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Taxonomy and systematics

Morphology of two species of the thecate dinoflagellate genus *Blepharocysta* (Dinophyta) from the tropical Mexican Pacific

Morfología de dos especies de dinoflagelados tecados del género Blepharocysta *(Dinophyta) del Pacífico tropical mexicano*

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Abstract

The genus *Blepharocysta* Ehrenberg currently comprises 5 species of truly marine planktonic thecate dinoflagellates, which are solitary, globose, subspherical and heterotrophic cells, lack typical depressed cingulum and sulcus, and are mainly distributed in tropical areas. The morphology of 2 species, *Blepharocysta splendor-maris* and *B. paulsenii*, found in net samples from the tropical Mexican Pacific, was studied by LM and SEM, especially plate arrangement and theca ornamentation. *Blepharocysta splendor-maris* showed more ovoid cells, developed sulcal lists, and theca with shallow poroids having a relative low density, whereas *B. paulsenii* had more spherical cells, shorter epitheca, reduced and slightly ornamented sulcal lists, and theca with deep poroids having a relatively higher density. The thecal tabulation in both species agrees with conventional tabulation, including the anterior intercalary plate in *B. paulsenii*. A comparison of both morphological and ecological characteristics among all species of the genus is done, outstanding shape of cells, relative size, development of sulcal lists, and especially the theca ornamentation as important morphological characters to distinguish species. *Blepharocysta splendor-maris* is the only species distributed from tropical to Antarctic waters, whereas the others are limited to tropical and subtropical waters.

Keywords: *Blepharocysta*; Planktonic thecate dinoflagellates; Podolampadaceae; Tropical Mexican Pacific

Resumen

El género *Blepharocysta* Ehrenberg comprende 5 especies de dinoflagelados tecados, planctónicos marinos. Incluye células solitarias, globulares, subesféricas y heterótrofas, que carecen de cingulum y sulcus típicos, de distribución principal en áreas tropicales. Se estudió la morfología de las especies *Blepharocysta splendor-maris* y *B. paulsenii*, encontradas en muestras de red del Pacífico mexicano tropical, usando ML y MEB, particularmente el

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arreglo de placas y ornamentación de la teca. *Blepharocysta splendor-maris* mostró una forma ovoide, aletas sulcales desarrolladas, teca con poroides poco profundos y mediana densidad, mientras que *B. paulsenii* tuvo forma más esférica, epiteca más corta, aletas sulcales reducidas, ligeramente ornamentadas y teca con poroides profundos, de mayor densidad. La tabulación de placas en ambas especies concuerda con la tabulación convencional, incluida la placa intercalar anterior en *B. paulsenii*. Se comparan las características morfológicas y ecológicas de las especies del género, destacando forma de células, tamaño relativo, desarrollo de las aletas sulcales y especialmente la ornamentación de la teca como caracteres morfológicos para distinguir especies. *Blepharocysta splendor-maris* es la única especie distribuida desde aguas tropicales a antárticas, mientras que las otras están limitadas a aguas tropicales y subtropicales.

Palabras clave: *Blepharocysta*; Dinoflagelados tecados planctónicos; Podolampadaceae; Pacífico tropical mexicano

Introduction

Dinoflagellates are a very important protist taxonomic group in the marine plankton. Dinoflagellates include thecate, athecate and "thin-walled" forms, depending on the type of cell covering. Most members of the family Podolampadaceae (Podolampaceae) Lindemann have the unique characteristic among other thecate dinoflagellates of lacking the typical depressed cingulum and sulcus, such as species of the genera *Blepharocysta* Ehrenberg, *Lissodinium* Matzenauer and *Podolampas* Stein (Abé, 1966; Balech, 1963, 1988; Carbonell-Moore, 1991, 1994a; Fensome et al., 1993; Gómez et al., 2010; Steidinger & Tangen, 1997), but there have been new additions including *Gaarderiella* Carbonell-Moore, *Heterobractum* Carbonell-Moore, and *Mysticella* Carbonell-Moore (Carbonell-Moore, 1994a, 2010; Gómez et al., 2011).

More recently, with the addition of the genus *Roscoffia* Balech to the family (Gómez, 2012), the podolampaceans might include 7 genera, but there are other positions, considering only 6 genera (with *Roscoffia* out of the family) (Hoppenrath, 2017). *Roscoffia* has the same tabulation and clades together with *Blepharocysta* sp. in molecular phylogenies, although it differs in many other aspects like the presence of a typical cingulum and the benthic habits (Gómez et al., 2010, 2011, 2019; Yamaguchi et al., 2018). The genus *Lessardia* Saldarriaga et Taylor was also considered to be part of that family (Saldarriaga et al., 2003), although this classification remains controversial, as *Lessardia* has been proposed to belong to the family Lessardiaceae Carbonell-Moore based on morphological differences (Carbonell-Moore, 2004). Recently, the marine benthic species *Cabra matta* Murray et Patterson also formed a clade together with *Roscoffia* and the podolampaceans in molecular phylogenies (Yamaguchi et al., 2018).

The genus *Blepharocysta* currently comprises 5 species (Guiry & Guiry, 2020), all of them marine and planktonic, of warm-water, tropical to subtropical affinity, and having a globose, spherical or subspherical shape, mostly apparently smooth, with no neck or spines but conspicuous sulcal lists usually present at the antapex of cells (Abé, 1966; Balech, 1963; Carbonell-Moore, 1994a; Guiry & Guiry, 2020; Steidinger & Tangen, 1997). Although chloroplasts have been mentioned to be present in *Blepharocysta* (Steidinger & Tangen, 1997), there is strong evidence that its species do not have chloroplasts and they have a feeding mechanism by extruding a pallium, as other heterotrophic thecate dinoflagellates (Carbonell-Moore, 2004; Jacobson, 1999).

Classic papers on dinoflagellates have included observations of certain species of the genus *Blepharocysta*, redescribing well-known species (*Blepharocysta splendor-maris* (Ehrenberg) Ehrenberg, the type species) or describing new species (*B. paulsenii* Schiller, *B. denticulata* Nie, *B. okamurae* Abé) and their tabulation, basically using Light Microscopy (LM) (e.g., Abé, 1966; Balech, 1963, 1988; Gaarder, 1954; Nie, 1939; Schiller, 1937). Later, another new species (*Blepharocysta hermosillae* Carbonell-Moore) was described and illustrated with light microscopy and Scanning Electron Microscopy (SEM) (Carbonell-Moore, 1992), and the species *B. compressa* Gaarder and *B. schilleri* (Gaarder) Ballantine in Parke et Dixon (= *B. matzenaueri* Gaarder) were found to be synonyms of the type species of the closely related genus *Lissodinium*, *Lissodinium schilleri* Matzenauer (Carbonell-Moore, 1991).

Other studies are the revisions on taxonomy and biogeography of the family Podolampadaceae (Carbonell-Moore, 1994a, b), including the illustrations of whole cells in lateral or ventral view from the 5 *Blepharocysta* species by SEM (Carbonell-Moore, 1994b, plate I, Figs. 1-5); additionally, 3 new genera of the family were proposed (*Gaarderiella*, *Heterobractum* and *Mysticella*), with taxonomic changes of species originally described as *Blepharocysta*, as *B. striata* Schütt transferred to *Mysticella striata* (Schütt) Carbonell-Moore (Carbonell-Moore, 1994a). The taxonomic revision of the family

Podolampadaceae also included a new interpretation on the plate formula: APC, 3', 1a, 5", 3C, 4S, 4-5"', 1"", considering 5 postcingular (5"') plates instead of the 3 traditional ones, with no intercalary posterior plate, which adjusts to the *Blepharocysta* species (Carbonell-Moore, 1994a). Additionally, molecular phylogenies of Podolampadaceae members, including *Blepharocysta* and *Podolampas* (and *Lessardia*) species have been provided (Gómez et al., 2010; Saldarriaga et al., 2003). The species *Blepharocysta splendor-maris* was recently documented by SEM micrographs from China (Yang & Li, 2014). Finally, there has been a large debate on a nomenclatural and taxonomic issue derived from the name *Peridinium splendor-maris* Ehrenberg, on which the type species of the genus *Blepharocysta* is based (Carbonell-Moore, 2018; Elbrächter et al., 2018, 2019).

During an oceanographic cruise along the coasts of the central Mexican Pacific, net samples were taken off the coast of Acapulco, and the microscopical analysis yielded a number of tropical forms of dinoflagellates, including 2 *Blepharocysta* species. This study shows details of the plate arrangement and ornamentation of the theca of *Blepharocysta splendor-maris* and *B. paulsenii*, using LM and SEM, and discusses important morphological and ecological characters to distinguish the species of the genus, providing a comparative table.

Material and methods

This study is based on material collected during the oceanographic cruise "MareaR VI" carried out from 10-21 June, 2014, along coasts of the central Mexican Pacific. Net samples (mesh 64 µm) were obtained by vertical hauls, the hauling depths depending on the depth of the fixed stations; a set of samples were fixed with formalin (4%) and another set with ethanol (70%). One sample obtained from 40 m depth to surface from station 42 (off the coast of Acapulco, State of Guerrero, 16°49' N, 99°53' W), yielded many tropical forms of dinoflagellates, including the *Blepharocysta* species studied herein. The total depth of the station was 56 m, the surface temperature was 29.8 °C, and a chlorophyll *a* maximum was recorded at 20 m.

Specimens belonging to *Blepharocysta* were isolated by micropippeting either from raw or rinsed samples, and analyzed by LM (Olympus BX 40, attached camera Hitachi KP-D50 Color digital) for identification, measuring and making the theca transparent, using sodium hypochlorite, following recommendations by Taylor (1978) and Taylor et al. (2003), whereas other additionally isolated specimens were studied by SEM (JEOL JSM6360LV) for details of the fine morphology, after rinsing, air-drying and coating with gold. General terminology for dinoflagellates follows recommendations by Fensome et al. (1993) and Steidinger and Tangen (1997), and more specific for the tabulation of podolampadacean members by Balech (1963), Abé (1966) and Carbonell-Moore (1994a) (Figs. 3, 15). Poroids density was calculated from SEM micrographs considering poroids numbers in 10 μm^2 at low magnifications, especially focused in the plates C1 and C3, and poroids numbers in 5 μm^2 at high magnifications, only in postcingular plates.

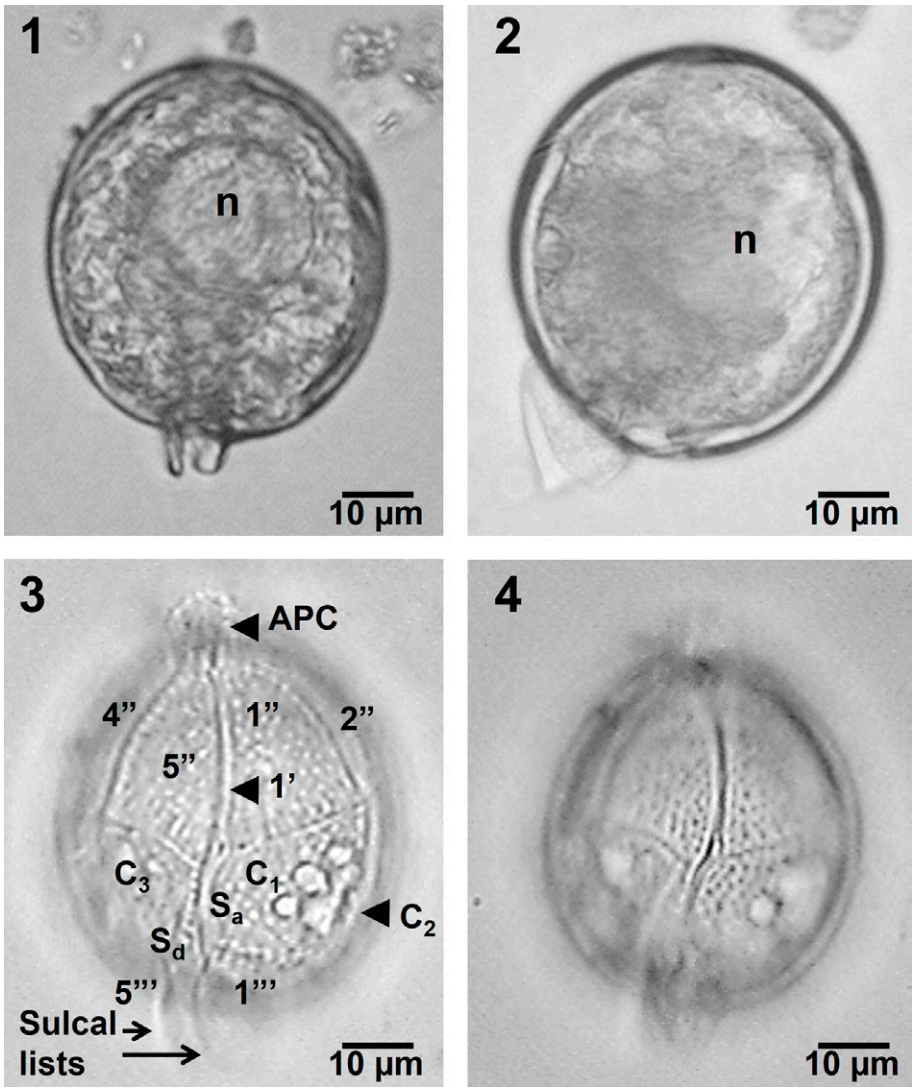
Results

Blepharocysta splendor-maris (Ehrenberg) Ehrenberg (Figs. 1-12)

References: Schiller, 1937, p. 477, Figs. 550 a-c; Nie, 1939, p. 31, text-Figs. 1, 2, pl. 1, Figs. 1-19; Balech, 1963, p. 16, pl. III, Figs. 34-44; Wood, 1963, p. 51, Fig. 188; Abé, 1966, p. 141, Figs. 21-32; Pesantes, 1978, p. 6, pl. 2, Fig. 6; Dodge, 1982, p. 254, Fig. 33H; Balech, 1988, 125, pl. 52, Figs. 16-19; Carbonell-Moore, 1994b, pl. I, Fig. 1; Steidinger and Tangen, 1997, p. 533, pl. 7, Fig. 49; Konovalova, 1998, p. 168, Fig. 36 6a, 6b; McMinn and Scott, 2005, p. 231, Fig. 3.17c; Iwataki et al., 2012, p. 127; Yang and Li, 2014, p. 162, 163.

Only solitary cells of medium size were found, with a globose appearance, spherical, subspherical or ovoid in shape, some specimens slightly more elongate (larger than wide) and wider at the posterior hypotheca (Figs. 1-4). No conspicuous structures, apart from the sometimes salient sulcal list, which may be variable in length and development, and the apical pore, which may be completely unapparent or observed as a short and truncated knob (Figs. 1-4). Untreated cells showed a slightly eccentric and large nucleus, but no trace of chloroplast (Figs. 1, 2). Conversely, a specimen made transparent in ventral view showed the epitheca considerably larger than the hypotheca, the plates of the apical (1'), precingular (1" to 4", except 3"), cingular (C1 to C3), and postcingular series (1"' and 5"', only), 2 sulcal platelets (Sa and Sd) and also the apical pore complex (APC) at the anterior apex and the sulcal lists at the posterior apex (Figs. 3, 4). The theca is completely covered by small poroids (Figs. 3, 4).

Observations by SEM confirmed the proportions of the epi- and hypotheca and plate arrangement of the species in ventral view, although postcingular plates (1"' to 5"') and the sulcal lists can be also visible (Fig. 5). The small postcingular plates constitute the hypotheca (Fig. 5), together with the antapical plate (1"", not shown). The plate 1' is very narrow and long (Figs. 5-7, 9). Another specimen in lateral view made possible to observe the only intercalary plate (1a), which is rectangular and small (6.2

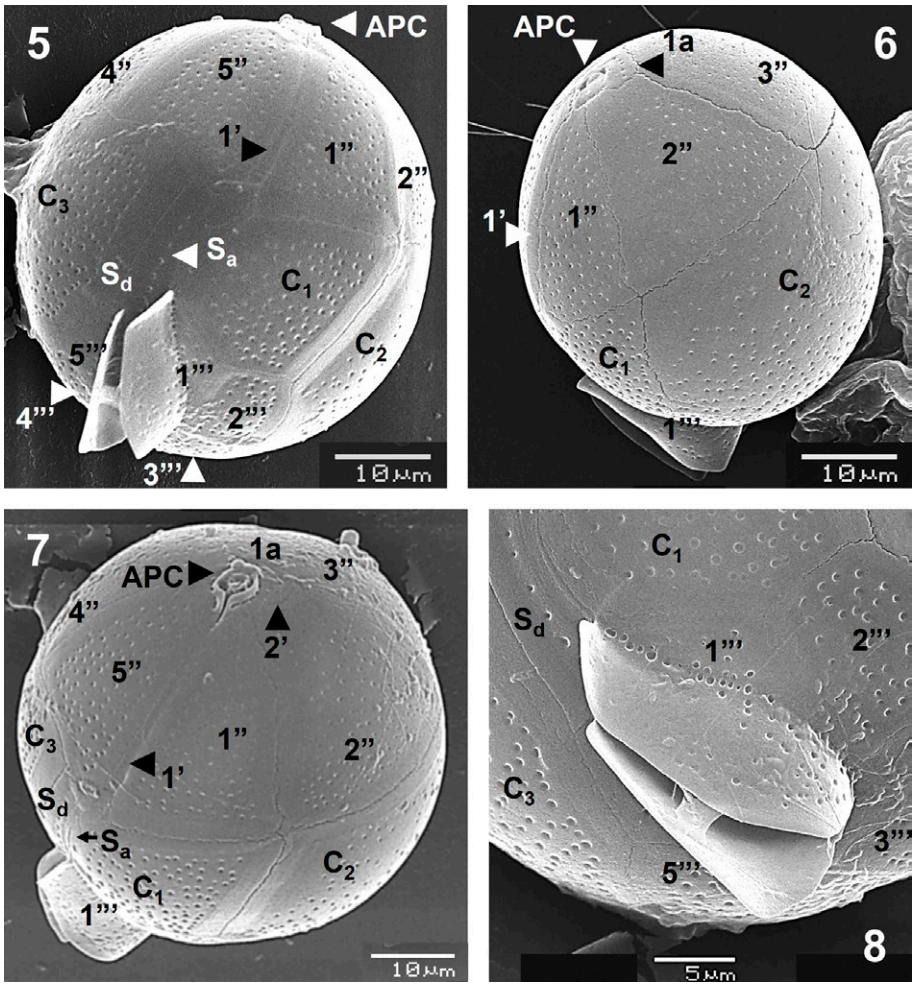


Figures 1-4. *Blepharocysta splendor-maris*, LM. 1, 2. Two complete cells with cellular content, in ventral (1) and left lateral view (2), showing the slightly eccentric nucleus (n). 3, 4. A treated cell with transparent theca in ventral view, showing the apical pore complex (APC), some plates of the apical, precingular, cingular, sulcal and postcingular series, and the sulcal lists (3), and with transparent theca exhibiting the ornamented theca (4).

× 3.5 µm), close to the APC, the third precingular plate (3”) and the cingular plate C2, the largest of the species (Fig. 6).

An apical view showed the APC, 1a and the second apical plate (2’), with the conspicuous sulcal plates (Sa and Sd) (Fig. 7), whereas a detailed view of the posterior part showed the sulcal list, the postcingular plates and the Sd platelet (Fig. 8). The APC, the first apical (1’), the intercalary (1a) and all precingular plates are seen in apical view (Fig. 9), whereas a detail of that view showed platelets of the APC: Po, Pt (cover platelet) and X (canal platelet) and the third apical plate (3’) (Fig. 10); the 3 apical plates (1’ to 3’), 2 being very small (2’ and 3’), and the APC are also shown (Fig. 11). The sulcal lists fairly developed and almost smooth, some plates (cingular and postcingular) and the anterior sulcal platelet (Sa) at the posterior zone, in lateral view, can be observed (Fig. 12).

The theca is covered by regularly distributed small and shallow poroids, which have a relative low density (39-43 pores in 10 µm^2, in plates C1 and C3, and 12-16 pores in 5 µm^2, in postcingular plates) (Figs. 5-12), and only few poroids have pores (perforations in the theca) (Fig. 10). The small, rectangular intercalary plate (1a) has usually a row of 5 pores (perforated poroids) (Figs. 6, 9, 10).



Figures 5-8. *Blepharocysta splendor-maris*, SEM. 5. Cell in ventral view, with relevant plates, APC and sulcal lists. 6. Cell in left lateral view, showing plates, APC and prominent sulcal lists. 7. Apical view with details of the APC and the anterior intercalary plate (1a), among others. 8. Antapical view, with some postcingular plates and the sulcal lists.

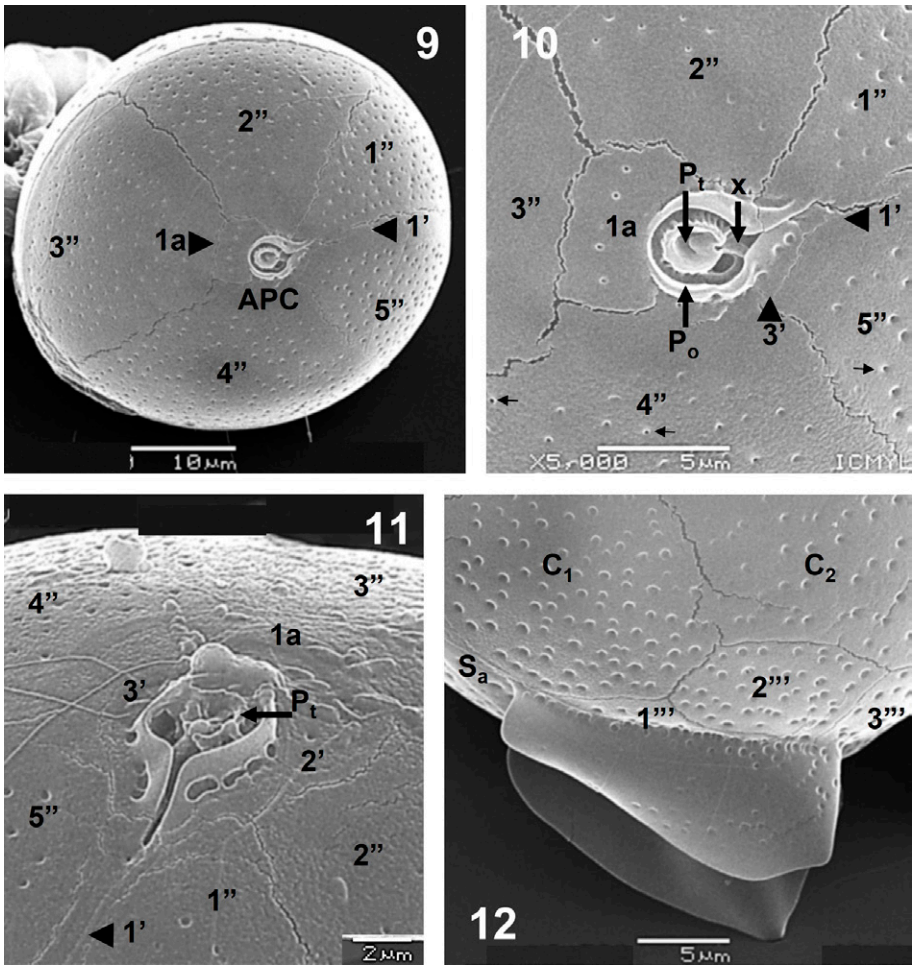
Measurements: 41-50 µm length, 39-46 µm width, 9-10 µm length of sulcal lists.

Blepharocysta paulsenii Schiller (Figs. 13-20)

References: Schiller, 1937, p. 478, Figs. 552 a-i; Gaarder, 1954, p. 7, Figs. 6-8; Hallegraeff, 1988, p. 96; Carbonell-Moore, 1991, Fig. 20; Delgado and Fortuño, 1991, p. 9, Fig. 5 X, pl. XXV c; Carbonell-Moore, 1994b, pl. I, Fig. 2; Carbonell-Moore, 2004, Fig. 3.

Solitary cells of medium size with a globose and spherical shape (Figs. 13-16). A very short apical protrusion indicating the APC and slight salient sulcal lists were apparent (Figs. 13-16). An untreated specimen showed a large, eccentrically located nucleus and no chloroplasts (Figs. 13, 14). Treated, transparent specimens in ventral and dorsal views showed the more reduced proportion of the epitheca in this species, and the plates arrangement (especially the precingular and cingular plates), very similar to the precedent species, but also showing the large cingular plate, C2 (Figs. 15, 16). The theca appeared covered by thick poroids and pores (Figs. 15, 16).

Cell shape and conspicuous plates of a specimen in ventral view are confirmed by SEM (Figs. 17, 18). The epitheca and the sulcal lists appeared more reduced than in *Blepharocysta splendor-maris* (Figs. 17, 18, 20). Shape and proportions of all plates seem similar to the precedent species, although the first apical (1’) is more evident in this species, and the sulcal platelets and the sutures of the plates are not as conspicuous as in *Blepharocysta splendor-maris* (Figs. 17-19). However, it is possible to observe the complete precingular plate series (Fig. 18).



Figures 9-12. *Blepharocysta splendor-maris*, SEM. 9. Apical view with apical and precingular plates, the anterior intercalary plate (1a) and the APC. 10. Detail of the APC (Pt, Po, x), the anterior intercalary plate (1a), apical and precingular plates, and the poroids and pores (arrowed) scattered on the theca. 11. Another view of the APC, also showing the 3 apical plates (1’ to 3’). 12. Left lateral view of the antapical portion, with the sulcal lists and some postcingular plates.

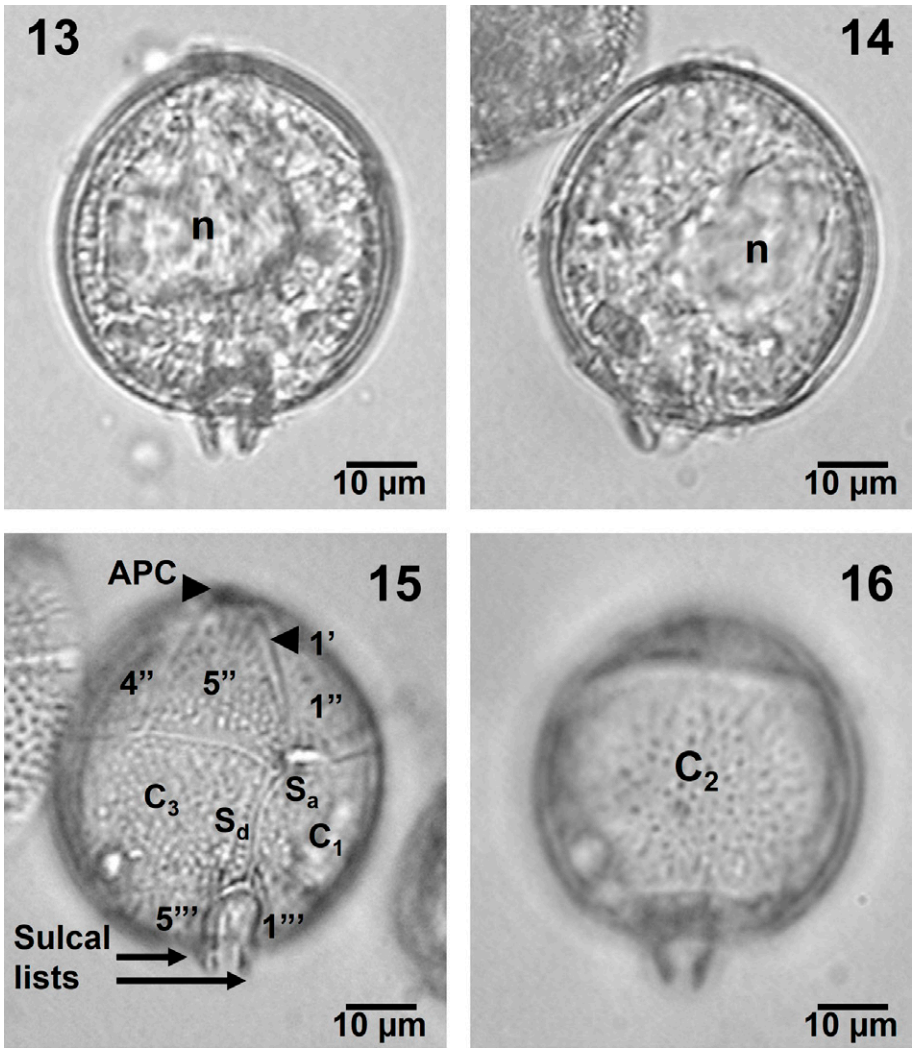
The APC is not prominent (Figs. 17, 18) and the small platelets forming it were not completely seen, although the 3 apical plates (1’ to 3’) were observed (Fig. 19). The intercalary plate (1a) has, as in *Blepharocysta splendor-maris*, a row of 5-6 small pores (perforated poroids) (Fig. 19). An antapical view allowed to see the complete series of postcingular plates (1”’ to 5”’), the antapical plate (1””), and 3 sulcal platelets (Sa, Sd and Sp), plus the sulcal lists well ornamented with poroids (Fig. 20). The theca is covered by well-defined and deep poroids, which are fairly homogeneously distributed, with a relative high density (52-56 pores in 10 µm^2, in plates C1 and C3, and 18-19 pores in 5 µm^2, in postcingular plates), and some are perforated by small pores (Figs. 19, 20).

Measurements: 44-46 µm length, 42-45 µm width, 6-7 µm length of sulcal lists.

Discussion

Morphology, taxonomy and phylogeny

Species of the genus *Blepharocysta* are mainly distributed in warm, tropical and subtropical waters, but they have never been reported as frequent or abundant. The characteristic shape of all the species, globose forms with slightly prominent sulcal lists, make their recognition and posterior positive identification not always easy, for they may be easily ignored or confused with a range of different organisms (Balech, 1988; Carbonell-Moore,

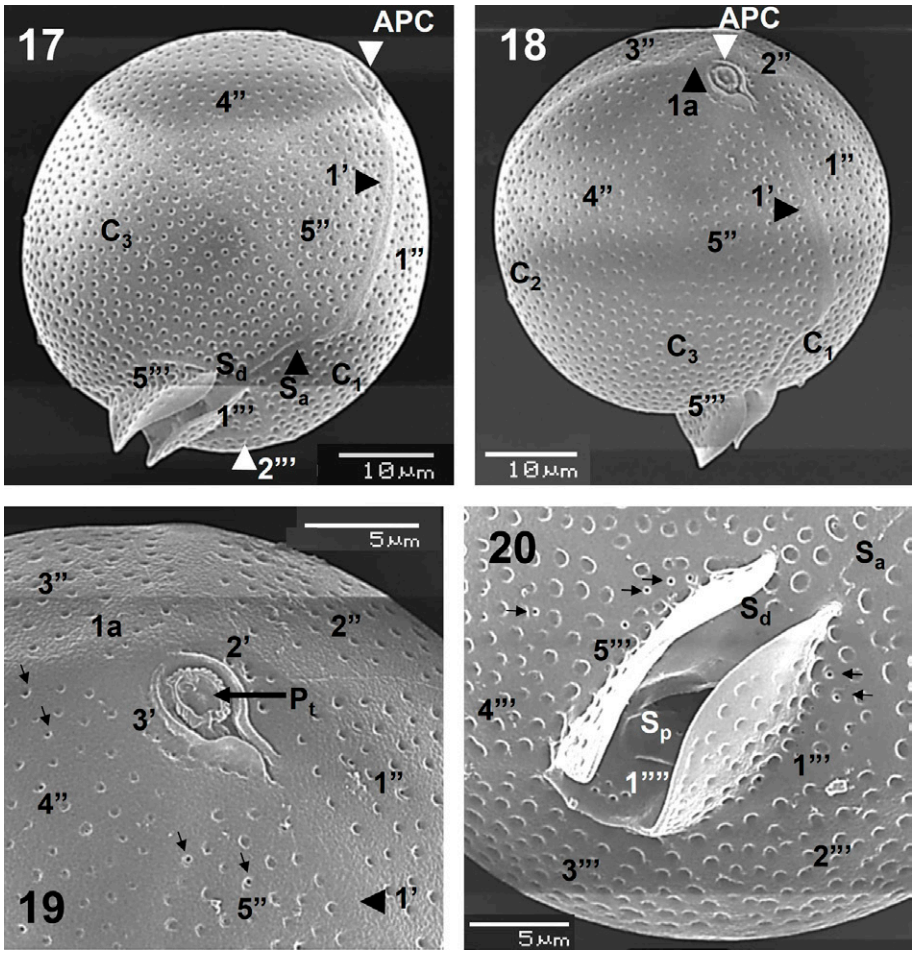


Figures 13-16. *Blepharocysta paulsenii*, LM. 13, 14. A complete cell with cellular content, in ventral (13) and left lateral view (14), showing the large nucleus (n). 15, 16. Treated cell with transparent theca in ventral view (15) showing plates and sulcal lists, and dorsal view (16) showing the large cingular plate C2 and the ornamented theca.

1994a; Taylor, 1976). This fact partially explains the inaccurate knowledge of their diversity and distribution all over the world.

We confirmed previous descriptions and observations in the literature mainly using LM (e.g., Abé, 1966; Balech, 1963, 1988; Dodge, 1982; Gaarder, 1954; Nie, 1939; Schiller, 1937; Steidinger & Tangen, 1997) for both species, particularly the theca tabulation, which applies to members of the family Podolampadaceae: APC, 3', 1a, 5", 3C, 4S, 4-5"', 1"". More recent observations by SEM were also comparable with those shown here (Carbonell-Moore, 1994b, 2004; Delgado & Fortuño, 1991; McMinn & Scott, 2005; Yang & Li, 2014).

In general terms, *Blepharocysta splendor-maris* appears subspherical to ovoid (larger than wide) than *B. paulsenii*, with large epitheca, fairly developed sulcal lists, and theca ornamented with shallow poroids (and pores) having a relative low density (39-43 poroids in 10 µm^2 at cingular plates). On the other hand, *Blepharocysta paulsenii* has a more spherical (globose) shape, with a shorter epitheca, reduced and slightly ornamented sulcal lists, and the theca is covered regularly by deep and marked poroids (and especially pores) with a relative high density (52-56 poroids in 10 µm^2 at cingular plates); additionally, the anterior intercalary plate, 1a, was also detected in this study (Figs 17, 18) in *B. paulsenii* (Carbonell-Moore, 1994a, pl. I, Fig.



Figures 17-20. *Blepharocysta paulsenii*, SEM. 17. Cell in ventral view with the APC, some plates of the apical, precingular, cingular, sulcal and postcingular series, and the ornamented sulcal lists. 18. Apical view of the same cell also showing the anterior intercalary plate (1a). 19. Detail of the apical view, with the plate Pt, the apical, precingular plates, the anterior intercalary plate (1a), and the poroids and pores (arrowed) covering the theca. 20. Antapical view with some sulcal plates, the postcingular and antapical (1””) plates, ornamented sulcal lists, and the poroids and pores (arrowed) covering the theca.

1). Measurements of the 2 species were among the ranges given in the literature (Abé, 1966; Balech, 1963, 1988; Dodge, 1982; Gaarder, 1954; Schiller, 1937; Steidinger & Tangen, 1997) (Table 1).

There are different opinions on the recognition and validity of some species of *Blepharocysta*, especially *B. paulsenii* and *B. okamurae*, which are superficially similar to the best known species and type of the genus, *B. splendor-maris*. Nie (1939) considered that *B. paulsenii* is conspecific with *B. splendor-maris*, with the latter having priority, whereas Balech (1988) stated that *B. okamurae* should be considered as a synonym of *B. splendor-maris*. Taylor (1976) illustrated a specimen (pl. 28, Fig. 289) of *Blepharocysta okamurae* which is very similar to *B. paulsenii* as shown here; Hallegraeff (1988) showed a specimen of *B. paulsenii* almost identical to the current specimens in this work, and more recently, Iwataki et al. (2012) provided 2 LM illustrations with a specimen (p. 127) of *B. okamurae*, which also resembles those given here for *B. paulsenii*. This shows the difficulty to positively identify the species of *Blepharocysta*, only based on LM observations.

Most probably, due to its morphological variability, Gaarder (1954) proposed 2 forms of *B. paulsenii*, f. *bullata* Gaarder and f. *depressa* Gaarder, 2 rare variants of the species, and based on one report of *B. paulsenii* by Gaarder (1954) a new species, *B. hermosillae*, was described (Carbonell-Moore, 1992). Gárate-Lizárraga et al. (2007) reported *Blepharocysta paulsenii* in a coastal lagoon of the western coasts of Baja California, in the northern Mexican

Pacific, following the plates' arrangement (presumably the epitheca proportion), but judging from their illustration (pl. 5, Fig. 17) the specimen most possibly represents *B. splendor-maris*, as the shape of the cell is ovoid and the sulcal lists are very prominent and conspicuous.

Regarding the rest of recognized species, *Blepharocysta denticulata* may be distinguished by the position and structure of the short sulcus, and especially for its dentate plate sutures, lack of one postcingular plate and the sulcal lists extremely reduced (Balech, 1963; Carbonell-Moore, 1994b; Nie, 1939), *Blepharocysta okamurae* has a drop-shape, conspicuous apical pore, poroid theca, and short and poroid sulcal lists which are close to the cingulum (Abé, 1966, Carbonell-Moore, 1994a), and finally, *Blepharocysta hermosillae* which is the largest species, almost perfectly spherical, with wide apical plate 1', and asymmetric sulcal lists, the left one being more developed (Carbonell-Moore, 1992, 1994b) (Table 1).

We gathered important morphological characters to distinguish all *Blepharocysta* species, and some distribution information is additionally included (Table 1), in order to compare these characters, facilitate the identification of the species, and emphasize their importance. From this information it is interesting to evaluate which morphological characters should be considered valuable, outstanding the "traditional" characters such as: *1)* the shape of cells, *2)* the relative size, *3)* the development of sulcal lists, *4)* position and structure of the sulcus, *5)* the theca arrangement (including plates which may be lacking), and *6)* type of plate sutures (linear or dentate).

Table 1

Comparative morphological and distribution characters of the 5 recognized species of the genus *Blepharocysta*. 1: Nie (1939), 2: Balech (1963), 3: Schiller (1937), 4: This study, 5: Balech (1988).

Character	*B. denticulata*	*B. hermosillae*	*B. okamurae*	*B. paulsenii*	*B. splendor-maris*
Outline (Shape)	Subspherical to ellipsoidal	Spherical, globose	Drop-shape, conspicuous APC	Spherical, globose	Subspherical to ovoid
Size	57-65 µm L, 51-55 µm W[1]; 38-47 µm L, 38-46 µm W[2]	56-79 µm L, 59-72 µm W Largest species	56-63 µm L, 53-60 µm W	50-65 µm W[3]; 44-46 µm L, 42-45 µm W[4] (6-7 µm sulcal lists)[4]	40.5-71 µm L, 37-65 µm W[5]; 41-50 µm L, 39-46 µm W[4] (9-10 µm sulcal lists)[4]
Proportion of epitheca or hypotheca	Epitheca reduced	Larger hypotheca: postcingular and antapical plates	Epitheca large	Shorter epitheca	Epitheca large
Tabulation and sutures	Only 4''''. 1a quadrangular. Sutures denticulate (serrate)	APC small. 1' wider than other species. 1a quadrangular	Similar to the type species	Similar to the type species. 1a present, rectangular	Typical tabulation: Po Pt X 3' 1a 5'' 3C 4S 5''' 1''''
Sulcal lists	Inconspicuous, extremely reduced	Reduced and asymmetric, left one more developed	Reduced, ornamented with poroids	Reduced, smooth or fairly ornamented with poroids	More developed, apparently smooth
Theca ornamentation	Poroids (?) and pores numerous	Deep pores of different sizes. No poroids apparently	Strong ornamentation: poroids (?) and pores marked and numerous	Deep poroids and pores, high density, regularly distributed	Shallow poroids and pores, low density. Variable character
Global distribution	Warm-water: subtropical Pacific, north Atlantic (Gulf of Mexico, Caribbean), Indian Ocean,	Warm-water: equatorial Pacific, subtropical and north Atlantic (Gulf Stream, Mediterranean), Indian Ocean	Warm-water: equatorial and tropical Pacific, north Atlantic	Warm-water: Pacific, north Atlantic (Mediterranean), Indian Ocean	Widely distributed, from tropical to Antarctic waters

In this work, we have also observed that the character of the theca ornamentation, type, distribution and density of poroids and pores should be an additional, important and useful morphological character. This character was already suggested by Steidinger and Tangen (1997, p. 533), who stated that "plate pore patterns may help in differentiating species". Some specimens of *Blepharocysta splendor-maris*, the most spread and best known species of the genus, observed with SEM show different patterns of poroids and pores, and possibly the most remarkable example could be the illustrations provided by Carbonell-Moore (2018, Fig. 1 B-E), exhibiting a rather smooth theca with scarce pores (and no poroids) of different sizes, including some few and considerable large pores distributed in the hypotheca, particularly in the postcingular plates 2"' and 4"', and the antapical plate 1"", where they resemble the couple of rows of large pores at the postcingular plates in species of the closely related genus *Podolampas* (Andreis & Andreoli, 1975, Burns & Mitchell, 1982; Dodge, 1985; Hernández-Becerril, 1988; Yang & Li, 2014). Additionally, the intercalary plate 1a shows only 3 small pores, which are scattered but not in a row (as found in this study) (Carbonell-Moore, 2018, Fig. 1 C). Unfortunately, this character should be considered only for "mature" cells and it can be viewed only using SEM facilities.

Molecular phylogenies of members of the family Podolampadaceae, basically the genera *Blepharocysta* and *Podolampas*, have been provided (Gómez et al., 2010; Yamaguchi et al., 2018), with the species *Cabra matta* Murray et Patterson and *Roscoffia capitata* Balech, cladding together with *Blepharocysta* sp., but differing considerably in morphological (presence of a typical cingulum) and ecological characters (benthic habits) (Gómez et al., 2010, 2011; Yamaguchi et al., 2018).

There has been a large debate on a nomenclatural and taxonomic issue derived from the name *Peridinium splendor-maris* Ehrenberg, on which it is based the type species of the genus *Blepharocysta* (Carbonell-Moore, 2018; Elbrächter et al., 2018, 2019). We are still using the concept and names of all *Blepharocysta* species from the literature until a reliable conclusion may be achieved.

Distribution and ecology

Blepharocysta splendor-maris is the only species of the genus with a wide distribution from tropical to Antarctic waters (Balech, 1988; Carbonell-Moore 1994b; McMinn & Scott, 2005), whereas the others seem to be limited to tropical and subtropical waters (Table 1). All species appear in the Pacific Ocean, the Atlantic Ocean (except the southern Atlantic, where only *B. splendor-maris* is present), and the Indian Ocean (where only *B. okamurae* is absent) (Carbonell-Moore, 1994b) (Table 1).

Precisely, according to the habitat preferences of most, if not all, species of *Blepharocysta*, which is relatively deep in the water column (Carbonell-Moore, 1994b), where light should be of very low intensity and quality or rather absent, we can confirm that the 2 species studied here, *Blepharocysta splendor-maris* and *B. paulsenii*, do not contain chloroplasts, despite they were mainly found in subsurface or shallow sampling depths (42 m depth), and they should be regarded as heterotrophic forms (Carbonell-Moore, 2004; Jacobson, 1999). In the closely related species *Podolampas bipes* Stein, Schweikert and Elbrächter (2004) detected the presence of endocytobionts with chloroplasts. This fact may count for Steidinger and Tangen's (1997) statements that *Blepharocysta* (and *Lissodinium* and *Podolampas*) have chloroplasts.

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