

***Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen (Cymatosiraceae, Bacillariophyceae): First report of the species from the Southeastern Pacific with comments on the variability of some of its morphological features**

***Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen, (Cymatosiraceae, Bacillariophyceae): Primera cita para el Pacífico Suroriental, con comentarios acerca de la variabilidad de algunas de sus características morfológicas**

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ABSTRACT

During the taxonomic analysis of strains from marine phytoplankton samples, it was found that a sample contained isolated or short chains of 2-4 small cells. Observations under the electron microscopes revealed the presence of *Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen, a minute species until now not reported for the Southeastern Pacific Ocean. In this paper we describe the characteristics of the Chilean cells, comment on the variability of some of its morphological features, and discuss about the differences between the two known species of the genus.

KEYWORDS: Diatom, morphology, taxonomy, distribution, Chile.

RESUMEN

Durante el análisis de cepas provenientes de muestras de fitoplancton marino se observó que una de ellas contenía células muy pequeñas, aisladas o formando cortas cadenas de 2-4 individuos. Observaciones realizadas con microscopía electrónica revelaron la presencia de *Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen, una diminuta especie no señalada hasta ahora para el Pacífico Suroriental. En el presente trabajo se describen las características de las células chilenas, se comenta acerca de la variabilidad de algunas de sus características morfológicas, y se discute sobre las diferencias entre las dos especies conocidas del género.

PALABRAS CLAVE: Diatomeas, morfología, taxonomía, distribución, Chile.

INTRODUCTION

In 1983, Hasle *et al.* established the family Cymatosiraceae, characterized basically by the presence of two polar elevations of the valves, bearing each one an ocellulus (defined as a small ocellus). The new family consists of the heterovalvate subfamily Cymatosiroideae and the isovalvate subfamily Extubocelluloideae, which lacks other features such as pili and the process present in the Cymatosiroideae. For the subfamily Extubocelluloideae they described the type genus *Extubocellulus*, structured by two species: *E. spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen, referred to *Bellerochea* Van Heurck by Hargraves and Guillard (1974), and by the new species

E. cribriger Hasle, Stosch & Syvertsen. According to the literature, the principal morphological differences between both species are the size of the poroids, the absent/presence of vela, and the morphology of the spines. While *E. spinifer* seems to have a wide distribution in coastal marine waters, *E. cribriger* has been reported only from the type locality, Helgoland, and from Leybucht, German Bight, Germany.

During the taxonomic analysis of strains from marine phytoplankton samples, one of them contained isolated and short chains of 2-4 small cells that showed little features of a diatom species. However, electron microscopy observations revealed the presence of *Extubocellulus spinifer*, a minute species until now not reported for the Southeastern Pacific. In this paper we describe the characteristics of the Chilean

cells, comment on the variability of some of its morphological features, and discuss differences between the two known species of the genus.

MATERIAL AND METHODS

The species was isolated from a marine surface plankton sample taken on May 2009 from the locality of Huenao, Chiloé Island (42°28'S-73°38'W), using a net with a mesh size of 25 µm. This sample is deposited at the Diatom Collection, Department of Botany, University of Concepción with the numbers DIAT-CONC M-3357, 3365 and DIAT-CONC 7318-7319. Then, isolated cells were cultured in a Walne + Si medium (Walne 1970) at the Microalgae Laboratory of the University of Concepción. Cultures were maintained under a temperature of 15 ±2° C with a photoperiod of 16 hours light and 8 hours dark and light of 10-20 µmol photons per m² per second. The sample was treated for the removal of organic matter according to the method described by Hasle & Fryxell (1970). The light microscope (LM) used was a Zeiss Photomicroscope III; the Jeol JSM-6380LV scanning electron microscope (SEM) and the Jeol 1200 EX II transmission electron microscope (TEM) were used for electron microscopy. Critical point drying, as described by Anderson (1951), was used. Terminology used is that suggested by Hasle *et al.* (1983).

RESULTS

Extubocellulus spinifer (Hargraves & Guillard) Hasle, Stosch & Syvertsen (Figs. 1-2).

Basionym: *Bellerochea spinifera* Hargraves & Guillard, Phycologia 13(2): 167, Figs. 1-8, 1974.

Hasle, Stosch & Syvertsen, Bacillaria 6: 70, Figs. 362-390, 392, Text fig. 13, 1983.

In girdle view the cells are rectangular, with a perivalvar axis 4.0-8.2 µm long, solitary or joined in chains of no more than 3-4 cells (Fig. 1 A-B) by spines present on their valves (Fig. 1C). The cingulum has many open bands, described by Hasle *et al.* as "quasifrac" due to its general appearance of being broken in small units (Fig. 1 C-D); the valvocopula and the next bands have a row of pores at the advalvar side, 10-11 in 1 µm (Fig. 1 E). The valves are oval (Fig. 2 A) to almost circular (Fig. 2 B), 2.3-2.8 µm long and 1.9-2.4 µm wide, with the valve face almost flat and with a well defined mantle, free of pores and spines (Figs. 1 D-E, 2 A-B). A small raised ocellulus is located at each valve apex (Figs. 1 F, 2 A-B), perforated by 7-9 porelli (Fig. 2 D-E). On the valve face, the poroids (no vella seen), 5-6 in 1 µm, are irregularly distributed, except those on the most external row that are parallel to the valve margin (Fig. 2 A-B). The spines are

irregularly distributed principally on the submarginal zone of the valves (Fig. 2 B), but also in the central part in some valves (Fig. 2 A). They are 0.2-2.0 µm long and always present a globular basal part (Figs. 1 E-F, 2 G).

DISCUSSION

Although many individuals of the species under discussion were analyzed, specific features of the components of the cingulum were very difficult to observe, even though the critical point drying method was used. This study did not include specimens from other geographical areas, nor identified material from other institutions; however, there is no doubt about the identity of the taxon examined. The morphological characteristics of the specimens studied agree well with those of *Extubocellulus spinifer*. Nakata (1987) and Riaux-Gobin & Chrétiennot-Dinet (2000) have also provided information about the morphology of this taxon.

In general, the Chilean specimens are slightly smaller compared to the material observed by other authors. The form, size, number and distribution of the spines on the valve face vary widely (commented also by Riaux-Gobin & Chrétiennot-Dinet (2000), studying material from North Brittany, and by Hasle *et al.* (1983), from the type locality). They always exhibit a globular basal part of variable diameter, characteristic until now distinctive for *E. spinifer* and commonly only the basal globule is present (Figs. 1 F, 2 A). Bifurcated spines, reported for this species by Hargraves & Guillard (1974) and Hasle *et al.* (1983), were not found in the Chilean material. The spines are abundantly scattered at the marginal area of the valve, at some distance from the valve margin (Fig. 1 E), but in some cases they are also present at the valve centre (Fig. 2 A-B), and in some individuals they were very scanty on the valves (Fig. 2 C). The occurrence in the studied material of valves with three ocelluli was sporadic (Fig. 2 F). The submarginal tubular process found by Hargraves & Guillard (1974) on the valve face of some cells of *Bellerochea spinifera*, was neither observed in the Chilean material nor by Hasle *et al.* (1983).

The second known species of the genus, *E. cribriger*, differs from *E. spinifer* principally by having larger areolae (a velum is present) and spines without a globe-shaped basal part (Hasle *et al.* 1983). However, Nakata (1987) reported *E. spinifer* from material collected in the Japanese coast, bearing areola with rota type vella and spines with basal globules. Poroids with or without vella could be the consequence of culture conditions, or variability in techniques of electron microscopy. A very thin layer of silica like a cribrum, breaks and disappears easily when it is treated for removal of organic matter, and to avoid it, the critical point drying method (Anderson 1951) can be used.

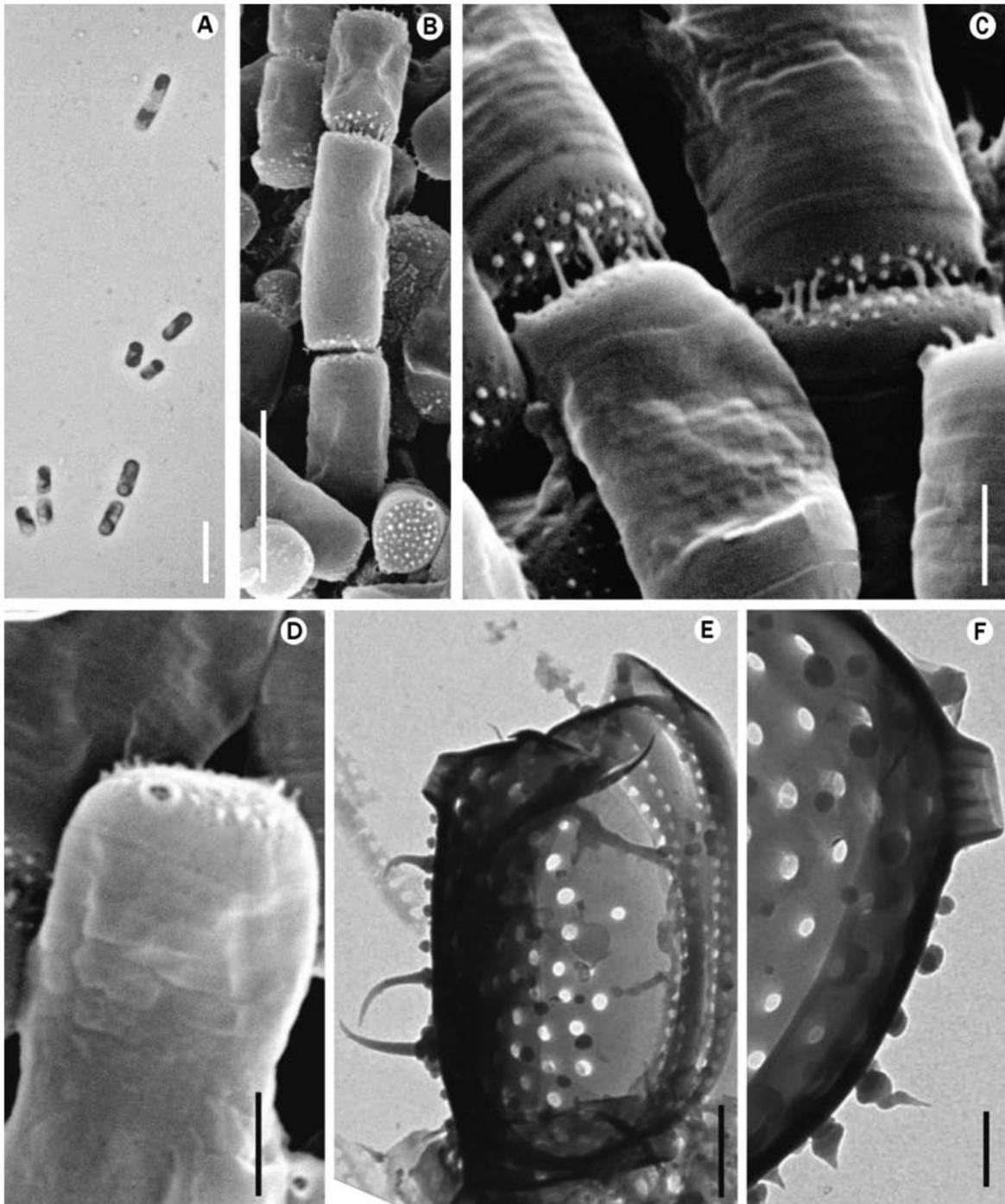


FIGURE 1. *Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen. Fig. A LM, Figs. B-D SEM, Figs. E-F TEM. A. Cultured cells. B. Short chains. C. Cells joined by spines; cingulum with many quasifract bands. D. Quasifract bands. E. Spines on the valve and a row of pores at the advalvar side of the bands. F. Raised ocellulus and spines with a globular basal part. Scale bars: A = 10 μ m, B = 5 μ m, C-D = 1 μ m, E = 0.5 μ m, F = 0.2 μ m.

FIGURA 1. *Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen. Fig. A LM, Figs. B-D SEM, Figs. E-F TEM. A. Células en cultivo. B. Cadenas cortas. C. Células unidas por espinas; cingulum con muchas bandas quasifractas. D. Bandas quasifractas. E. Espinas sobre la valva y una fila de poros en el lado advalvar de las bandas. F. Ocellulus elevado y espinas con una parte basal globular. Escala: A = 10 μ m, B = 5 μ m, C-D = 1 μ m, E = 0.5 μ m, F = 0.2 μ m.

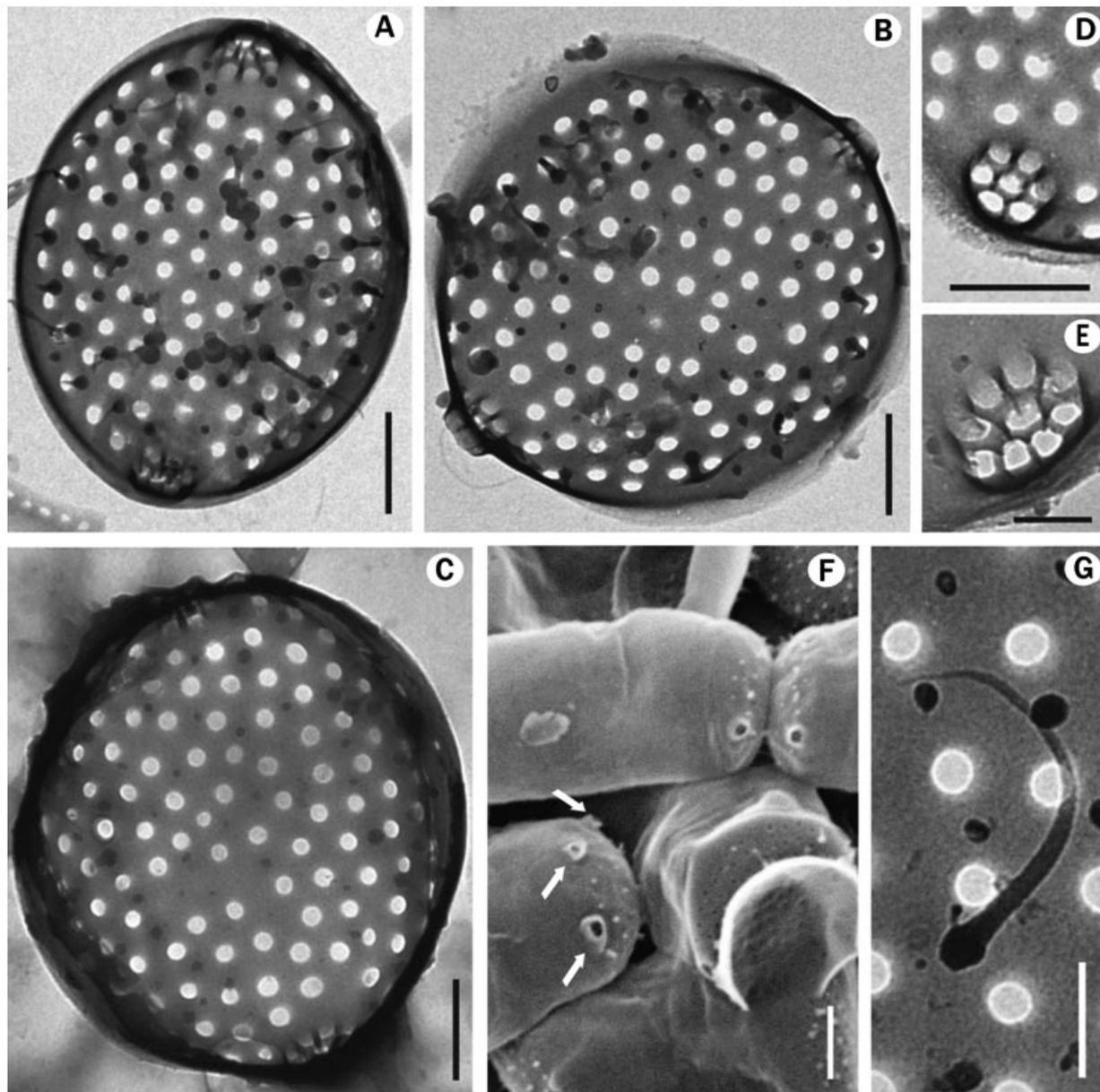


FIGURE 2. *Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen. Figs. A-E, G TEM, Fig. F SEM. A. Oval valve with spines distributed in submarginal and central areas. B. Subcircular valve with poroids irregularly distributed. C. Spines very scanty on the valve surface. D. Ocellulus with 7 porelli. E. Ocellulus with 9 porelli. F. Valve with 3 ocelluli (arrows). G. Long spine with a globular basal part. Scale bars: A-D = 0.5 μ m, E,G = 0.2 μ m, F = 1 μ m.

FIGURA 2. *Extubocellulus spinifer* (Hargraves & Guillard) Hasle, Stosch & Syvertsen. Figs. A-E, G TEM, Fig. F SEM. A. Valva ovalada con espinas distribuidas en las áreas submarginal y central. B. Valva subcircular con poroides distribuidos irregularmente. C. Espinas muy escasas sobre la superficie valvar. D. Ocellulus con 7 porelli. E. Ocellulus con 9 porelli. F. Valva con 3 ocelluli (flechas). G. Espina larga con una parte basal globular. Escala: A-D = 0.5 μ m, E,G = 0.2 μ m, F = 1 μ m.

In the present study the method was used but no cribrum was found. In conclusion, there is not yet sufficient information on the real magnitude of the morphological variation in both species of the genus *Extubocellulus*, so further observations are needed to clarify its taxonomy.

This is the first report of *E. spinifer* for the Southeastern Pacific Ocean. The species was previously reported from the North Atlantic (Hargraves & Guillard 1974, Hasle *et al.* 1983, Riaux-Gobin & Chrétiennot-Dinet 2000, Martin-Cereceda *et al.* 2007), Southeastern Atlantic (Adams *et al.* 1999), Northeastern Pacific (Hargraves & Guillard 1974, Hasle *et al.* 1983), Northwestern Pacific (Nakata 1987) and Southwestern Pacific (Stauber & Jeffrey 1988, Knuckey *et al.* 2002).

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