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Morphological study of two closely related marine planktonic diatoms: *Bellerochea malleus* and *Helicotheca tamesis*

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Abstract – In this paper, we show morphological features of two marine planktonic diatoms that are phylogenetically related, as suggested by molecular studies: *Bellerochea malleus* and *Helicotheca tamesis*. In order to study *Bellerochea malleus* specimens of the species were isolated from samples collected from marginal coral reefs located in the southern portion of Puerto Rico and cultured in the laboratory. Specimens of the species *Helicotheca tamesis* were isolated from net phytoplankton samples collected from the tropical Mexican Pacific and also cultured in the laboratory. The two species were examined by means of light and scanning electron microscopy. The morphology of *Bellerochea malleus* agrees well with previous reports: cells arranged in relatively long chains, united by the horn-shaped apical elevations, elliptical valves (not triangular) with depressed valve face bearing an eccentric annulus, from which numerous costae radiate, formation of costate ocelli, and one eccentric or marginal protruded and tubular rimoportula per valve. *Helicotheca tamesis* also shows the typical morphological features of this species: cells are solitary or form long and distinctly twisted chains, with no apparent apertures between sibling valves, valves are linear to elliptical, weakly silicified and flat. Furthermore, we found one conspicuous and protruded rimoportula per valve, located eccentrically or marginally (not central as previously described), consisting of an elliptic tubular structure. The cingulum is composed of numerous half-bands and segmented copulae. This study compares the morphology of these two species, with a special emphasis on the structure of the rimoportulae.

***Bellerochea* / Diatoms / *Helicotheca* / Morphology / Phytoplankton / Rimoportulae**

Résumé – **Étude morphologique de deux diatomées marines planctoniques : *Bellerochea malleus* et *Helicotheca thamesis*.** Dans cet article nous présentons les détails de la morphologie des deux espèces marines de diatomées planctoniques phylogénétiquement liés l'un aux autre, selon des études récentes de la biologie moléculaire : *Bellerochea malleus* et *Helicotheca thamesis*. Spécimens de *Bellerochea malleus* ont été recueillies au marginal récifs coralliens sur la côte sud de Puerto Rico et cultivées en laboratoire. D'autre part, spécimens de *Helicotheca thamesis* ont été recueillies dans le Pacifique tropical du

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Mexique et aussi cultivées en laboratoire. Les deux espèces ont été étudiées par microscopie optique et la microscopie électronique à balayage. La morphologie de *B. malleus* est similaire à celle décrite précédemment par d'autres : les cellules formant des chaînes, réunis par des sommets valvaires en forme de corne, les valves de forme elliptique (pas triangulaire), face valvaire excentrique et déprimé, et sommets élevée formant un type nervures d'ocelle et de nombreux nervures rayonnant à partir de l'annulus, un rimoportula par valve, excentrique ou marginale et tubulaire. *Helicotheca thamesis* montre également les aspects décrits précédemment : les cellules formant des chaînes, qui peut être long et le plus souvent tordu, sans ouvertures visibles entre les cellules, qui sont rectangulaires et fragile, souvent tordu, des valves linéaires à elliptiques, faiblement silicifié, et faces valvaires planes. Nous trouvons que le cingulum est composé de multiples bandes comme copulae (bandes segmentè) et qu'il y a un rimoportula par valve, excentrique ou marginal (pas au centre), avec un tube elliptique, très remarquable. Cette étude montre des différences entre les deux espèces, et quelques similitudes, telles que la position et la forme des rimoportulae.

***Bellerochea* / Diatomées / *Helicotheca* / Morphologie / Phytoplancton / Rimoportulae**

INTRODUCTION

The marine planktonic diatoms *Bellerochea malleus* (Brightwell) Van Heurck *emend*. Von Stosch and *Helicotheca tamesis* (Shrubsole) Ricard are common and widespread in coastal waters of tropical and temperate zones. Both species form relatively long chains and are phylogenetically related, according to recent molecular studies (Medlin and Kaczmarska, 2004; Kaczmarska *et al.*, 2005).

The genus *Bellerochea* Van Heurck currently comprises three species: *B. malleus*, *B. horologicalis* von Stosch and *B. yucatanensis* von Stosch (von Stosch, 1977; Round *et al.*, 1990). However, it has been suggested that *B. horologicalis* is a junior synonym of *B. malleus* (see Algaebase: http://www.algaebase.org/search/species/detail/?species_id=37288). On the contrary, the genus *Helicotheca* Ricard is monospecific; *Helicotheca tamesis* was originally described as *Streptotheca tamesis* by Shrubsole, and subsequently transferred to *Helicotheca* because of nomenclatural reasons (Ricard, 1987).

Previous studies on these genera and their species (von Stosch, 1977; Round *et al.*, 1990) have identified several unusual morphological characters, e.g. the structure of the areolae and the apical ocelli in *Bellerochea malleus* (von Stosch, 1977), and the apical pore fields present in *Helicotheca tamesis* (von Stosch, 1977).

In this paper, we present morphological features of these two diatoms based on cultured materials collected from Puerto Rico (*Bellerochea malleus*) and Mexico (*Helicotheca tamesis*), with special emphasis on structure of the rimoportulae.

MATERIALS AND METHODS

Sampling and culturing techniques

Chains and cells of *Bellerochea malleus* were isolated from marine macroalgae from a coral reef near Ponce, Puerto Rico. They were isolated and cultured in f/2 medium and in a light-darkness cycle of 14:10, at a temperature of 24 °C.

Alternatively, phytoplankton samples collected by net (54 µm mesh) were obtained from the Mexican Pacific coast. The samples were kept unfixed until observations in the laboratory were performed. Individual cells or short chains of *Helicotheca tamesis* were isolated from living samples, using a micropipette under an inverted microscope (Invertoskope Zeiss), and placed in a 24-well Petri-dish with 1.5 mL f/2 culture medium. Unialgal cultures were grown in a light-darkness cycle of 12:12, at a temperature of 20 °C ± 2 °C.

Morphology examination techniques

Live specimens of *Bellerochea malleus* were photographed using a Nikon inverted microscope. For examination in Scanning Electron Microscopy (SEM), critical point-dried specimens were ion beam-coated with platinum and examined with a low-voltage SEM (Hitachi S-900) operated at 1.5 kV.

Culture samples of *Helicotheca tamesis* were examined after rinsing with distilled water. Fresh material was examined using an Olympus BX 40 optical microscope, equipped with a Hitachi KP-D50 color digital camera, or a Zeiss Axiolab microscope. For SEM examination, critical point-dried material was put on coverslips, air-dried and mounted on aluminum stubs, coated with gold (Ion Sputter JFC 1100, JEOL) and examined using a JEOL JSM6360LV SEM.

Terminology

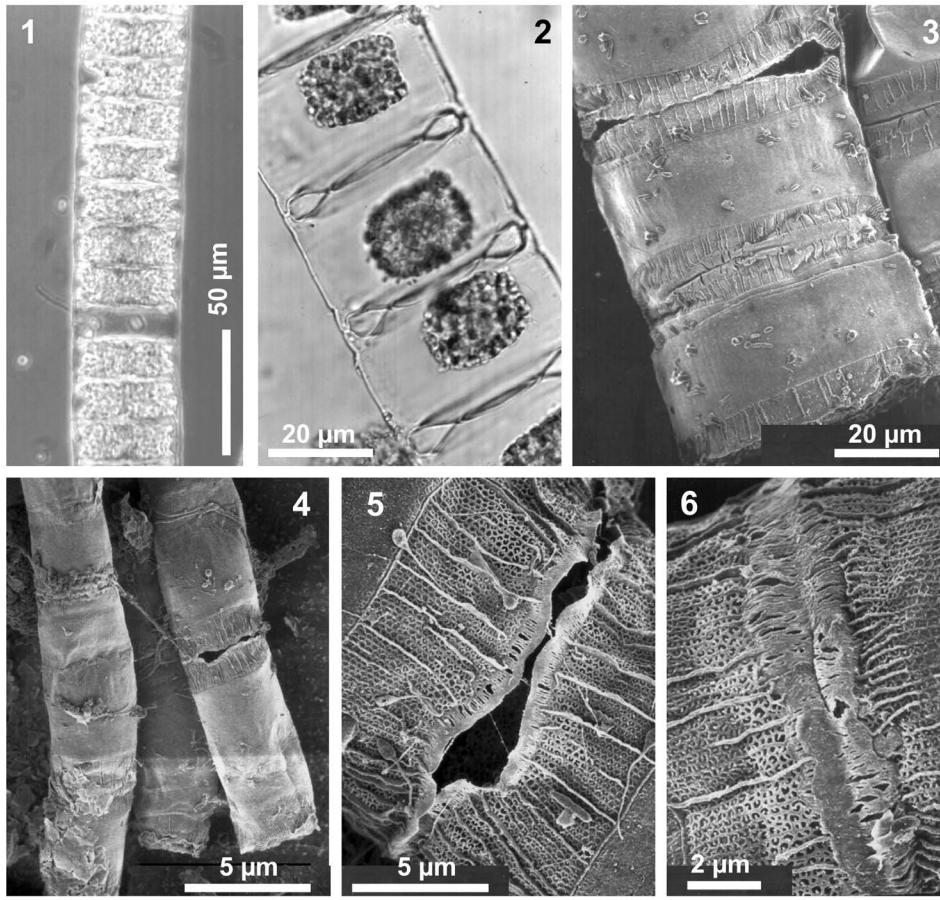
The terminology used in this paper follows the guidelines of Anonymous (1975), Ross *et al.* (1979), Sims (1986), Round *et al.* (1990) and Navarro (2009).

OBSERVATION

Bellerochea malleus (Brightwell) Van Heurck *emend.* von Stosch

Basionym: *Triceratium malleus* Brightwell
References: Hustedt, 1930, p. 781, fig. 456; Hendey, 1964, p. 122, pl. 6, fig. 5; von Stosch, 1977, p. 129, text fig. 1a, figs. 1-25, 43; Hasle & Syvertsen, 1997, p. 227, pl. 48, table 57; Bérard-Therriault *et al.*, 1999, p. 32, pl. 17 d, f; Hoppenrath *et al.*, 2009, p. 91, figs 38 t-z; Kraberg *et al.*, 2010, p. 90.
Description: This species usually forms straight and relatively long ribbon-shaped chains, with teardrop-shaped apertures between siblings located next to the horn-shaped apical elevations (Figs 1-3). The cells are weakly silicified, nearly rectangular in girdle view (Figs 2-4) and broadly elliptical in valve view (Fig. 8), with low elevations at both apices. Sibling valves are linked by apical elevations that are in contact with those of the sibling valves (Figs 2, 3, 5). There are numerous small rounded chloroplasts per cell (Fig. 1).

Observations in SEM show that the valve face is pierced by small poroid areolae that are occluded by modified rotae with one peg (Figs 7, 8, 12). Patches of areolae are interrupted by costae radiating from the central annulus to the valve mantle (Figs 5-8). Some shorter costae also occur on the valve mantle (Figs 5-10). Sometimes the marginal ridge is fused or interlocks with the marginal ridge of the sibling valve (Figs 5, 6). There is a costate ocellus at each apical elevation with longitudinally orientated costae (Figs 9, 10). Ocelli are intersected by the marginal ridge (Figs 7-10).



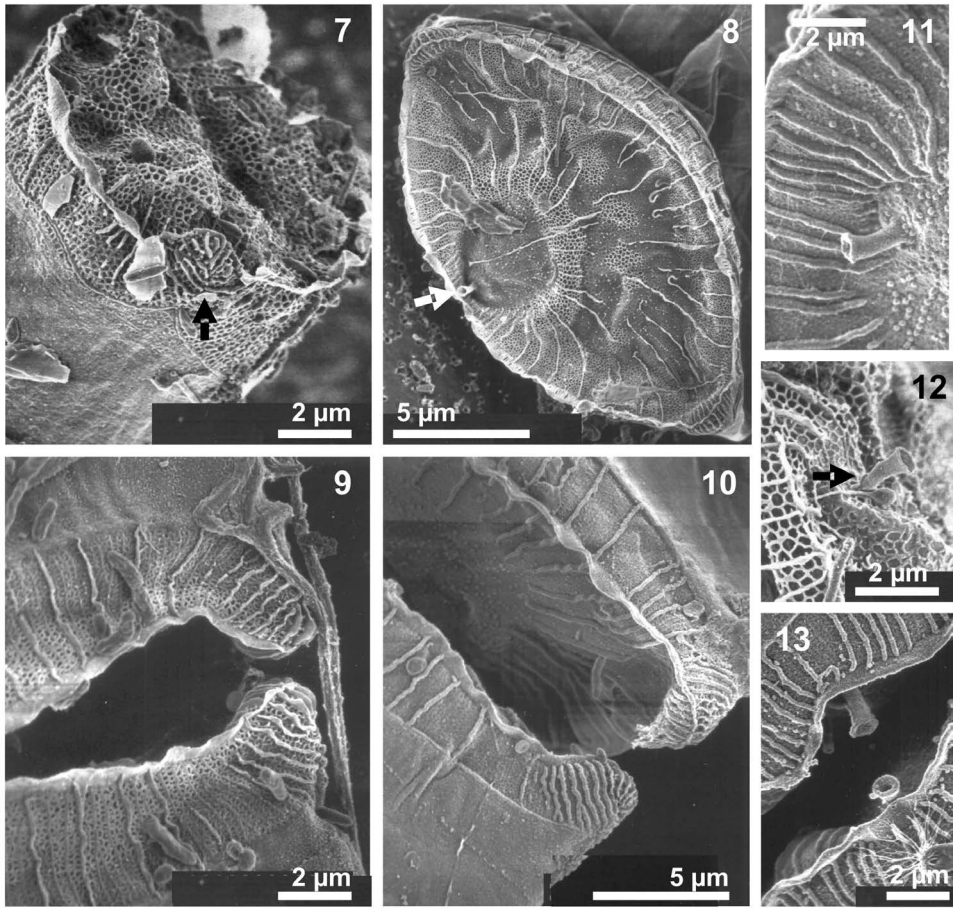
Figs 1-6. *Bellerochea malleus*, LM and SEM, external view. **1.** One long and straight chain in girdle view, LM. **2.** Another chain in girdle view showing three complete cells and teardrop apertures between cells in the chain, LM. **3.** Part of a chain with cells in girdle view, SEM. **4.** Three long chains in girdle view with cells strongly transapically elongate, SEM. **5.** Two sibling valves joined by the horn-shaped apical elevations, SEM. **6.** Two separated sibling valves, girdle view, SEM.

There is one rimoportula per valve, eccentrically located or even marginally within the annulus (Figs 8, 11). Rimoportulae have long external tubes, slightly curved and wider at the tip (Figs 11-13). The rimoportulae of the sibling valves in the chain occur at same side of the chain (Fig. 13). No observations of the internal labiate structure were made. Elements of the cingulum are fused and they are more heavily silicified than the valves, apparently with no areolae (Figs 3, 4), but with a row of relatively large poroids at the abvalvar side of the valvocopula (Fig. 7).

Measurements: 18-45 µm pervalvar axis, 20-55 µm apical axis, 36-40 areolae in 10 µm on both valve face and mantle.

***Helicotheca tamesis* (Shrubsole) Ricard**

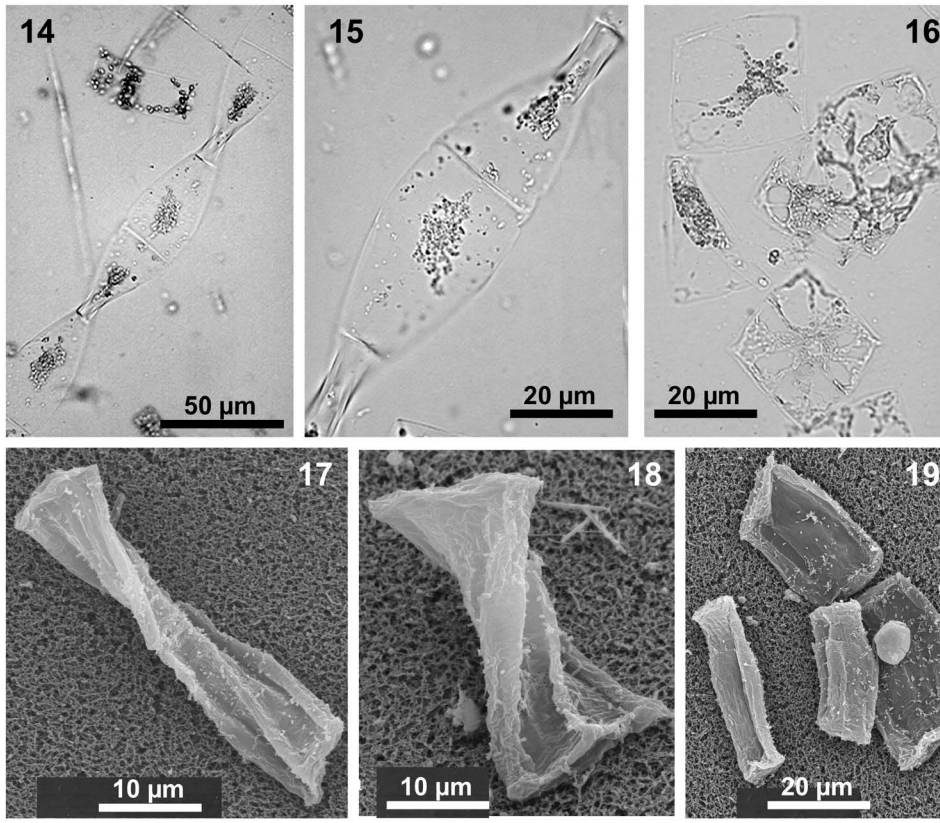
Basionym: *Streptotheca tamesis* Shrubsole



Figs 7-13. *Bellerochea malleus*, SEM, external view. **7.** Apical view of a valve, showing a costate ocellus (arrow), and cingular bands. **8.** Valve view of a specimen with a slightly eccentric annulus and a marginal rimoportula (arrow). **9.** Detail of two sibling valves with horn-shaped apical elevations. Note the teardrop-shaped aperture. **10.** Sibling valves showing details of the valve face and mantle costae, and the costate ocelli. **11.** Detail of valve face, with the external tube of the rimoportula. **12.** Detail of another rimoportula (arrow). **13.** Two rimoportulae on intercalary valves.

References: Hustedt, 1930, p. 778, fig. 455; Cupp, 1943, p. 147, fig. 106; Hendey, 1964, p. 113, pl. 7, fig. 2; Hasle, 1975, p. 126, figs 131-141: von Stosch, 1977, p. 134, figs 71-84; Ricard, 1987, p. 75; Moreno *et al.*, 1996, p. 125, pl. 5, fig. 11; Hasle & Syvertsen, 1997, p. 234, pl. 48; Throndsen *et al.*, 2007, p. 182; Hoppenrath *et al.*, 2009, p. 87, figs 37 d-g; Kraberg *et al.*, 2010, p. 88.

Description: This species is found either forming long, distinctly bow-shaped, twisted chains (Fig. 14), with no apparent apertures between sibling valves (Fig. 15), or as solitary cells that are square, rectangular or more irregular in shape, transapically flattened (Fig. 16). The cells are usually strongly elongate in the pervalvar axis, and contain many small chloroplasts (Figs 15, 16).

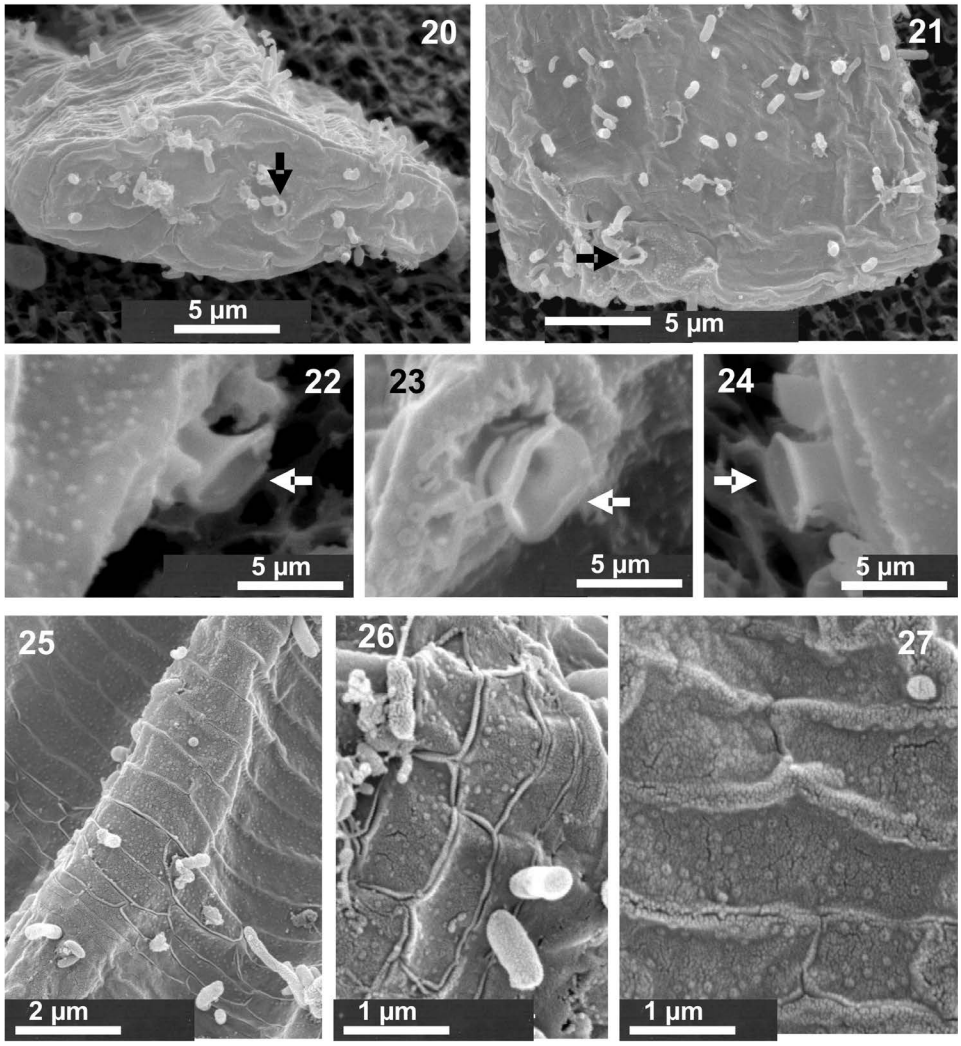


Figs 14-19. *Helicotheca tamesis*, LM and SEM. **14.** A long, twisted chain, LM. **15.** Detail of the chain showing no apertures between cells, LM. **16.** Living cultured single cells with chloroplasts, LM. **17.** A short and twisted chain of two frustules, SEM. **18.** A single, twisted frustule, SEM. **19.** Frustules of various sizes, SEM.

Short straight or twisted chains composed of two cells were examined in SEM (Figs 17, 19). In addition, intact frustules of various sizes and shapes (rectangular, strongly elongate and even twisted) were seen in SEM (Figs 18, 19). Valves are weakly silicified, elliptical to linear, with flat valve face (Figs 17, 18, 20, 21). No areolae were apparent in the valve face or the mantle, but the valves are finely perforated by pores, with tiny, randomly distributed granules were also observed on the valves (Figs 20-24).

There is one rimoportula per valve, observed only on terminal valves, eccentric or marginal (not central) (Figs 20, 21). Externally, it takes the form of a conspicuous, slightly protruded elliptical tube that is widened at the tips (Figs 22-24). No rimoportula was seen on intercalary valves, and no internal labiate structure was observed. The cingulum is formed by numerous half-bands (Fig. 25), which may become segmented copulae, and are randomly perforated (Figs 26, 27).

Measurements: 35-88 µm pervalvar axis, 38-68 µm apical axis.



Figs 20-27. *Helicotheca tamesis*, SEM. **20.** Valve view of a complete frustule, showing an eccentric rimoportula (arrow). **21.** Another frustule with a marginal rimoportula (arrow). **22-24.** Details of three rimoportulae (arrows), showing slightly protruded external tubes widening at the tips. **25.** Cingular bands. **26, 27.** Details of cingulum, with segmented and perforated bands.

DISCUSSION

Morphology

This study shows morphological features of both *Bellerochea malleus* and *Helicotheca tamesis*, and generally confirms the observations made by previous workers (Hasle, 1975; von Stosch, 1977; Round *et al.*, 1990).

The genus *Bellerochea* was studied, illustrated and discussed in great detail by von Stosch (1977), basically using LM and TEM. Then, Round *et al.* (1990), described the genus by LM, TEM and SEM, and more recently, Yahia-Kéfi *et al.* (2005) studied the morphology and seasonality of the species *B. horologicalis*, and Allaway *et al.* (2008, p. 3, Fig. 2) showed an external view of a very lightly silicified valve. Round *et al.* (1990) commented on the possible formation of an ocellus in *Bellerochea*, and Yahia-Kéfi *et al.* (2005) defined this ocellus as pseudocellus in *B. horologicalis*, but we confirm here the presence of a costate ocellus (a certain modified costate ocellus of the *Eucampia* type, Sims, 1986; Navarro, 2009).

The straight chains of *Bellerochea malleus* seem to be a useful morphological character that separates this species from *B. horologicalis*, which forms curved chains, or *B. yucatanensis* which occurs in shorter and separable chains, or even as solitary cells (von Stosch, 1977). Yahia-Kéfi *et al.* (2005) mentioned the presence of slightly curved chains in *B. horologicalis*, but there seems to be no more additional morphological differences between both *B. malleus* and *B. horologicalis*, thus the possibility that these two species may be conspecific, as mentioned earlier (see Algaebase: http://www.algaebase.org/search/species/detail/?species_id=37288), and that can be demonstrated following molecular studies.

Our observations on *Helicotheca tamesis* are generally consistent with previous studies on the species. However, in this study, we were able to observe the structure and location of the rimoportulae in terminal valves of *H. tamesis*. Furthermore, we also documented the general pattern of cingular bands by means of SEM. Hasle (1975) had previously showed some details of the centrally-located (not marginal or eccentric) rimoportulae, which are very similar in shape to those observed here, but she was able to observe the internal labiate structure. We were unable to observe the apical pore fields in *Helicotheca* that were previously documented by von Stosch (1977). Their possible function is joining sibling valves in chains. Cingular bands in *H. tamesis* appear as both half bands and segmented copulae, perforated by randomly distributed pores. This is similar to the illustrations given by Hasle (1975, figs 136, 137), but different from the illustration provided by Round *et al.* (1990, p. 285, Fig. i) where perforations are more regularly distributed.

Molecular studies indicate that *B. malleus* and *H. tamesis* are phylogenetically related (Medlin & Kaczmarska, 2004, Kaczmarska *et al.*, 2005). This is supported by numerous morphological features: forming chains, location and structure of the rimoportulae, similarities in sexual reproduction modes (von Stosch, 1977). Additionally, Hasle (1975) mentioned that the marginal ridge of *Bellerochea* "may be present although poorly developed in *Streptotheca* (sic)", another morphological similarity.

However, both species can not be confused even in LM and there are some noticeable morphological differences between them. The shape of the chains is different, as *Bellerochea malleus* forms straight chains, with teardrop-shaped apertures close to the apices, and cells united in chain (generally interlocked) by the costate ocelli located at the horn-shaped apical elevations; conversely *Helicotheca tamesis* forms distinctive bow-shaped chains, with no apertures between sibling cells, and cells in the chain are possibly joined by the apical pore fields (von Stosch, 1977). Besides, *B. malleus* has areolated and costate valves and mantles, while the valves in *H. tamesis* are only sparsely perforated, and bear no costae.

Rimoportulae are similar in both species, however, those in *H. tamesis* are shorter and wider at their tips. Kaczmarska *et al.* (2005) have used the term

"bilabiate portula" for the rimoportula of both genera *Bellerochea* and *Helicotheca* (*Streptotheca*), assuming that the internal structure is as described by von Stosch (1977). Also Yahia-Kéfi *et al.* (2005) used this term describing *B. horologicalis*, but they were not able to show internal labiate structures; we made no observations of the internal labiate structure of the rimoportula. Finally, there are considerable differences in the structure of the cingula of both species, as described above.

From all references given for each species, it is possible to note that *B. malleus* and *H. tamesis* are common in coastal temperate and tropical waters, and their chains and cells are easily recognizable even in LM.

Taxonomy

According to Round *et al.* (1990), the genus *Bellerochea* is currently placed within the Family Bellerocheaceae Crawford, together with *Subsilicea* von Stosch. *Helicotheca* (*Streptotheca*) and *Neostreptotheca* von Stosch *emend.* von Stosch are included in the Family Streptothecaceae Crawford (Round *et al.*, 1990).

Apart from *Neostreptotheca*, other morphologically similar genus that is probably related to *Helicotheca* is *Mediopyxis* Medlin *et* Kühn. *Neostreptotheca* comprises two species, *N. subindica* von Stosch and *N. torta* von Stosch, while *Mediopyxis* is monospecific, with only *M. helysia* Kühn, Hargraves *et* Halliger described as yet (Kühn *et al.*, 2006).

The fragile *Neostreptotheca* species have square or rectangular cells bearing numerous chloroplasts. These cells form straight or twisted chains, with no apertures between siblings. Even in LM, the characteristic bow-shaped chain of *H. tamesis* is probably the major difference between *Neostreptotheca* and *Helicotheca*. However, solitary cells of *H. tamesis* are difficult to distinguish. The two species of *Neostreptotheca* are distributed in warm waters (Hasle & Syvertsen, 1997).

Mediopyxis helysia can be separated from *Helicotheca tamesis* because the former forms less arcuate chains, and there are gaps between sibling cells in the chains. Other morphological features revealed by electron microscopy provide additional characters for the separation of *M. helysia* from *H. tamesis*. These include: radially arranged poroid areolae, valve apices bearing pseudo-ocelli, the presence of submarginal ridges, and the location, number and structure of the rimoportula in *Mediopyxis* (Kühn *et al.*, 2006). *Mediopyxis* and *Helicotheca* also appear to differ in distribution: *M. helysia* is considered a cold-water species, while *H. tamesis* is widely distributed in more temperate and tropical areas.

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