Morphological variability of the planktonic diatom *Thalassiosira delicatula* Ostenfeld emend. Hasle from the Mexican Pacific, in culture conditions

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Cultures of marine microalgae have greatly contributed to the better understanding of the morphology, phylogeny, life cycles, physiology and ecology of these species. In this paper, the planktonic diatom *Thalassiosira delicatula* Ostenfeld emend. Hasle, originally isolated and cultured (in f/2 medium, a light-darkness cycle of 12:12, and at temperature of 20 °C ± 2 °C) from material obtained from the tropical, southern Mexican Pacific, was studied by LM, SEM and TEM. General morphology of the species agrees well with previous descriptions, including the formation of chains and colonies embedded in mucilage, the chitin threads connecting cells in the chain, the shape of the cells (in girdle view rectangular with a concavity in the valve centre), and processes (with a large rimoportula between valve face and mantle, a marginal ring of occluded ones, and two or three marginal rings of fultoportulae) and areolae arrangement. However the shape and distribution of chloroplasts and threads produced by marginal fultoportulae in living cells, and the presence of more than one central fultoportula, close to a larger areola (two and three fultoportulae were detected in this study) had never been described. Urn-shaped marginal fultoportulae were also very common in most specimens studied, whereas in the literature this character was found only once for the species. Additionally, two rimoportulae were detected in at least one valve. This morphological variability is discussed. This species is a new record for the Mexican Pacific and also the first record of the northern Pacific Ocean.

**Keywords:** diatom, phytoplankton, *Thalassiosira*, culture, morphology, ultrastructure, variability

**Introduction**

Cultures of marine microalgae have been and are still very useful to gain knowledge on several aspects of the morphology, phylogeny, life cycles, physiology and ecology of these species. Some complex life cycles and cryptic species have been recently resolved and recognized from cultured material, notably marine planktonic dinoflagellates *Scrippsiella trochoidea* (MONTRESOR et al. 2003), *Alexandrium taylori* (FIGUEROA et al. 2006) and diatoms *Pseudo-nitzschia* (LUNDOHLM et al. 2003) and *Skeletonema* (SARNO et al. 2005).

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In Mexico, historical studies on marine phytoplankton from the Pacific Ocean have yielded recognized diverse floras, with important tropical and subtropical components, and continuous findings of new records, especially of diatoms and dinoflagellates, and even new species (e.g. HERNÁNDEZ-BECERRIL and TAPIA PEÑA 1995, AKÉ-CASTILLO et al. 1999, HERNÁNDEZ-BECERRIL and BRAVO-SIERRA 2004, HERNÁNDEZ-BECERRIL et al. 2008). However, it has also been recommended that future investigations should consider new and modern study methods and concepts (HERNÁNDEZ-BECERRIL 2003), including culturing of species and molecular studies.

In this paper, the morphology and the morphological variation of the marine planktonic diatom *Thalassiosira delicatula* were studied, based on cultured material obtained from the Mexican Pacific Ocean. Despite the wide geographic distribution of this species, this is its first record from the Mexican Pacific and also from the northern Pacific Ocean.

**Material and methods**

**Cultures**

Phytoplankton samples (either collected by 54 µm net and 1.5 L bottle) were obtained during the cruise TEHUA IV on board R/V »El Puma«, from the tropical, southern Mexican Pacific (15° 07' N, 93° 59' W, in the Gulf of Tehuantepec, 5 September, 2006) and they were kept unfixed and living until observations in the laboratory. Individual cells or short chains of *Thalassiosira delicatula* were isolated from living samples, with a micropipette in an inverted microscope (Invertoskope Zeiss), and placed in a 24-well Petri dish with 1.5 mL f/2 culture medium (GUILLARD and RYTHER 1962). All wells were observed after some days to check growth and conditions of cells, and when cell density was high enough the cultures were transferred to plastic culture bottles and/or sterile plastic Petri dishes with 15–25 mL of f/2 medium. Unialgal cultures were obtained and studied. Conditions of the cultures were a light-darkness cycle of 12:12, and room temperature of 20 °C ± 2 °C.

**Study of the morphology**

Specimens of *Thalassiosira delicatula* were studied from a single culture, mainly in exponential growth, by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Living specimens were observed and measured in LM (Olympus BX40), in fresh slides; cleaned material (acid treated, following recommendations by HASLE 1978) was also observed by LM. For SEM (JEOL JSM-6360LV) cells were critically point dried, after rinsing and dehydration in an alcohol series, and/or acid treated to get only cleaned valves, with no organic remnant. This cleaning treatment was also followed for observations in TEM (JEOL TEM1200 EXII).

**Results**

*Thalassiosira delicatula* Ostenfeld emend. Hasle


Description: Living cells were most commonly joined in short to long chains (2–6 cells), separated from each other 2–3 times the length of cells (or even more), and united by chitin threads (Figs. 1, 2). Occasionally, cells formed chains with sibling cells connected by their valves, with no thread (Fig. 8), as they are possibly recently divided cells as yet without any thread development; alternately, colonies were embedded in mucilage. Cells in girdle view were cylindrical, rectangular in shape (1.5 to 3 times the diameter), 16–18.5 µm in pervalvar length, with rounded corners and a conspicuous concavity in the valve centre.
In valve view cells were completely circular, 8.2–13.9 µm in diameter (Figs. 2, 5, 6). Living cells also showed numerous and long chitin threads from the margin of the valves, directed in different angles to the main axis, but always oblique (Figs. 1–3). Various discoid to elongate chloroplasts were found all over the cell (Figs. 1–3).

(Figs. 1–3, 8–10). 

Pl. 2. *Thalassiosira delicatula*, SEM. Fig. 8 – Two cells joined together, critical point drying (CPD). Fig. 9 – A complete cell with remaining of chitin threads from marginal fultoportulae, CPD. Fig. 10 – Another complete cell showing concavity in the valve centre, marginal processes and structure of the girdle (same symbols as Fig. 4), CPD. Fig. 11 – Valve with one central fultoportula, two marginal rings of fultoportulae and one ring of occluded processes. Fig. 12 – Another valve with two central fultoportulae. Fig. 13 – Valve with three central fultoportulae.
The valves are convex with a concavity in the center (Figs. 10–14). Areolae are fine (30–35 areolae in 10 µm) and displayed in a radial, fasciculate pattern (Figs. 11–17), and in general they are very irregular, from oblong, rectangular, square, pentagonal to hexagonal in shape (Figs. 13–20, 23). The valves usually have one central fultoportula (Figs. 5, 10, 11,
14–17), adjacent to a larger areola (Figs. 14, 16, 18, 19), but also two or three central fultoportulae can be found (Figs. 12, 13, 20), with short to relatively long external protrusions and 4–5 satellite pores (Fig. 19). This central fultoportula produces the chitin thread that connects cells in the chains (Figs. 11, 18).

Pl. 4. *Thalassiosira delicatula*, SEM and TEM. Fig. 21 – Internal view of a single valve showing a central fultoportula, two scattered fultoportulae, two marginal rings of fultoportulae, and a rimoprtula (arrowed), SEM. Fig. 22 – Another valve from internal view, with three central fultoportulae, two scattered fultoportulae, two marginal rings of fultoportulae, and two rimoprtulae (arrowed), SEM. Fig. 23 – Marginal processes of the valve: occluded ones (arrows) and urn-shaped fultoportulae (arrowheads), SEM. Fig. 24 – Mantle of a valve and girdle bands: valvocopula (vc), copula (c) and pleurae (p), SEM. Fig. 25 – Details of valvocopula (vc), copula (c) with ligula and one pleura (p), SEM. Fig. 26 – Valvocopula with areolae, TEM. Fig. 27 – A single pleura with pores in vertical rows, TEM.
MORPHOLOGY OF THALASSIOSIRA DELICATULA

Some fultoportulae may be found scattered in the valve face (Figs. 21, 22). There are also two or sometimes three marginal rings of fultoportulae between valve face and mantle, or on the mantle (Figs. 5, 6, 10–17, 23), with external tubes. Many of these marginal fultoportulae have an urn shape and shorter external tubes, which were very common in most specimens studied (Figs. 11, 12, 14, 15, 17, 23, 24). These fultoportulae were found with remnants of chitin threads (Fig. 9). A rimoportula (in one specimen two rimoportulae were found, Fig. 22) is present between valve face and mantle (Figs. 5, 16, 21, 22) with a fairly large external tube. Additionally, there is a marginal ring of occluded processes, between valve face and mantle, with long tubes (Figs. 9–17, 23). Internally, the rimoportula shows a short neck and a slit orientated perpendicularly to the margin (Figs. 21, 22).

The girdle is composed of valvocopula, copula and a number of pleurae, of which there can be up to 10 (Figs. 4, 9, 10, 24). The valvocopula is broad and has irregular areolae, arranged in vertical rows (4–5 areolae in 1 µm), which become smaller at the abvalvar margin (Figs. 25, 26). The copula is less broad than the valvocopula. In the case of smaller areolae that become smaller at the abvalvar margin, a ligula was present (Fig. 25). Pleurae have very small pores arranged in vertical rows (Figs. 24, 27).

Discussion

Morphology and morphological variation

Almost all morphological details depicted here have been previously described for Thalassiosira delicatula: formation of chains connected by chitin threads, shape of cells in girdle and valve views, details of valves, with their convex shape and a concavity in the centre, areolae arrangement and different processes (one central fultoportula, one or two marginal rings of fultoportulae, one ring of occluded processes, and one rimoportula between valve face and mantle or on the mantle) (e.g. Hasle 1980, Rivera 1981).

Many details by EM were provided by Hasle (1980), so that led her to amend the original descriptions by Ostenfeld and also to consider Thalassiosira coronata Gaarder, conspecific to Thalassiosira delicatula, and hence a synonym of the latter. Later, the morphological variability showed in specimens of Thalassiosira delicatula from Chilean material and revisions made on the type material, led Rivera (1981) to synonymize another species, Thalassiosira chilensis Krasske.

However, no living material had been studied, to show the shape and distribution of the chloroplasts, which indeed are similar to other species of the genus (Horn 2002), and the long chitin threads or fibers that not only join the cells in the chains, but are also present in numbers at the margin of the valves (also a common character in many species of the genus). Complete cells in chains from fixed and mounted material show remains of the protoplasm with only traces of chloroplasts and no marginal threads (Hasle 1980, Rivera 1981). Here we illustrate and depict both elongate to discoid chloroplasts and long marginal threads in living cells.

The central chitin thread is obviously associated to the chain formation, by joining cells together in the chain, and the marginal chitin threads are related to the decrease of cell sinking velocity by increasing form resistance, as in another species of the genus, Thalassiosira fluviatilis Hustedt (Walsby and Xypolyta 1977). All living cells observed in culture had
long marginal threads and were suspended in the medium within the Petri dish, whereas dead cells, with very short, smaller threads or no threads were found sunk at the bottom of the Petri dish.

Almost intact cells (critically point dried) are also shown here in SEM, with some of the sibling valves joined together, with no threads. These complete cells show the marginal rings of fultoportulae with the remains of chitin threads, occluded processes, and a significant number of pleurae (up to 10 in some specimens) in the girdle bands. Rivera (1981) mentioned 3–4 pleurae with no particular structure, but we found some small pores arranged in vertical rows (Fig. 27).

The presence of more than one central fultoportula, close to a larger areola had never been described: all available descriptions illustrate and depict only one fultoportula at the centre. We found valves with two and three fultoportulae in this study. This feature cannot be explained easily but we can speculate that those cells showing this condition were not in exponential growth or they were in rather old cultures experiencing significant variations or even producing theratological forms, either by deficiency in nutrients that could affect »normal« morphogenesis or because of incomplete cell division. Structure and function of fultoportulae (also called strutted process) were discussed by Schmid (1984), regarding wall morphogenesis of Thalassiosira eccentrica (Ehrenberg) Cleve, indicating that »locally exerted stress« affects valve patterns, occurring »only during morphogenesis«, in this case we could appeal this reason in the formation of »extra« fultoportulae in the valve centre, but see also below.

The marginal fultoportulae were usually urn-shaped, with short external tubes, a character that was illustrated for this species only once in the literature (Rivera 1981: Pl. 16, fig. 103). The presence of this kind of fultoportulae has been very rarely reported, although Fryxell (1978) found these in Thalassiosira punctigera (Castracane) Hasle (synonym, Thalassiosira angstii (Gran) Makarova). It is very difficult to relate this shape to any possible function or culture conditions, and it is probably not a useful, consistent taxonomic character, but future experimental investigations on environmental conditions should be conducted. Kaczmarska et al. (2006) have made a hypothesis that central and marginal fultoportulae are non-homologous and they have different origins; this was also largely discussed by Theriot (2008).

We detected two rimoportulae in at least one valve, but it is possible that other valves may also develop more than one rimoportula per valve. No clear explanation exists, but we may also consider different culture conditions that cause this variation, or as in the case of numerous central fultoportulae, theratological forms. Interestingly, the valve we found with two rimoportulae had in addition three central fultoportulae (Fig. 22). In cultures of species of the »pennate« genus Pseudonitzschia, cells display a different chain arrangement than usual, possibly because »The raphe of these cells has apparently stopped functioning« (Lundholm et al. 2002); unusual forms have been found in »old cultures« (e.g. cells with one or two prominences at their margins, personal unpublished data). In other diatoms, development of abnormal structures or absence of the normal, usual number of structures, related to the frustules, are found even in field samples, not only in cultures: e.g. terminal or intercalary setae in the »centric« planktonic diatom Chaetoceros similis Cleve (Hernández-Becerril 2009).

A minor source of variation was detected in the sizes of cells we studied. Whereas we found relatively smaller specimens (8.2–13.9 µm in diameter), other authors have reported
larger cells in diameter: 9–25 µm (Rivera 1981), 9–30 µm (Hasle 1980, Hasle and Syvertsen 1997). The parvalvar length was 16–18.5 µm (this study), against 11–26 µm detected by Hasle (1980). Areolae density was higher in our study: 30–35 areolae in 10 µm, than reported elsewhere: 24–33 (Rivera 1981), 22–26 (Hasle 1980), and 20–33 µm (Licea 1994).

The presence of occluded processes is a unique character occurring only in species of the family Thalassiosiraceae (including the genus *Lauderia* Cleve), although even in this group there are not many species possessing this character. Fryxell (1978) listed 8 species having occluded processes, without including *Thalassiosira delicatula*. Her study was preceded that of Hasle (1980), where the presence of occluded processes in the species was shown. Fryxell (1978) had already discussed some hypotheses to explain the presence and possible functions of occluded processes, according to (1) valve size (larger valves in some species do not have occluded processes), (2) salinity ranges in relation to the number of these processes, (3) physical balance, (4) »initial step toward multiple labiate processes«, and (5) »defense mechanism against grazers«. Hypotheses 3 and 5 are more convincing to us, although we have to test them. The character of possession of occluded processes was not considered »a consistent feature taxonomically« by Fryxell (1978) and not a regular pattern for the genus by Hasle and Syvertsen (1997).

**Distribution and ecology**

*Thalassiosira delicatula* is now considered to be a cosmopolitan species, excluding to polar regions (Hasle and Syvertsen 1997). It was originally found in the Benguela Current and since then it has been reported from all around the world (also as *Thalassiosira chilensis* and *T. coronata*), as in the Atlantic Ocean: north east Atlantic Ocean, Norwegian west coast (ca. 60° N) and east coast of North America (Hasle 1980), Scotland (56° 32’ N) (Harris et al. 1995), Gulf Stream and Sargasso Sea (Herzig and Fryxell 1986), southern Gulf of Mexico (Licea 1994), and the southwestern Atlantic Ocean and Argentinean waters (Lange 1985, Sar 1996, Sar at al. 2002), as in the Pacific Ocean: southern Pacific Ocean, Chilean coasts (Rivera 1981), Sidney, Australia (Hallegraeff 1984). Here we recognize the first record of the species from the Mexican Pacific and also from the northern Pacific Ocean.

This species has been found mainly in coastal systems, and we can consider it a coastal species. No high abundances have been recorded for the species.

**Acknowledgements**

We are grateful to Yolanda Hornelas (SEM service, ICMyL, UNAM) for her skilled assistance at SEM. Partial support for this work was provided by PAPIIT (DGAPA, UNAM) (Project IN223206-2).

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