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**NOTE**

# FIRST REPORT OF FOSSILIZED CYSTS PRODUCED BY THE BENTHIC *BYSMATRUM SUBSALSUM* (DINOPHYCEAE) FROM A SHALLOW MEXICAN LAGOON IN THE GULF OF MEXICO[1]

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**Cysts belonging to the benthic dinoflagellate *Bysmatrum subsalsum* were recovered from palynologically treated sediments collected in the Alvarado Lagoon (southwestern Gulf of Mexico). The cysts are proximate, reflecting the features of the parent thecal stage, and their autofluorescence implies a dinosporin composition similar to the cyst walls of phototrophic species. This finding is important for our understanding of *B. subsalsum* life cycle transitions and ecology. Encystment may play an important role in the bloom dynamics of this species as it can enable the formation of a sediment cyst bank that allows reinoculation of the water column when conditions become favorable. This is the first report of a fossilized cyst produced by a benthic dinoflagellate recovered from sub-recent sediments.**

*Key index words:* **Alvarado Lagoon; dinosporin; palynology; red tide; resting cyst**

Benthic dinoflagellates belonging to the genus *Bysmatrum* are photosynthetic marine species that are typically observed in shallow tropical and subtropical environments (Lombard and Capon 1971, Horiguchi and Chihara 1983, Horiguchi and Pienaar 1988, Faust and Steidinger 1998), where they have been associated with the formation of red tides (Lombard and Capon 1971, Horiguchi and Pienaar 1988, Faust 1996). Although originally classified as *Scrippsiella* (Faust 1996, Steidinger and Tangen 1996), the species were subsequently transferred to the genus *Bysmatrum* on the basis of their reticulated thecal surface and plate configuration: the intercalary plates 2a and 3a are separated from each other by the apical plate 3′, a morphological feature that is not present in *Scrippsiella* (Faust and Steidinger 1998, Murray et al. 2006). In addition to these morphological differences, molecular sequencing analyses recently confirmed that *Bysmatrum* is genetically different from *Scrippsiella,* and forms an isolated and uncertain position outside the Thoracosphaeraceae (Gottschling et al. 2012, Jeong et al. 2012).

About 15% of living planktic dinoflagellate species is known to produce fossilizable organic-walled or calcareous resting cysts (Head 1996). However, cysts produced by benthic dinoflagellates have only been reported from culture experiments, but never from sub-surface sediments. For example, hyaline division cysts have been described in cultures of *Prorocentrum* species, but resting cyst descriptions are considered questionable (Hoppenrath et al. 2013). For *Ostreopsis* species, only pellicle cysts have been reported (Bravo et al. 2012), and the cysts described from the related species *Coolia monotis* by Faust (1992) are also probably pellicle cysts (Bravo and Figueroa 2014). In the genus *Bysmatrum,* an ovoid cyst was reported for *Bysmatrum subsalsum* by Ostenfeld (1908) (as *Peridinium subsalsum*). However, he did not provide a drawing or photograph, and doubt still persists whether this was indeed a resting cyst instead of pellicle cyst. More recently, coccoid stages of *B. subsalsum* were reported from culture experiments (Gottschling et al. 2012) and an unidentified *Bysmatrum* species was germinated from an undescribed cyst in (sub-)surface sediments from

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an estuarine bay in the Mediterranean (Satta et al. 2013). Here, we report well-preserved cysts of *B. subsalsum* extracted from sediments that show autofluorescence and withstand standard palynological processing.

Samples were taken at a 1 cm interval from a 50 cm sediment core recovered from the Alvarado Lagoon (1.5 m water depth) in 2011 (Veracruz, Mexico; 18.7979° N; 95.8579° W). A sampling resolution of ~2–4 years was calculated from average sediment accumulation rates (0.03–0.61 cm/year) from $^{210}$Pb measurements (Fig. 1). Sampling was performed using a gravity corer (UWITEC) with a clear PVC liner (9 cm diameter) and samples were kept refrigerated at 4°C until analyses. During palynological treatment, ~5 g of dried sediment was wet sieved between 106 and 10 μm to remove the coarse and fine fraction. The remaining fraction was then submitted to repeated macerations with warm HCl (10%) and warm HF (49%) to remove carbonate and silicate particles, respectively. This treatment includes maceration with HF overnight (for details see de Vernal et al. 1996). The organic residue was sieved again on a 10 μm nylon mesh and the retained fraction was mounted in glycerine gelatin for observation under a Leica DMR light microscope (Wetzlar, Germany) and a Leitz Orthoplan fluorescence microscope. Color staining was not applied to avoid imparting artificial fluorescence. One specimen was isolated using a micropipette onto a glass slide, subsequently air-dried, and observed with a Hitachi S-3400N scanning electron microscope (SEM, Chiyoda, Tokyo, Japan; Fig. 2). Specimens were not coated before observation.

Twenty-six cysts were observed in samples during routine palynological counting from the upper 22 cm of the core. This interval corresponds to ~170 years (Fig. 1). According to the fairly low- to-moderate sedimentary organic carbon contents (0.4%–1.2%) and palynological observations, there is no reason to believe that the presence of the observed cysts is related to exceptionally good preservation of organic material (Fig. 1). All specimens exhibit yellowish autofluorescence under excitation by blue light and this allows visualization of the paratabulation (Fig. 3, micrographs 6, 12 and 14). While autofluorescence in living cells is generally associated with chlorophyll or other intracellular bodies (Lessard and Swift 1986), the thecal plates of living cells are not autofluorescent, and need staining with Calcofluor White to observe the bright blue fluorescence of cellulose (Fritz and Triemer 1985, Sekida et al. 1999). On the other hand, the wall material of organic-walled dinoflagellate cysts consists of dinosporin, which in phototrophic species is likely a complex carbohydrate-based polymer (Versteegh et al. 2012, Bogus et al. 2014) that exhibits autofluorescence in the yellow to green spectrum (Brenner and Biebow 2001). All dinoflagellate cysts that show autofluorescence are considered phototrophic (Brenner and Biebow 2001, Bogus et al. 2014). Accordingly, the observed autofluorescence pattern in our specimens is likely due to the carbohydrate composition of the outer wall, which suggests it is a cyst stage produced by a phototrophic species.

Observations with the transmitted light microscope and SEM reveal that the cysts belong to the benthic dinoflagellate *B. subsalsum* (Ostenfeld 1908, Steidinger and Balech 1977, Faust and Steidinger 1998), a species that was first observed and described in the Gulf of Mexico by Steidinger and Balech (1977; as *Scrippsiella subsalsa*) (Figs. 2 and 3). The main characters differentiating *B. subsalsum* from other *Bysmatrum* species (*B. teres*, *B. granulosum*, *B. arenicola* and *B. caponii*) are its general pentagonal morphology, the presence of striations and cross-reticulation on the paraplates, the pentagonal shape of paraplates 1a, 2a and 3a, and the apical pore complex morphology, which resembles a teardrop (see Murray et al. 2006, their table 1 for a comparison of the five *Bysmatrum* species). Cysts observed herein, are proximate and pentagonal in ventral view with the epicyst and hypocyst being more or less conical and trapezoidal, respectively, and of equal length (Fig. 3, micrographs 9–10). There is a pronounced apical horn and two antapical horns. The cysts are ~35.2–48 μm in length (average 44.0 μm; SD: 5.65; $n = 5$) and ~35.2–53 μm in width (average 42 μm; SD: 5.01; $n = 6$). In some cases, the remainder of a red body is observed inside the cyst (Fig. 3, micrograph 4). The formation of such red bodies has been associated with the breakdown of the cell's metabolic activity (Bravo and Figueroa 2014). The cyst wall is composed of two layers that are often tightly appressed. The paratabulation is readily observable on the outer layer and is characterized by a prominent, teardrop-shaped apical pore complex and tabulation formula typical for the genus: Po, X, 4′, 3a, 7″, 6c, 5‴, 2⁗. Intercalary paraplates 2a and 3a, which are separated by apical paraplate 3′, are pentagonal in shape. The paracingulum is relatively narrow and deep, but its vertical displacement is hard to estimate due to compression. Details of the parasulcus and paracingulum are difficult to observe because of the "principle of minority suppression" in cysts (Evitt 1985, p. 157). The surface of the paraplates bears longitudinal striations and sometimes shows smaller cross-reticulations (Fig. 3, micrographs 3, 5, 11, 13). Parasutures are relatively large and also bear striations. The archeopyle is epicystal.

Our results suggest that, over the course of its life cycle, the benthic dinoflagellate *B. subsalsum* produces organic-walled cysts that are preserved in the sediment for at least 170 years. The autofluorescence of the wall suggests that a phototrophic dinoflagellate produced the cysts. Moreover, a good preservation of the cysts after palynological treatment implies some degree of higher preservation potential and suggests a more robust dinosporin

composition, similar to what has been described for other resistant phototrophic cysts belonging to planktonic dinoflagellates. Further research will be needed to determine whether encystment occurs during sexual or asexual reproduction (Bravo and Figueroa 2014). However, in both cases, it may allow survival during adverse conditions and play an important role for the seeding strategy of this species. Since *Bysmatrum* species have been associated with the formation of non-toxic red tides, the ability to form cysts could be important with respect to the initiation, dispersion and termination of blooms

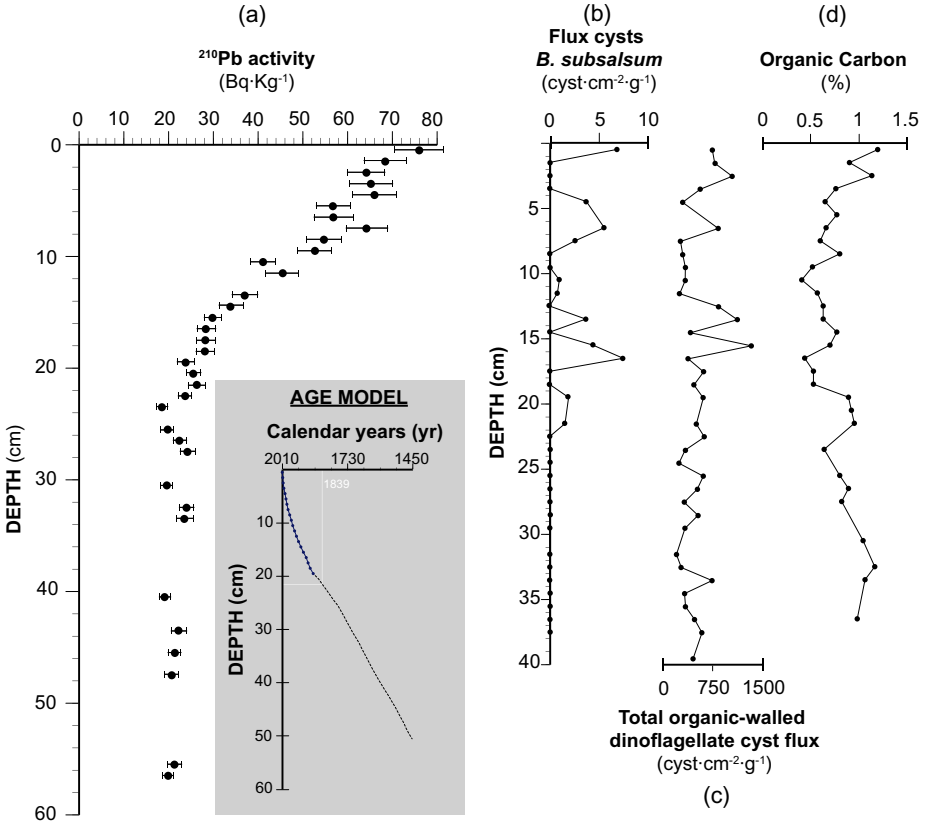


FIG. 1. (a) $^{210}$Pb activity (Bq · Kg$^{-1}$) over depth, and age model, (b) Flux of *Bysmatrum subsalsum* cysts (cysts · cm$^{-2}$ · year$^{-1}$), (c) Flux of total organic-walled dinoflagellate cysts (cysts · cm$^{-2}$ · year$^{-1}$) and (d) sedimentary organic carbon (%).

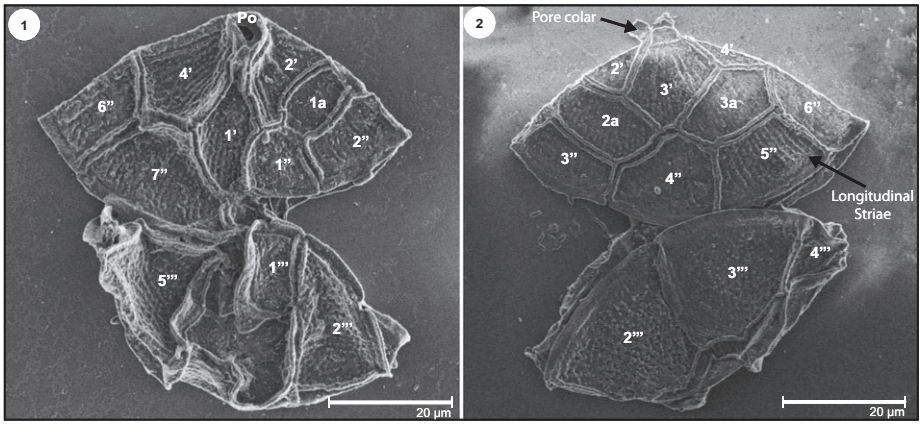


FIG. 2. Scanning electron micrographs (SEM) of one specimen of a collapsed cyst of *Bysmatrum subsalsum*, rotated 180°. (1) Ventral view showing apical pore complex and paratabulation. Note that paraplate 1a is pentagonal in shape. (2) Dorsal view showing intercalary paraplates 2a and 3a separated from each other by apical paraplate 3′. Note that paraplates 2a and 3a are pentagonal in shape. Both micrographs clearly illustrate the striated ornamentation of the paraplates. Abbreviations: $n'$: apical plates, $n''$: precingular plates, $n'''$: postcingular plates. *na*: anterior intercalary plates, Po: apical pore plate.

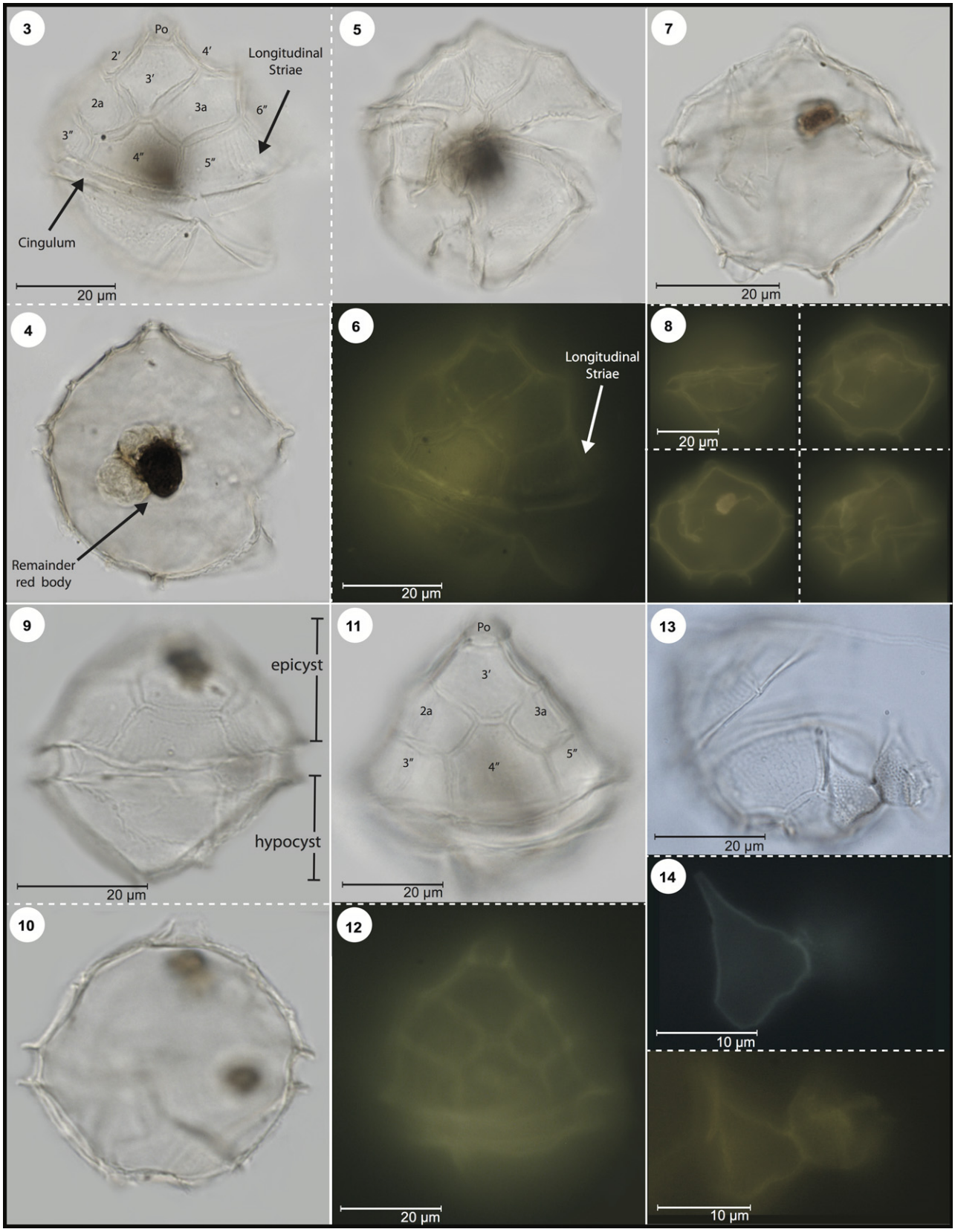
3
Po
2′
4′
3′
2a
3a
6″
3″
4″
5″
Longitudinal Striae
Cingulum
20 µm
5
7
20 µm
4
Remainder red body
6
Longitudinal Striae
20 µm
8
20 µm
9
epicyst
hypocyst
20 µm
11
Po
3′
2a
3a
3″
4″
5″
20 µm
13
20 µm
14
10 µm
10
12
20 µm
10 µm

FIG. 3. Bright and dark field light micrographs of cysts of *Bysmatrum subsalsum* from palynological slides. (3–6) Specimen from sample 4–5 cm (slide 1, England Finder (EF): Q47-02). 3: Dorsal view, high focal plane. Paratabulation and ornamentation of paraplates (striation) can be seen clearly under optical microscope. 4: Cross-section showing general shape and the remainder of a red body. 5: Ventral view, low focal plane. 6: Fluorescence image. Ornamentation, outline of paraplates and both walls of the cyst show clear yellow autofluorescence. (7–8) Specimen from sample 4–5 cm (slide 1; EF: U47-02). 7: Cross-section. 8: Fluorescence images from high to low focal plane. (9–10) Specimen from sample 0–1 cm (slide 1; EF: R65-02). 9: Dorsal view, high focal plane. Note that epicyst and hypocyst are of equal length. 10: Cross-section. (11–12) Specimen from 7 to 8 cm (slide 1; EF: R65-02). 11: Dorsal View. High focal plane. 12: Fluorescence image. (13–14) Collapsed specimen from sample 6–7 cm (slide 1: EF: F41-02). 13: High focal plane. Note ornamentation (striations and cross-reticulation) on hypocystal plates and rupture along the cingulum of the epicystal archeopyle. 14: Fluorescence images with narrow field-of-depth on the two paraplates shown on the lower right of micrograph 13. Outline and ornamentation of paraplates show clear autofluorescence. Abbreviations: see Figure 2.

(Anderson et al. 2004). Finally, the good preservation of these cysts would potentially also enable their use for coastal paleoenvironmental studies.

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