Morphology of three marine epizoic diatoms from the Mexican Pacific: *Protoraphis hustedtiana*, *Pseudohimantidium pacificum* and *Sceptronema orientale* (Bacillariophyta)

Morfología de tres diatomeas epizoicas marinas del Pacífico mexicano: *Protoraphis hustedtiana, Pseudohimantidium pacificum* y *Sceptronema orientale* (Bacillariophyta)

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Abstract

Diatoms are a successful taxonomic group of microbes exhibiting a high diversity in marine habitats. Marine epibiont diatoms also display a vital diversity of symbiotic associations. Net phytoplankton samples collected in the Mexican Pacific were analyzed, and apart from finding planktonic species, three epizoic diatom species were additionally observed: *Protoraphis hustedtiana*, *Pseudohimantidium pacificum*, and *Sceptronema orientale*. The three species, usually found attached to copepods, were studied by light, scanning, and transmission electron microscopy (ML, SEM, and TEM), showing morphological characteristics such as the shape of the valves, central (and sigmoid) sternum, fine striae, areolae shape, apical and aggregated rimoportulae, and slit fields at the apices (or poles). Morphological details found in *Pseudohimantidium pacificum* and *Sceptronema orientale* include velum in the areolae (rota type) and the external openings of the rimoportulae, respectively. *Protoraphis hustedtiana* and *Sceptronema orientale* are new records for the Mexican Pacific.

Palabras clave:
caracteres morfológicos; diatomeas; microscopía electrónica; nuevos registros; ticoplancton

Resumen

Las diatomeas constituyen un grupo taxonómico de microbios exitosos, que exhiben una alta diversidad en hábitats marinos. Las diatomeas epibiontes marinas también despliegan una diversidad importante de asociaciones simbióticas. Se analizaron muestras de red de fitoplancton de localidades del Pacífico mexicano, que estuvieron representadas por especies planctónicas, y adicionalmente se observaron tres especies de diatomeas epizoicas: *Protoraphis hustedtiana*, *Pseudohimantidium pacificum* y *Sceptronema orientale*. Las tres especies, usualmente adheridas a copépodos, fueron estudiadas mediante microscopía de luz, electrónica de barrido y transmisión (ML, MEB y MET), cuyas observaciones mostraron caracteres morfológicos como forma de las valvas, sternum central (y sigmoideo), delicada estriación, forma de las areolas, rimopórtulas agregadas en los ápices, y campos apicales (o en los polos) de ranuras, con lo que se confirman estudios previos. Algunos detalles morfológicos encontrados en *Pseudohimantidium pacificum* y *Sceptronema orientale* incluyen el tipo de velum de las areolas (del tipo rota) y las aperturas externas de las rimopórtulas, respectivamente. *Protoraphis hustedtiana* y *Sceptronema orientale* son nuevos registros para el Pacífico mexicano.

Diatoms belong to a very successful taxonomic group of microbes in aquatic environs worldwide (Round *et al.,* 1990; Medlin and Kaczmarska, 2004). Marine diatoms exhibit a remarkable diversity in size, shape, and life histories, and planktonic diatoms are a significant contributor to the biomass and productivity of the world's oceans (Kooistra *et al.,* 2007). Littoral, benthic, and epibiont marine diatoms have also been studied, although they display a high morphologic complexity (Round, 1971; Round *et al.,* 1990). Various diatom species used to live as epizoic forms on small planktonic animals as copepods and others, showing particular ways of attachment (*e.g.,* developing stalks or peduncles, producing mucilage.) (Hiromi and Takano, 1983; Tiffany, 2011; Totti *et al.,* 2011; Gárate-Lizárraga and Esqueda-Escarcega, 2018).

During several oceanographic expeditions carried out along coasts of the Mexican Pacific, the high diversity of planktonic diatoms has been confirmed (Hernández-Becerril *et al.,* 2010, 2013), but additionally epipsammic forms have been also reported, including descriptions of two new species (Hernández-Becerril and Barón-Campis, 2008; Hernández-Becerril *et al.,* 2012).

Three species of araphid diatoms, *Protoraphis hustedtiana Simonsen*, *Pseudohimantidium pacificum* Hustedt et Krasske, and *Sceptronema orientale Takano*, are considered as epizoic, living on certain species of copepods. There are some modern studies of these three species, especially showing the way they attach to the copepods, some morphologic details (including the formation of chains), and their biological affinities (symbiosis), distributions, and relative abundances (Simonsen, 1970; Gibson, 1978; Hiromi and Takano, 1983; Rivera *et al.,* 1986; Skovgaard and Saiz, 2006; Gómez *et al.,* 2018, 2020; Li *et al.,* 2020).

The main purpose of this work was to study the detailed morphology of these three species to contribute to the knowledge of the diversity of marine diatoms in the Mexican Pacific litorals.

This study is based on material collected during various oceanographic surveys along the coasts of the Mexican Pacific during the period 1985-2019. Main spots were located at: (1) 18° 12.943' N, 103° 20.094' W, (2) 17° 47.729' N, 102° 09.294' W, (3) 17° 08.293' N, 100° 54.078' W, (4) 16° 39.310' N, 99° 55.901' W. Net samples (mesh 64 and 54 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 %).

Either raw or rinsed (with distilled water) samples were analyzed by Light Microscopy (LM, Olympus BX 40, attached camera Hitachi KP-D50 Color digital), where preliminary identifications, measurements, and microphotographs were made. After air-drying and coating with gold, whole rinsed samples or isolated specimens were also studied by Scanning Electron Microscopy (SEM, JEOL JSM6360LV). Only cleaned material was observed by Transmission Electron Microscopy (TEM, JEOL TEM1200 EXII), once the acid treatment, recommended by Hasle (1978), was followed.

Terminology for diatoms and the genera *Pseudohimantidium*, *Protoraphis* and *Sceptronema* follows that recommended by Round *et al.* (1990) and Hasle and Syvertsen (1997).

The preliminary microscopical analysis yielded several planktonic forms of diatoms, and also, the species studied here are considered epizoic species, although they were not found attached to copepods or other small animals.

Description of species

Protoraphis hustedtiana Simonsen (figures 1-4)

References

Simonsen, 1970, p. 383, pl. 1; Hallegraeff and McWilliam, 1990, p. 39, figures 1-13; Li *et al.,* 2020, p. 121, figures 1 a-f, 2 a-f, 3 a-e.

The cells commonly adhere to copepods (as in the precedent species). Valves are lanceolate (slightly curved), diagonally symmetrical, with rounded apices (figure 1). The valve is convex, curving into the mantle (figure 1). The sternum is conspicuous, linear to slightly sigmoid, especially at the apices, where it becomes slightly bent at opposite sides of the rimoportulae (figure 1, 2). The striae are uniseriate, perpendicular to the central axis, and formed by rounded areolae (figure 2). There is a deep perpendicular groove near each apex of the valve, at the end of the sternum, with four external openings representing the rimoportulae (figure 1, 3, 4). Striae are radial at the end of the valve, and apical slits fields are also apparent (figures 3, 4).

Measurements: Apical axis 45-60 µm, transapical axis 6-10 µm, 30-32 striae in 10 µm, 3-4 areolae in 1 µm.

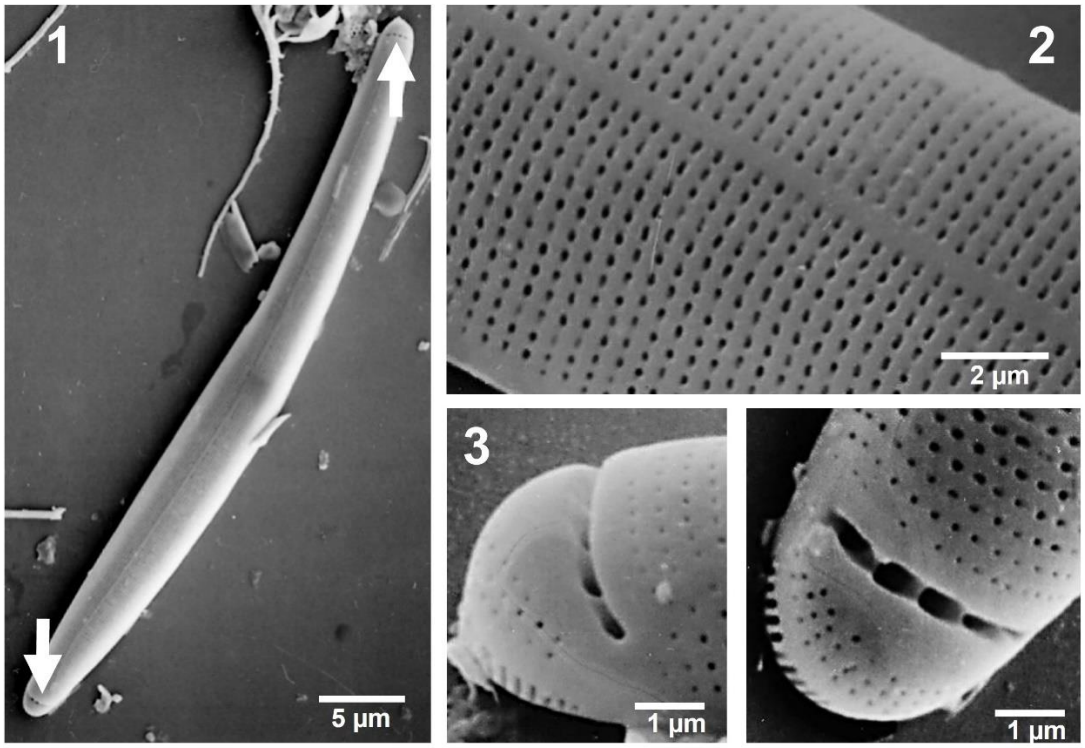
Pseudohimantidium pacificum Hustedt et Krasske (figures 5-8)

References

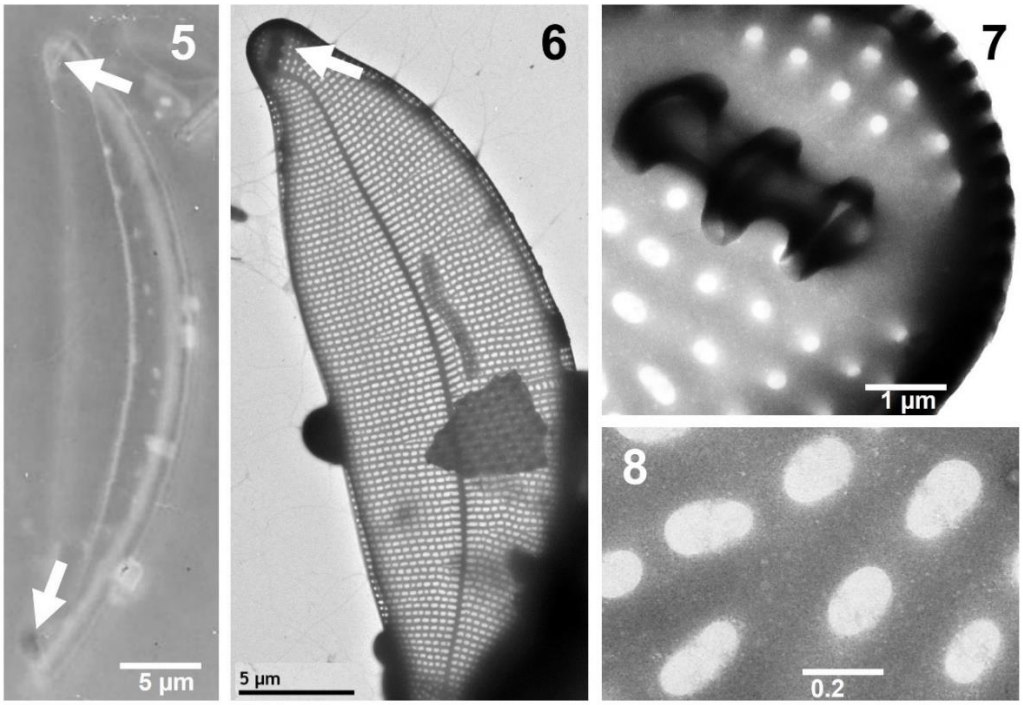
Krasske, 1941, p. 272, pl. 5, figure. 8; Simonsen, 1970, p. 385, figures 5-22; Gibson, 1979a, p. 149, figure 1-13; Rivera *et al.,* 1986, p. 21, figure 1-35; Round *et al.,* 1990, p. 446, figure a-g; Fernandes and Calixto-Feres, 2012, p. 837, figures 1-17; Sunesen *et al.,* 2015, p. 268, figure 23-40.

Living cells have been usually found attached to copepods by mucilage stalks produced at the apices of the frustules. Only loose valves were encountered in this study, which could be positively identified as all morphological characters fit well with previous descriptions. Valves are arcuate with subrostrate apices (figure 5, 6). The valve is slightly convex and curves into the mantle. There is a curved, slightly sigmoid, and narrow sternum, at one apex curving more abruptly, ending in the apical rimoportulae and at the other apex ending in the other side of the rimoportulae (figure 5, 6). The striae are uniseriate, perpendicular to the central axis, and turning radiate toward the

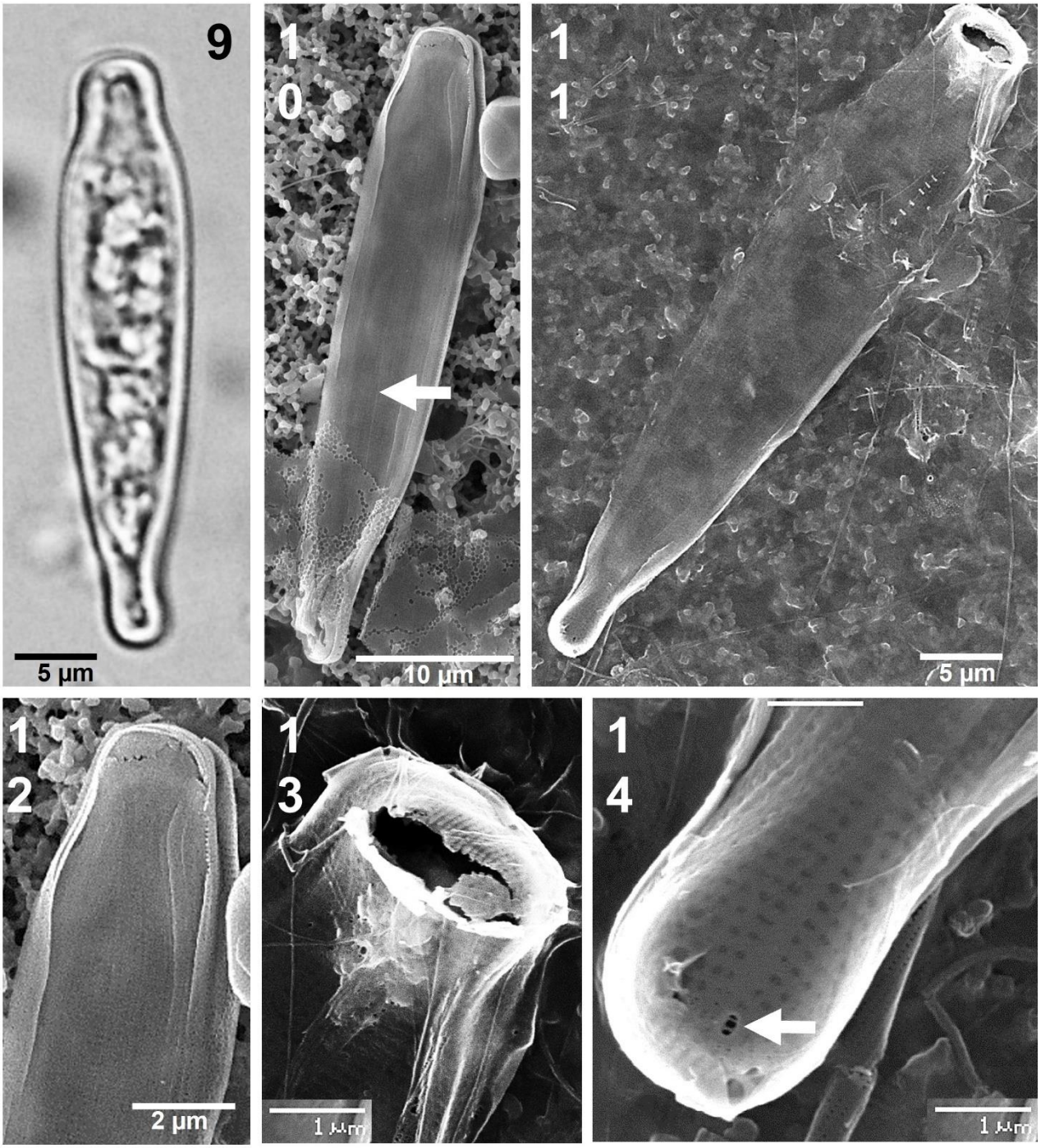
apices (figure 6); areolae have a rota type (figure 8). The rimoportulae are located at the apices of the valves, with three to six rimoportulae aligned next to the other, orientated to the valve's ventral side (figure 5, 7).



Figures 1-4. *Protoraphis hustedtiana*. Fig. 5. Lanceolate valve with rimoportulae (arrows), SEM. Figure. 6. Detail of the central part of the valve, showing sternum and striae, SEM. Fig. 7. Detail of the apical groove, with the external opening of the rimoportulae, SEM. Figure. 8. Another view of the apical groove, SEM.



Figures 5-8. *Pseudohimantidium pacificum*. Figura 1. A complete valve showing the central sternum and the rimoportulae (arrows), LM. Figure. 2. Another valve with the sternum, striae and rimoportulae (arrow), TEM. Figure. 3. Detail of the rimoportulae, TEM. Figure. 4. Detail of the areolae, TEM.



Figures 9-14. *Sceptronema orientale*. Figure. 9. Solitary cell with a distinct valve shape, LM. Figure. 10. Complete frustule, exhibiting the central sternum (arrow), SEM. Figure. 11. Another frustule, slightly broken at the head-pole, SEM. Figures 12, 13. Details of the head-pole, with the slit field, SEM. Figure. 14. Foot-pole showing the sternum, areolae and the external opening of a rimoportula (arrow), SEM.

***Sceptronema orientale* Takano (Figures 9-14)**

References

Takano, 1983, p. 26, figures 2, 15-20; Sar and Sunesen, 2014, p. 3, figures 2-28; Gómez *et al.,* 2020, p. 26, figures 2 A-U.

Complete, living cells have been detected joined by a mucilaginous pad to copepods. Solitary cells were found in this study (Figures 9-11). Cells in valve view have a distinct shape of an Egyptian sarcophagus (Figures 9-11). Valves are distinctly heteropolar, narrowly obovate, with a head-pole and a narrow capitate foot-pole (Figures 9-11). The valves are flat, and the

sternum is central and straight (Fig. 10). The striae are uniseriate and are perpendicular to the sternum (Figures 10-12), whereas the areolae are round to elliptic (Figures 14). Apical slits with longitudinal bars are present at both poles, although this slit field is more prominent at the head-pole (Figures 12-14). These fields occur more from where the mucilage pads emerge to adhere to copepods. There are numerous rimoportulae close to the poles, especially the foot-pole, where their elliptical external openings are visible (Figures 14).

Measurements: Apical axis 31-48 µm, transapical axis 8-10 µm, 38-42 striae in 10 µm, 4-5 areolae in 1 µm.

Some morphological details of three epizoic marine diatoms found in plankton samples from the Mexican Pacific are provided in this study. These species usually live attached to copepods, although they were encountered as solitary cells or valves, most probably caught accidentally in phytoplankton net samples.

The three species studied here, *Protoraphis hustedtiana*, *Pseudohimantidium pacificum*, and *Sceptronema orientale*, are all araphid diatoms, which usually produce mucilage pads or stalks to adhere to copepods. There are reports of large infestations of these species (Rivera *et al.,* 1986; Fernandes and Calixto-Feres, 2012; Sar and Sunesen, 2014; Gómez *et al.,* 2020). They are well adapted to epizoic life, developing shapes and mechanisms to deal with the supposed turbulent habitat. They share some morphological characteristics such as the presence of a central sternum (instead of a raphe) and specialized rimoportulae in aggregations or large numbers (Simonsen, 1970; Gibson, 1978; Hiromi and Takano, 1983; Rivera *et al.,* 1986; Gómez *et al.,* 2020; *Li et al.,* 2020).

The morphological characteristics described here have been previously shown in the literature, particularly in studies using electron microscopy, and this is a confirmation of some details, such as the shape of the valves, the central (and sigmoid in two species) sternum, the fine striae and the shape of the areolae, the apical and aggregated rimoportulae (with particular orientation in *Pseudohimantidium pacificum*), and the slit fields at the poles (especially the *species Sceptronema orientale*) (e.g., Simonsen, 1970; Gibson, 1978; Rivera *et al.,* 1986; Fernandes and Calixto-Feres, 2012; Sar and Sunesen, 2014; Gómez *et al.,* 2020; Li *et al.,* 2020). An exceptional morphological detail found in Pseudohimantidium pacificum is the areola, a velum of the rota type, not previously described.

From these species, *Pseudohimantidium pacificum* has been historically recognized and is considered widely distributed and probably the best studied of the marine epizoic diatoms, whereas *Protoraphis hustedtiana* and *Sceptronema orientale* have been less reported and, consequently, less studied.

The original description of *Sceptronema orientale* included a protocol mentioning the absence of rimoportulae in this species (Takano, 1983, p. 26), although his illustrations (figures 2 C and 17) suggest the presence of various rimoportulae. Later, Sar and Sunesen (2014) provided details of these rimoportulae in the species. The external openings of the rimoportulae were also found in this study.

The species Pseudohimantidium pacificum has been already reported from the Mexican Pacific (Gárate-Lizárraga and Muñetón-Gómez, 2009; Gárate-Lizárraga and Esqueda-Escarcega, 2018), but the other species represent new records in the Mexican Pacific, *Protoraphis hustedtiana* and *Sceptronema orientale*. The recognition of these two records is not a surprise, considering the poor studies dedicated to epibiont diatoms in Mexico and the relatively few records worldwide (cited for each species), and widens the species' distribution in subtropical areas of the Mexican Pacific.

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