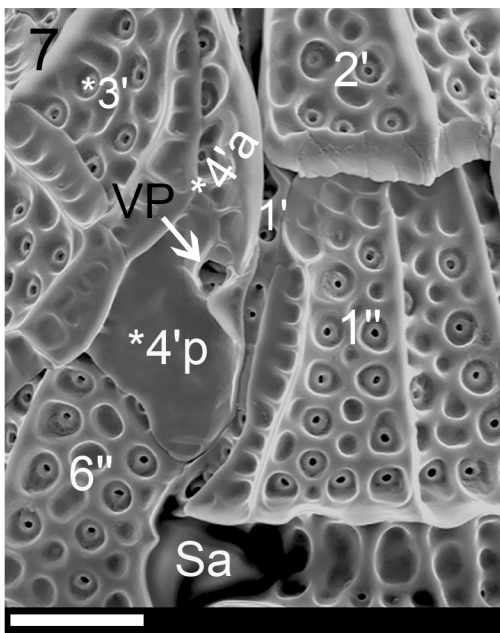
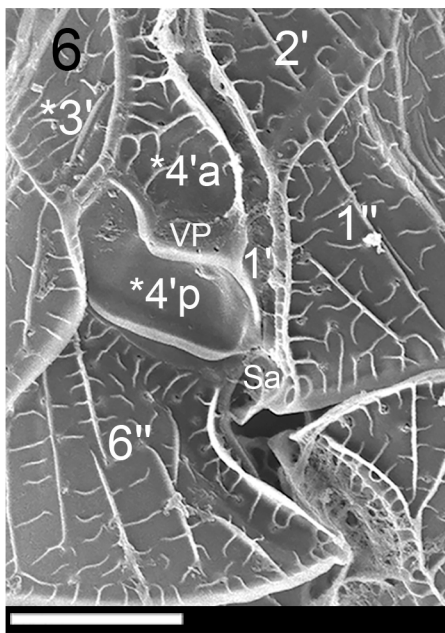
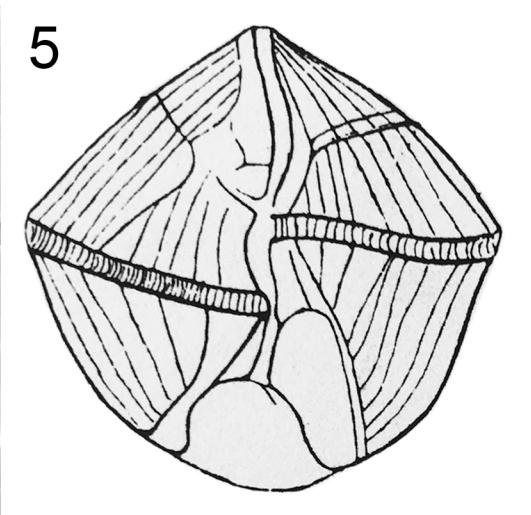
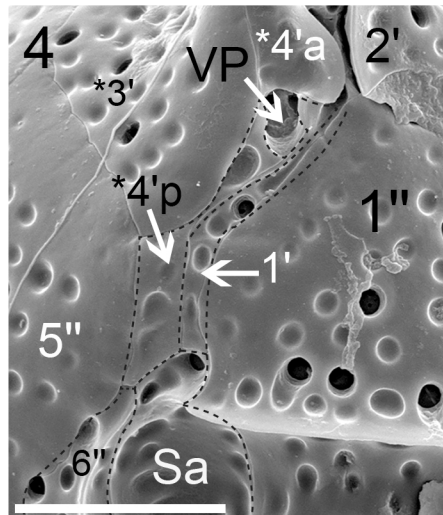
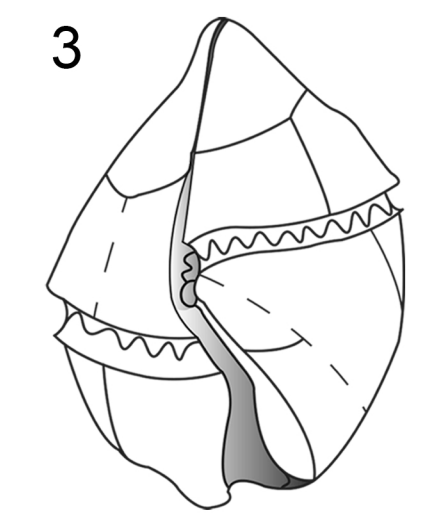
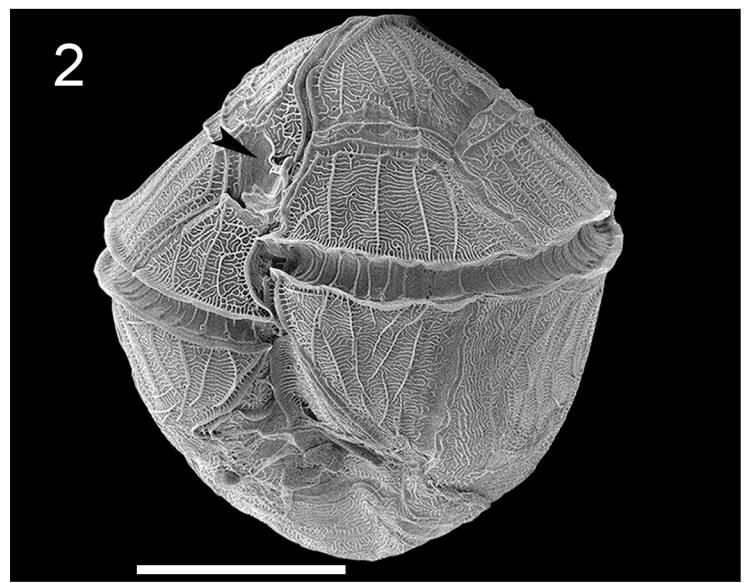
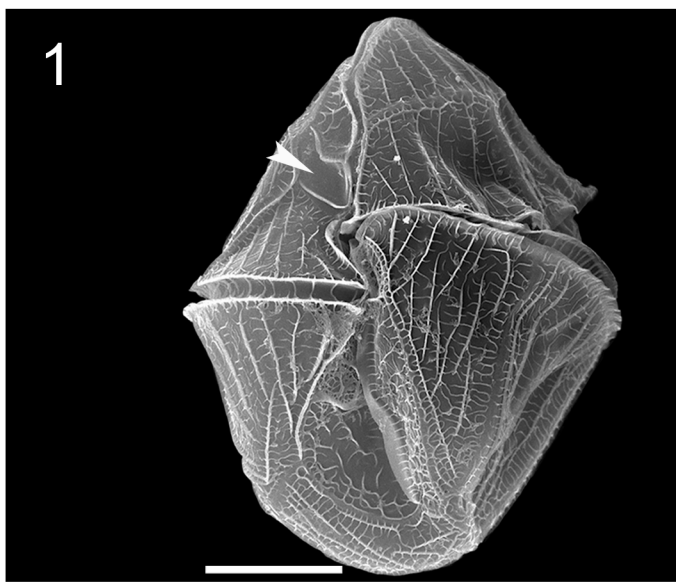
Fig. 11. Apical view of *G. fragilis*. Dash lines indicate where plate 1a should be, whose presence was not confirmed.

Fig. 12. Apical view of *G. hyalina* with conspicuous plate 1a. Epithecical plate nomenclature is the same used by Escalera *et al.* (2018). Hypothecal plate nomenclature follows that used by Mertens *et al.* (2018).

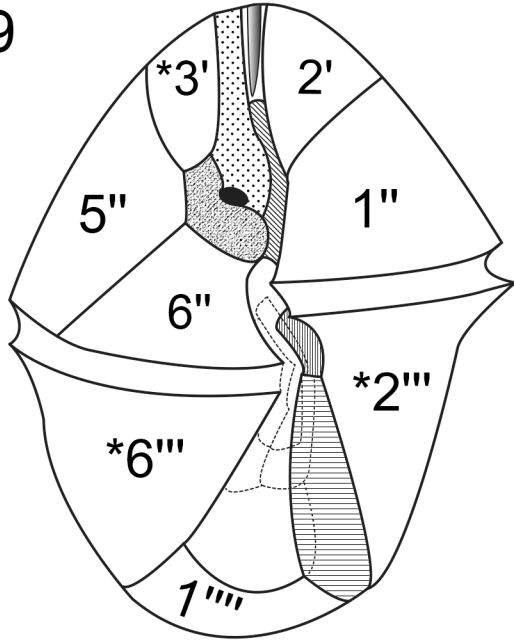
Fig. 13. Line drawings of the sulcal area of a typical gonyaulacoid as represented by the sulcus of *Gonyaulax fragilis* (similar in *G. hyalina*) with flagella pore area shown in grey. Dash lines indicate sutures not visible generally as they lay under sulcal list of plate 1p.

Sa: anterior sulcal plate; Sda: right anterior sulcal plate; Sdp: right posterior sulcal plate; Sp: posterior sulcal plate; Ssa: left anterior sulcal plate; Ssp: left posterior sulcal plate; 1p: first

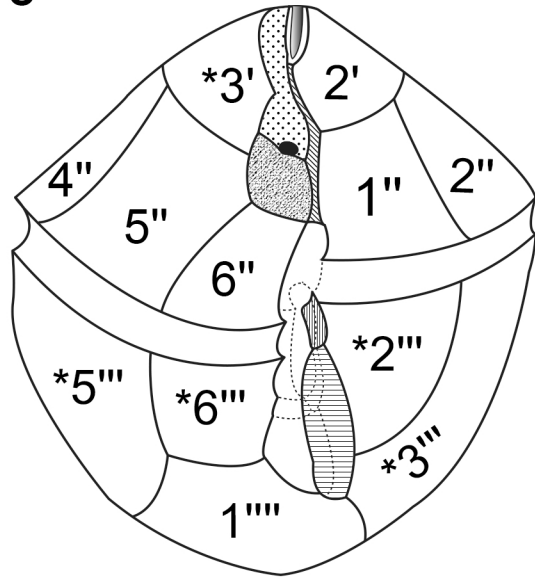
posterior intercalary plate; C_n : n cingular plate; *4' p: homologue of the posterior fourth apical plate.



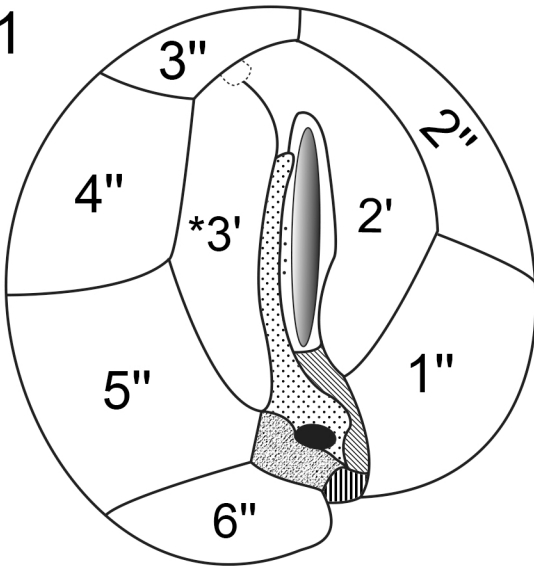
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