

Fryxelliella sepulvedana sp. nov. (Triceratiaceae, Bacillariophyta), a rare species from the Mexican Pacific coasts

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ABSTRACT – *Fryxelliella sepulvedana* n. sp., from the Mexican Pacific coasts is the second species hitherto known for the genus. As in the other *Fryxelliella* species, *F. sepulvedana* has a siphon marginalis bearing triangular apertures, three ocelli and three rimoportulae equidistantly placed near the margin of the valve. The ocelli are the most important diagnostic features of *F. sepulvedana* as they are large (3.5-5.0 µm in diameter) and are located in the submarginal region, at least four areolae distant from the valve margin. They differ from those of *F. floridana*, the other species in the genus, where the ocelli are marginal and are in contact with the siphon marginalis. While *F. floridana* was found in the Atlantic Ocean, *F. sepulvedana* was recorded in the eastern tropical Pacific. The new species was found in different coastal localities of Mexican Pacific Ocean, all close to the coast, and sampled in the water column.

Key words: Bacillariophyta, *Fryxelliella sepulvedana*, Mexican Pacific, taxonomy.

RESUMO – *Fryxelliella sepulvedana* sp. nov. (Triceratiaceae, Bacillariophyta), uma rara espécie do Pacífico Mexicano. *Fryxelliella sepulvedana* n. sp., encontrada no Pacífico Mexicano, é a segunda espécie conhecida até o momento para o gênero. Como na outra espécie de *Fryxelliella*, *F. sepulvedana* apresenta siphon marginalis com aberturas triangulares, três ocelos e três rimopórtulas colocadas equidistantes entre si e próximo à margem valvar. Os ocelos constituem as mais importantes características diagnósticas de *F. sepulvedana* pois eles são grandes (diâmetro 3,5-5,0µm) e localizados na região submarginal, aproximadamente quatro areolas de distância da margem valvar. Eles diferem daqueles de *F. floridana*, a outra espécie do gênero, onde os ocelos são marginais e em contato com o siphon marginalis. Enquanto *F. floridana* foi encontrada no Oceano Atlântico, *F. sepulvedana* foi registrada no Pacífico tropical. A espécie foi encontrada em diferentes localidades do Pacífico mexicano, próximo à costa, e sempre na coluna de água.

Palavras-chave: Bacillariophyta, *Fryxelliella sepulvedana*, Pacífico Mexicano, taxonomia.

INTRODUCTION

Fryxelliella A. K. S. K. Prasad has so far been a monotypic genus of centric diatoms, found in the marine plankton of Florida (Prasad *et al.*, 1997) and South Brazil (Fernandes *et al.*, 1999; Procopiak *et al.*, 2006). According to the classification of Round *et al.* (1990), it is placed in the Family Triceratiaceae Round (Prasad *et al.*, 1997; Fernandes, 2003). According to Prasad & Nienow (2008), its main characteristics are the 2-3 typical marginal ocelli, intercalated by rimoportulae located at the transition of the mantle and valve surface, loculate areolae and

the marginal circumferential tube (hereafter called *siphon marginalis*). The latter structure is a hollow tube occupying the entire mantle edge; in *Fryxelliella* it is perforated by subtriangular holes (Fernandes, 2003). In its original description, Prasad *et al.* (1997) advocated that the siphon marginalis be regarded as an exclusive character of *Fryxelliella*. Later, Sims (2001) pointed out that the siphon marginalis is in fact present in other Triceratiaceae like *Cerataulus* Ehrenberg, *Eupodiscus* J. W. Bailey, *Pseudoauliscus* A. Schmidt and *Triceratium* Ehrenberg. However, the subtriangular perforations are an exclusive character of *Fryxelliella*.

To date, there are some species presently placed in *Triceratium* that lack any kind of siphon marginalis, for instance, *T. moreirae* L. F. Fernandes & Souza-Mosimann and *T. dubium* Brightwell (Fernandes & Souza-Mosimann, 2001). Sims' interpretation of the siphon marginalis is that the outer wall of the tube may be modified to form specialized structures like the scutellum, a view we share with. Therefore, the definition and the very identity of *Fryxelliella* has become vague, unless another structure is selected as the diacritic character of the genus – for instance, the triangular apertures placed at the valve edge, extending across the mantle. Sims (2001) maintained this view and the main consequence was the retention of the type species *F. floridana* A. K. S. K. Prasad, but removing *F. inconspicua* (Rattray) A. K. S. K. Prasad to her new proposed genus *Praetriceratium* P. A. Sims under the name *P. inconspicuum* (Rattray) P. A. Sims, because this species has pseudoculate areolae and has no triangular apertures in the siphon marginalis.

During our investigation of the phytoplankton composition of the Mexican Pacific, a new species of *Fryxelliella* was found, closely related to the type species *F. floridana*. In this report, we describe this as *F. sepulvedana*, the second species hitherto known for the genus.

MATERIAL AND METHODS

Four hundred eighty six liquid samples collected since 1997 at 20 locations along the Mexican Pacific coasts (Fig. 1) were carefully reviewed to detect the new species. All the samples belong to the Marine Phytoplankton Laboratory at the Universidad Autónoma Metropolitana Iztapalapa (abbreviated as FpM). Collections were made with a standard phytoplankton net of 54 μm mesh through vertical or horizontal hauls during 5 minutes. The material was fixed with formaldehyde at a final concentration of 4%. For permanent slides, material was washed several times with distilled water and oxidized after Hasle & Fryxell's (1970) method (designated as UAMIZDIAT). The slides were examined with a Leica DMLB light microscope. For Scanning Electron microscopy (SEM), cleaned valves were dried on a glass slide, picked up with an acupuncture needle and gently placed on conductive carbon adhesive previously attached to SEM stubs. The stubs were sputter-coated with gold and examined with a JEOL JSM-5900LV microscope at 10-15 Kv acceleration voltages. For Transmission Electron Microscopy (TEM), cleaned material was placed on 200 mesh nickel grids covered with formvar/carbon and examined with a Zeiss EM910 electron microscope

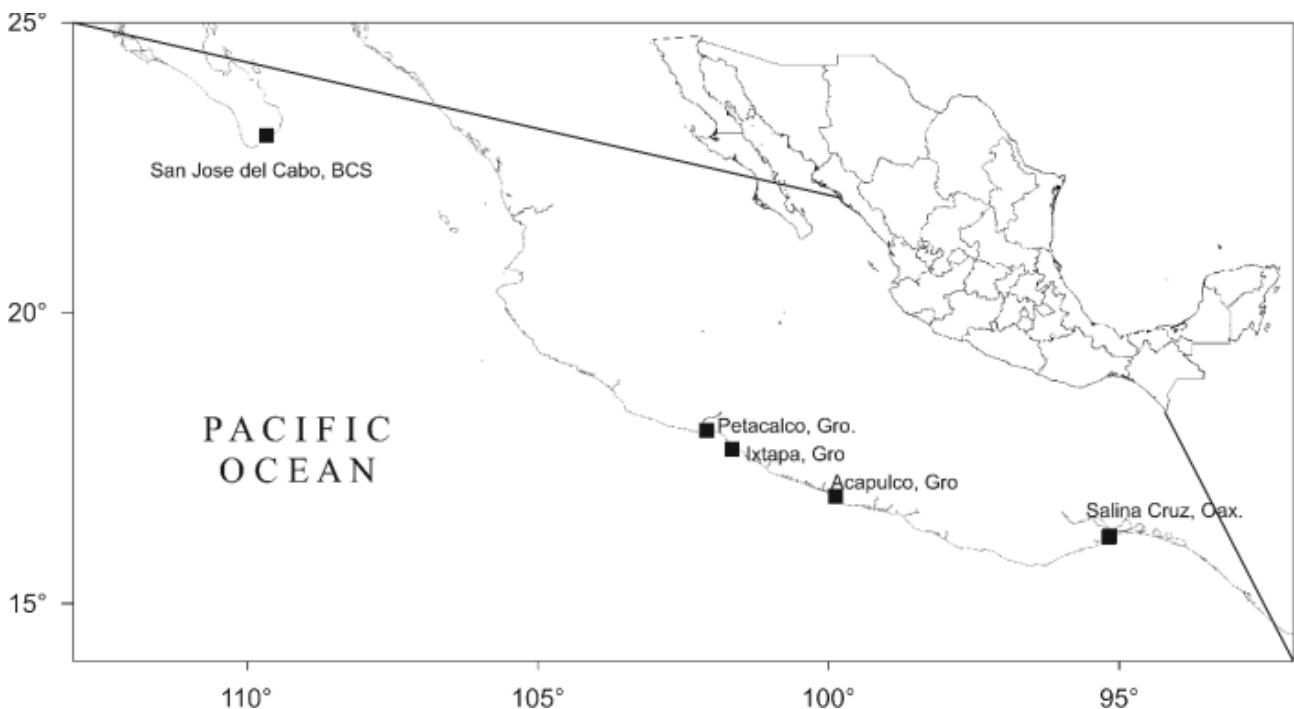


Fig. 1. Distribution of *Fryxelliella sepulvedana* in Mexican Pacific Ocean.

at 120kV acceleration voltage. Terminology followed Ross *et al.* (1979), Round *et al.* (1990) and that specifically proposed for *Eupodiscus* and *Fryxelliella* (Sullivan, 1986; Sullivan & Porguen, 1991; Prasad *et al.*, 1997; Sims, 2001). Considering that Sims' arguments about the structure and arrangement of the scutelli appear to be species-specific, it is proposed here that the palisades (Fernandes, 2003), the scutellum of *Eupodiscus* (Sullivan & Porguen, 1991), and the quadrangular plates of *Fryxelliella* and *Praetriceratium* (Prasad *et al.*, 1997; Fernandes *et al.*, 1999; Sims, 2001; Fernandes, 2003) should all be reunited under the name *scutellum*.

RESULTS

Fryxelliella sepulvedana Meave, Zamudio & L. F. Fernandes

(Figs 2-28)

Diagnosis: *Species nova haec ab omnibus allis speciebus distinguitur ocellis majoribus et haud marginem valvae attingentes atque siphone marginali continuo et non interrupto ut in F. floridana.*

Description: Valvae circulares 41.7-68.3 μm diametro areolis loculatis pentagonalibus hexagonalibusque, unaquaque cribra externo duobus annulis circularibus cribralium poris et uno poro marginali maiore composito. Areolae centrales illis majores (5-7 in μm) in margine (6-9 in 10 μm) et concentricae striae in centro dispositae, ex medio marginem versus radialescentes. Ocelli circulares 3.3-5.5 μm diametro, aequidistanter in regioni submarginali locati haud marginem valvae attingentes, ergo areolis omnino circumdati. Hae areolae alii leviter maiores in valva. Rimoportulae inter se aequidistantae in annulo marginali et per ocellis intercalatae. Extra ut brevis tubus et intra structura labiata fere sessili. Siphon marginalis circumferentialis continuus, 2.2 μm latus haud interruptus per ocellos or rimoportulas sed per fissuras apertularum triangularium incisus. Paries externus siphonis marginalis scutellorum compositus granulis et silicis ponticulo latitudine fere aequali illa scutellorum. Cingulum valvocopula angusta instructum et copula adiacenti ligula ornata.

Holotype: Specimen Figs 2-3 taken from the holotype slide MEXU 1837. The specimen is the only valve present in the slide and is indicated in color. The holotype slide is deposited at MEXU (Herbario

Nacional de México, Instituto de Biología, Universidad Nacional Autónoma de México) Apartado Postal 70-367, Delegación Coyoacán, 04510 México D.F., Mexico, from water sample FpM 413.

Isotypes: Permanent slides MEXU 1838, MEXU 1839, MEXU 1840, MEXU 1841, deposited at MEXU.

Type Locality: Salina Cruz, Oaxaca (16°03'30" N, 95°10'30" W). Mexican Coast, May 15 1998. Plankton sample.

Other Materials Examined: Liquid sample FpM 1506 from Acapulco (Guerrero) and samples from Petacalco (Guerrero) and Puerto La Libertad, El Salvador, not registered in herbarium.

Etymology: The species name is in honor of Physician José David Sepulveda Sánchez, specialized in electron microscopy, who has been fruitfully collaborating with the UAM-Iztapalapa phytoplankton group.

Light Microscopy Observations

Valves circular, 41.7-68.3 μm diameter. Areolae loculate, valve surface with areolae arranged in concentric striae at the center, becoming radial from the middle towards the margin (Figs 5, 7, 9, 10), 5-7 areolae in 10 μm in the center and 6-9 areolae in 10 μm in the edge. Three circular ocelli with 3.3-5.5 μm diameter (Table 1), equidistantly placed in the submarginal region, not reaching the valve margin, and thus entirely surrounded by areolae (Figs 3, 7, 9, 10). These areolae are slightly larger than the others on the valve (Fig. 7). Three rimoportulae compose a marginal ring, each one situated midway the distance between the ocelli (Figs 2, 8, 10); rarely two rimoportulae were observed between the ocelli (Fig. 6). Siphon marginalis continuous, that is, not interrupted by ocelli or rimoportulae (Figs 4, 6, 9, 10).

Electron microscopy Observations

Valves circular, slightly convex; surface areolated (Figs 11-13). Areolae loculate hexagonal (Figs 14-15), sometimes pentagonal, especially those on the margin (Fig. 16). Each one bears an external cribra composed of two circular rings of 24-34 cribral pores (Fig. 13). A small hyaline area is left at the center of the cribra. Most of the areolae have a larger marginal pore on the cribra (Figs 13, 17). Foramen large with thickened rim (Fig. 14). Three flat ellipsoid to circular ocelli of 4-5 μm long are placed in the submarginal region at equal distances to each other (Fig. 11). Porelli arranged in concentric rows enclosed by a thickened silica rim (Fig. 17). Ocelli about 4-5 areolae distant

TABLE 1 – Sample data, distribution and some measures of *Fryxelliella sepulvedana* in Mexican Pacific.

Locality and Record Number of Samples	Lat. N	Long. W	Date	Number of Slide	Diameter (µm)	Central areolae	Marginal areolae	Ocelli diameter (µm)
Salina Cruz, Oax. FpM 413 UAMIZDIAT-380	16°08'30"	95°10'30"	15/Sep/1998	MEXU 1837 Holotype	47.3	6	8	3.9
Salina Cruz, Oax. FpM 312, FpM 313 UAMIZDIAT-310a, 310b	16°09'40"	95°10'01"	15/Sep/1997	MEXU 1838 MEXU 1839 Isotypes	42.9-47.9	6-7	8-8.5	3.3-3.4
Ixtapa, Gro. FpM 453 UAMIZDIAT-1954	17°39'22"	101°39'41"	07/Nov/1998	MEXU 1840 Isotype	57.5	6	8	4
La Playita, San José del Cabo, B.C.S. UAMIZDIAT-1955	23°03'32"	109°40'09"	07/Oct/2006	MEXU 1841 Isotype	45.8-68.3	5-6	6-9	3.3-5.5
Acapulco, Gro. FpM 1506	16°50'44"	99°52'54"	20/May/2003		58.8-59.9	5-6	7.5-8	3.6-4.2
Petacalco, Gro.	17°58'49"	102°05'58"	30/Nov/1999		41.7	6	8	3.9
Puerto Libertad. El Salvador.	13°28'63"	89°19'20"	5/Mar/2006		45.8-55.3	6	8	4.0

from the valve margin (Figs 18-19). Three rimoportulae are present, each one intercalated midway between the ocelli (Fig. 11). The external circular opening of rimoportula is a short tube surrounded by small granules (Figs 17, 20). Internally, the labiate structure is nearly sessile, bearing an elongate fissure (Figs 21-23). Circumferential siphon marginalis continuous, 2.2 µm in breadth, located at the valve margin and continuing towards the mantle (Figs 12, 25-26). Very small granules occupy the outer part of the siphon marginalis on the valve margin (Figs 17, 27, 28); internally there are poroids arranged in 3 rows at a density of 23-25 pores in the perpendicular direction (Figs 21, 25, 26). Many subtriangular apertures (Figs 17, 27, 28), generally 10-11 in 10 µm, pierce the tube at the edge of the valve. In some regions they are more distant to each other, 6-8 in 10 µm (Fig. 17). The siphon ridge is a thick hyaline silica strip cut by the fissures of the subtriangular apertures (Figs 19, 24). The outer wall of the siphon marginalis is located in the mantle. It is composed of many quadrangular plates (altogether composing the scutellum) with granules (Fig. 24) and a silica ridge of almost of equal width as the plates (Figs 19, 24, 25). Cingulum simple; valvocopula thin, and adjacent band ligulate and thicker than the valvocopula (Fig. 28).

Remarks: Frustules of *F. sepulvedana* were found in only six samples from the Mexican Pacific (Table 1). These corresponded to phytoplankton collections from the coastal sites of Guerrero and Oaxaca states, as well as one collection from the beach of San José del Cabo at the extreme of the

Baja California Peninsula (Fig. 1). Valves of *Eupodiscus* spp. and sediments were common in the samples. In the Acapulco sample, a salinity of 31 and temperature of 30° C were recorded.

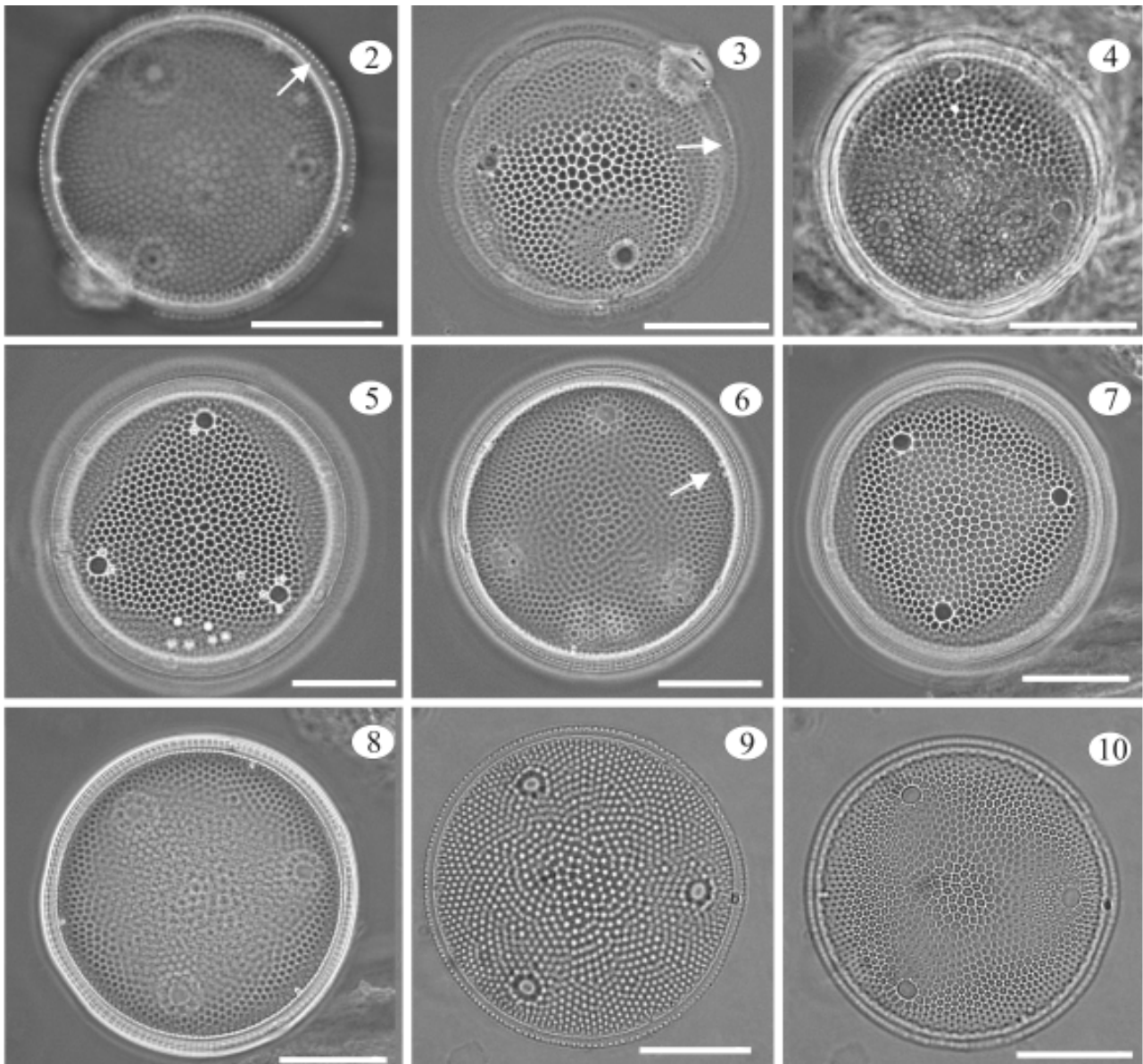
DISCUSSION

The presence of a siphon marginalis plus the associated subtriangular apertures, ocelli and loculate areolae (Prasad & Nienow, 2008) allowed the placing of the new species in the genus *Fryxelliella*. *Praeticeratium*, an apparently very closely related genus when viewed in the light microscope, also has submarginal ocelli and siphon marginalis, but the latter structure lacks any type of apertures and its areolae are pseudoloculate (Sims, 2001). *Eupodiscus* is another very closely related genus as it possesses marginal ocelli, loculate areolae and a siphon marginalis. The rimoportulae are equidistant and intercalated to the ocelli. Its internal aperture looks like that of *Fryxelliella* (Prasad & Nienow, 1988; Fernandes, 2003), but the external tube in *Eupodiscus* is longer, as in *E. paracaënsis* Sullivan & Porguen (Sullivan & Porguen, 1991) or flush with the scalloped extensions as in *E. radiatus* J. W. Baeley (Fernandes, 2003). However, there are no marginal subtriangular holes, and the mantle of *Eupodiscus* is very high when compared to *Fryxelliella*.

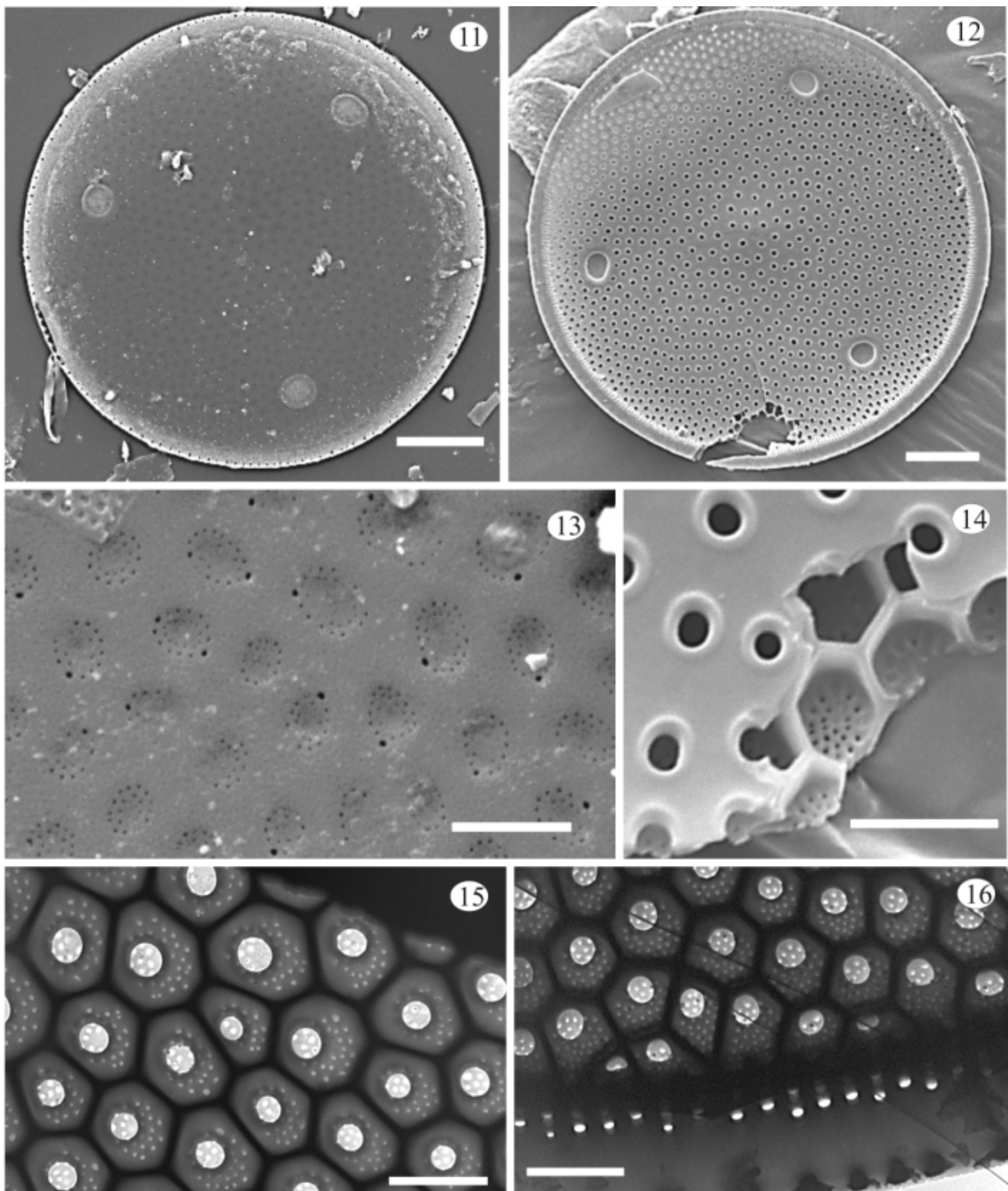
The ocelli are the most remarkable structure of *F. sepulvedana*, particularly when compared to *F. floridana*, for they are located at some distance from the valve margin, leaving 3-4 areolae between them. Moreover, they are large (4-5 µm) and barely raised. In *F. floridana*, the ocelli are marginal, almost on the

sipho marginalis, and they are raised and smaller, 1.4-1.6 μ m (Prasad *et al.*, 1997; Fernandes, 2003). Areolae in *F. sepulvedana* have two circular rings of cribral pores, and with a total of 22-36 pores, while *F. floridana* has areolae bearing one or more than two irregular rings of about 19-23 cribral pores (Prasad *et al.*, 1997; Fernandes, 2003). The sipho marginalis is similar in both species, its outer wall being composed of a scutellum bearing quadrangular plates covered by scattered granules. The number of subtriangular apertures is markedly higher (6-10 in 10 μ m) in

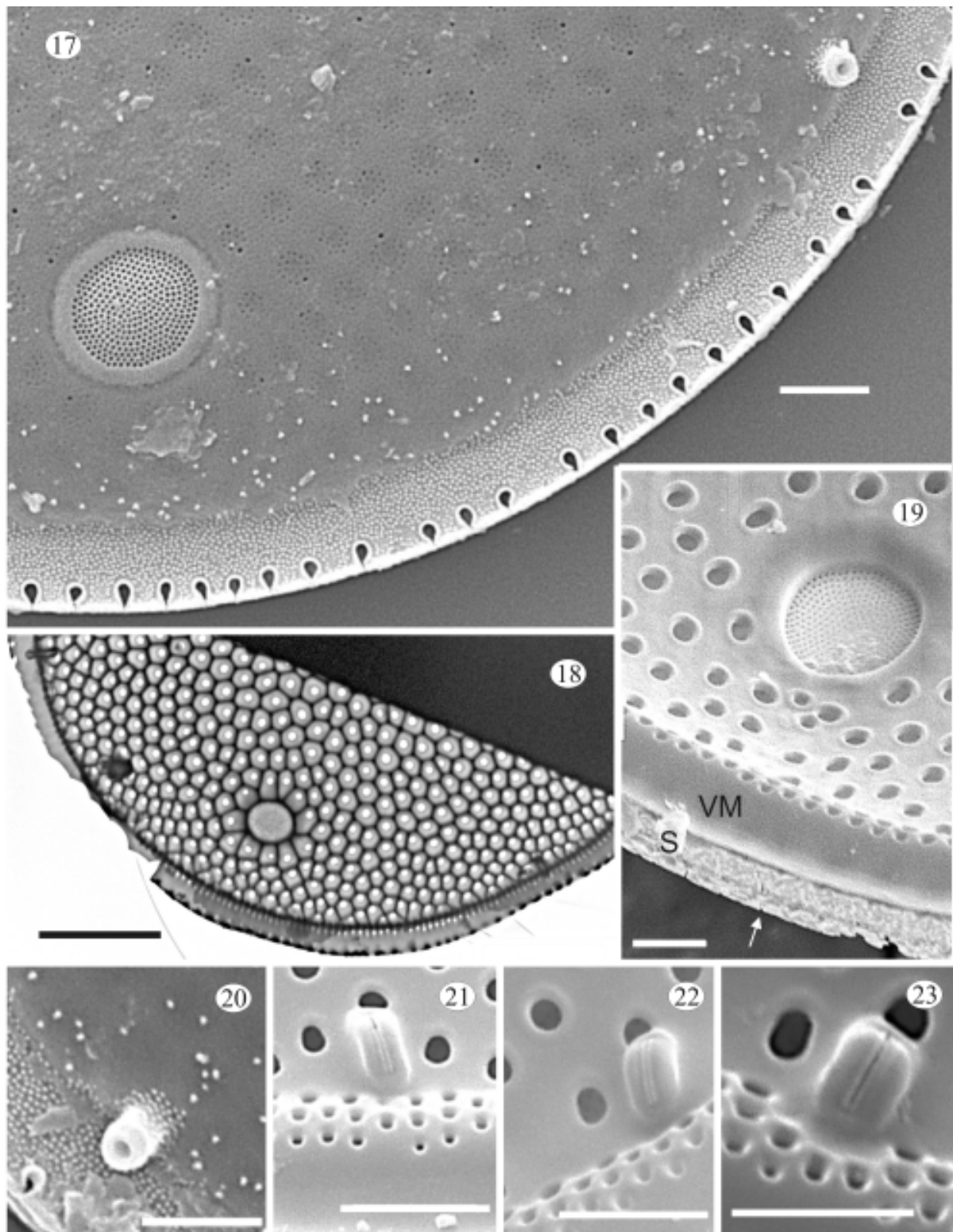
F. sepulvedana than in *F. floridana* (4 in 10 μ m). One interesting feature is that the sipho marginalis of *F. floridana* is definitely interrupted at the ocelli (see Fernandes, 2003, his Fig. 24), while it is continuous in *F. sepulvedana* and in all other species within the Triceratiaceae genera so far examined that possess a sipho marginalis. Unfortunately, we found only one valve that presented a limited view of the cingulum (Fig. 24). Therefore, we were not able to further describe its structure or compare it with the cingulum of *F. floridana* studied by Fernandes (2003).



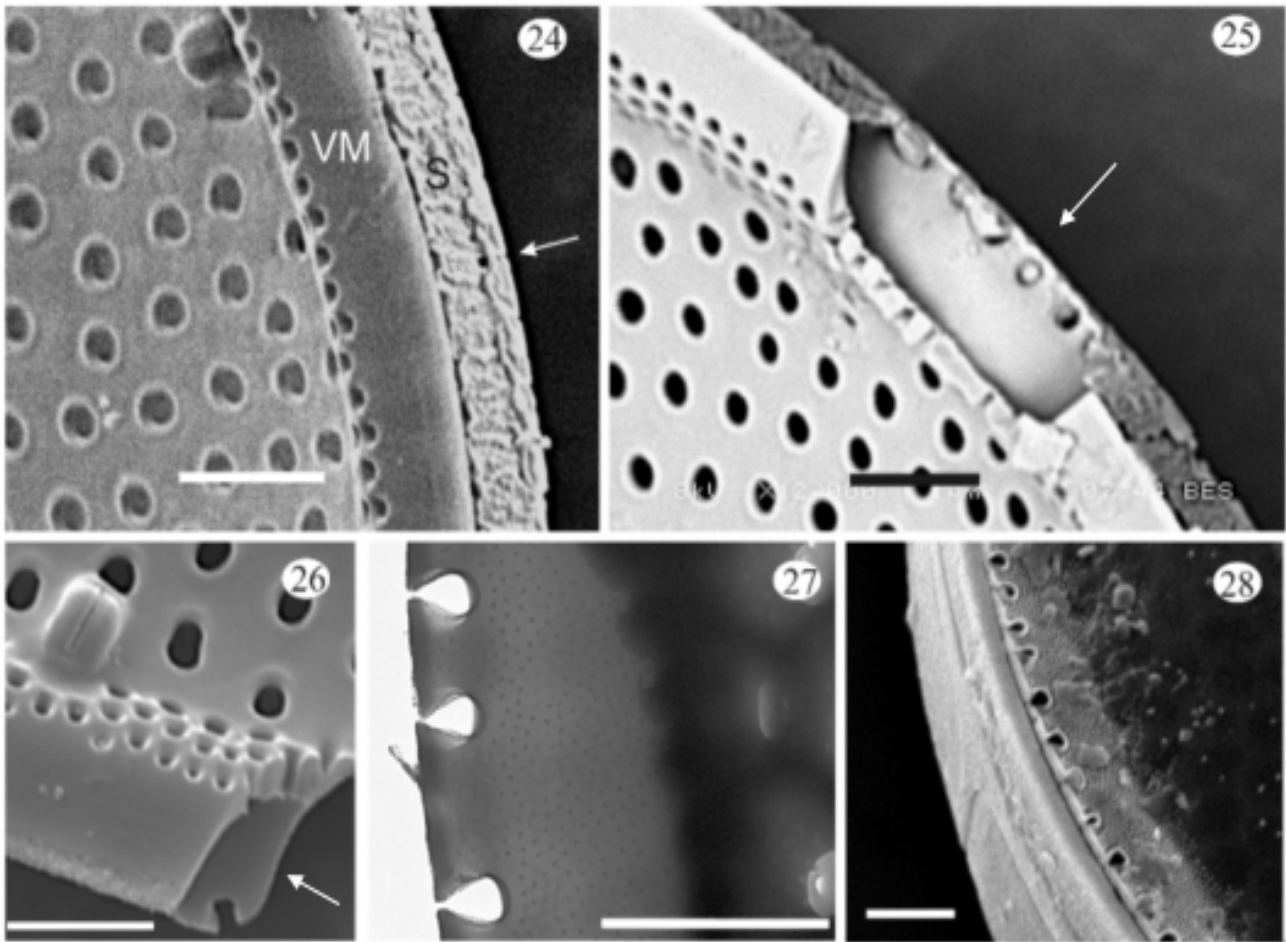
Figs 2-10. *Fryxelliella sepulvedana* from the Mexican Pacific (LM). **2, 3.** Phase contrast; holotype slide MEXU1837. Ocelli, rimoportulae and conspicuous apertures of circumferential sipho marginalis (arrow); **4.** Phase contrast; isotype slide MEXU 1839; **5, 6.** Bright field; isotype slide MEXU 1840. Note valve with two rimoportulae between the ocelli (arrow) in Fig. 6; **7-10.** Phase contrast; Material from Acapulco, Guerrero. Scales: **Figs 2-10** = 10 μ m



Figs 11-16. *Fryxelliella sepulveda* from Mexican Pacific— electron photomicrographs. **11.** SEM. External valve view. Material from Salina Cruz, Oaxaca; **12-16.** Material from La Playita, Baja California Sur, Mexico; **12.** SEM. Internal valve view; **13.** SEM. External surface, central part of the valve. Areolae have two circular rings of cribra; **14.** SEM. Internal valve view of areolae. Foramen and external cribra are clearly evidenced; **15.** TEM. Internal valve view; central region with pentagonal and hexagonal areolae; **16.** TEM. Internal view showing smaller areolae at the valve margin and *sipho marginalis* with rows of pores. Scales: **Figs 11, 12** = 10 μ m; **Figs 13-16** = 2 μ m.



Figs 17-23. *Fryxelliella sepulvedana* from Mexican Pacific; electron photomicrographs. Material from La Playita, Baja California Sur, Mexico. **17.** SEM. External view of valve edge with ocelli, rimoportula, and siphon marginalis covered with granules. Subtriangular apertures of *siphon marginalis* are evident; **18.** TEM. Internal view of valve showing ocelli surrounded by areolae slightly larger than the rest ones; **19.** SEM. Internal view of ocellus. The siphon ridge (arrow), the scutellum (S) and the expanded valve margin (VM) are also illustrated; **20.** SEM. External view of rimoportula and associated granules; **21-23.** SEM. Internal views of rimoportulae close to the *siphon marginalis*. Scales: **Figs 17, 19-23** = 2µm; **Fig. 18** = 10 µm.



Figs 24-28. *Fryxelliella sepulvedana* from Mexican Pacific; electron photomicrographs. Material from La Playita, Baja California Sur, Mexico. **24.** SEM. Internal view of valve. The siphon ridge (arrow), the scutellum (S) and the expanded valve margin (VM) are also illustrated. Note the tight junction between scutellum and internal wall of siphon; **25.** SEM. Internal view of valve and broken *siphon marginalis* exposing its lumen (arrow); **26.** SEM. Detail of *siphon marginalis* (arrow). Note row of pores and quadrangular lumen; **27.** TEM. Triangular apertures of *siphon marginalis*; **28.** SEM. Cingulum bearing narrow valvocopula, and first copula with ligula. Scales: **Figs 24-28** = 2 μm

Although the *siphon marginalis* is an interesting structure, its function is unknown (Sims, 2001). Prasad *et al.* (1997) suggest that this structure is similar to the tubes and canopea of other diatoms. Sims & Paddock (1979) showed hollow structures in many Naviculoid diatoms and mention that they are useful for bottom living diatoms. Sims (1994) also showed a central tubular cavity in species of *Benetorus* G. D. Hanna and *Entogonia* Greville. Paddock (in Sims, 2001) has considered the possibility that all these hollow structures lodge symbionts (Sims, 2001). The *siphon marginalis* is a common structure in species with ocellus, but not all of them have *siphon marginalis*, as is the case of *Auliscus* Ehrenberg, *Neohuttonia* Kuntze, *Glyphodiscus* Greville and

Rattrayella De Tony (Sims, 2001; Sims *et al.*, 2006). Additionally, the genus *Isodicus* Rattray has *siphon marginalis* but lacks ocellus. Based on this information it has been concluded that *siphon marginalis* is a polyphyletic character that evolved more than once along parallel lines (Sims, 2001). Notwithstanding, Prasad *et al.* (1997) mentioned that this is a useful character to define poorly defined genera within Eupodiscoideae. *Fryxelliella* is a genus that only includes living species, which suggests that this taxon is more recent than other closed related genera like *Eupodiscus* and *Pseudoauliscus*.

F. sepulvedana is a rare species, although it is widely distributed along the Mexican Pacific and toward the Central American Pacific (El Salvador).

As the samples containing it often came from coastal areas and contained many sand grains, a possible epipsammic habit should not be discarded. In addition, Sequeiros-Beltrones (2002) did not find *F. sepulvedana* during their study on the benthic diatoms of Baja California Peninsula, Mexico. Regarding the distribution of the only two known species of the genus *Fryxelliella*, it is significant that the generic type *F. floridiana*, has been so far restricted to subtropical and temperate Atlantic waters (inshore water off Fernandino Beach and estuarine waters nearby at Florida, and two estuaries of Southern Brazil), in contrast with *F. sepulvedana*, which only occurs in Mexican waters at the eastern tropical Pacific. This differential distribution reinforces the idea that they are distinctly different species.

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