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## A NEW DIATOM (BACILLARIOPHYCEAE) SPECIES FROM A THERMAL SPRING IN AZORES ARCHIPELAGO (SÃO MIGUEL ISLAND, ATLANTIC OCEAN)

# NUEVA ESPECIE DE DIATOMEA (BACILLARIOPHYCEAE) EN UNA FUENTE TERMAL EN EL ARCHIPIÉLAGO DE AZORES (ISLA DE SAN MIGUEL, OCÉANO ATLÁNTICO)

<sup>10</sup>CRISTINA DELGADO<sup>1,2\*</sup>,<sup>10</sup>VITOR GONÇALVES<sup>3,4</sup>,<sup>10</sup>SAÚL BLANCO<sup>5</sup>,<sup>10</sup>SALOMÉ F.P. ALMEIDA<sup>1</sup>

<sup>1</sup>Department of Biology and GeoBioTec - GeoBioSciences, GeoTechnologies and GeoEngineering Research Centre, University of Aveiro, Aveiro, Portugal

<sup>2</sup>Department of Ecology and Animal Biology, University of Vigo, Spain

<sup>3</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Pólo dos Açores, Universidade dos Açores, Ponta Delgada, Portugal.

<sup>4</sup>Faculdade de Ciências e Tecnologia, Universidade dos Açores, Ponta Delgada, Portugal.

<sup>5</sup>Departamento de Biodiversidad y Gestión Ambiental, Facultad de Ciencias Biológicas y Ambientales, Universidad de León, León, España. Current affiliation: Laboratorio de diatomología y calidad de aguas. Instituto de Investigación de Medio Ambiente, Recursos Naturales y Biodiversidad, León, España.

\*Author for correspondence: cdelgado.cristina@gmail.com

#### Abstract

**Background:** Due to their isolation, islands offer excellent areas for the study of distribution of benthic diatoms. On the other hand, diatoms bearing canal raphe have received less attention compared to other groups of diatoms such as *Navicula, Pinnularia* or *Amphora*.

Questions: Is it possible that thermal springs on islands offer a refuge for infrequent diatom species?

Studied species: Platichthys furnensis C. Delgado, V. Gonçalves & S.F.P. Almeida sp. nov.

Study site and dates: The species here described was collected in the epilithon of a thermal spring in São Miguel Island (Azores Archipelago, Portugal) in September 2015.

Methods: This new taxon was compared to other diatom species of the genera *Nitzschia*, *Tryblionella*, *Entomoneis* and *Hantzschia* and to the other species of the genus *Platichthys*. The morphology is documented by light and scanning electron images and discussed in detail.

**Results:** *Platichthys furnensis* was found in a thermal pool, a similar habitat to the one where *P. krammeri* type was collected in Chile in 1940. *P. furnensis* has many structures that are characteristic of the recently described genus *Platichthys*, including raised canal raphe and fibulae, compressed valve face, steep valve face and numerous open copulae.

**Conclusions:** The description of the new taxon is interesting because it is the first species within *Platichthys* to be described from the Northern Hemisphere.

Keywords: diatoms, epilithon, freshwater algae, Platichthys, thermal waters, volcanic island.

#### Resumen

Antecedentes: Debido a su aislamiento, las islas son zonas excelentes para el estudio de la distribución de diatomeas bentónicas. Por otro lado, las diatomeas con canal rafiano han sido menos estudiadas que otros grupos como *Navicula*, *Pinularia* o *Amphora*.

Preguntas: ¿Es posible que fuentes termales en islas ofrezcan un refugio para especies de diatomeas poco frecuentes?

Especies de estudio: Platichthys furnensis C. Delgado, V. Gonçalves & S.F.P. Almeida

Sitio y años de estudio: La especie aquí descrita fue recogida en el epiliton de una fuente termal en la isla de São Miguel (Azores Archipielago, Portugal) en Septiembre de 2015.

**Métodos:** Este nuevo taxón se comparó con las características generales de los géneros *Nitzschia*, *Tryblionella*, *Entomoneis* y *Hantzschia* y con otras especies del género *Platichthys*. Su morfología se documenta mediante imágenes de microscopio óptico y electrónico discutiéndose en detalle.

**Resultados:** *Platichthys furnensis* se encontró en una fuente termal, un hábitat similar a donde fue recogida el tipo de P. *krammeri* en 1940 en Chile. *P. furnensis* tiene características del género *Platichthys* incluido el canal rafiano elevado, fibulas robustas, la cara valvar comprimida y numerosas cópulas abiertas.

Conclusiones: La descripción de nuevos taxones es interesante porque es la primera especie dentro de *Platichthys* que se describe en el hemisferio norte.

Palabras clave: algas de agua dulce, aguas termales, diatomeas, epiliton, isla volcánica, Platichthys.

Diatoms bearing a canal raphe have received less attention compared to other groups of diatoms such as the *Navicula* Bory, *Pinnularia* Ehrenberg or *Amphora* Ehrenberg ex Kützing (Krammer 2000, Lange-Bertalot 2001, Levkov 2009). The Entomoneidaceae comprises two diatom genera, *Entomoneis* Ehrenberg and *Platichthys* Lange-Bertalot, Kulikovskiy, Witkowski, Seddon & Kociolek. The generitype *P. krammeri* Lange-Bertalot & Kulikovskiy was described from a thermal spring in the temperate climate zone of Chile (South America).

Its morphology includes a canal raphe and fibulae, and a compressed valve face, absence of a distinct valve mantle and presence of open porous copulae (Lange-Bertalot *et al.* 2015). Other characteristics of this genus include a raphe positioned on an elevated keel, uniseriate striae composed of simple areolae, very simple portulae and two raphe branches separated by a narrow central nodule whereas at the apices they terminate in a small helictoglossa (Lange-Bertalot *et al.* 2015).

Due to their isolation, islands offer excellent areas for the study of environmental factors determining the distribution of benthic diatoms (e.g., Flower 2005, Gonçalves et al. 2015). Some of the most remote oceanic islands in the Atlantic is the Azores archipelago. It is located in the North Atlantic and is composed of nine volcanic islands of recent origin (6.01-0.27 Myr; Larrea et al. 2014). The archipelago spans 615 km along a roughly WNW-ESE trend and is located approximately 1,300 km west of Portugal and 1,600 km east of North America. The volcanic origin of the Azorean islands explains the high abundance of hydro mineral discharges, including fumaroles and thermal springs, especially in São Miguel Island (Freire 2006). Furnas volcano, one of the three active volcanoes in São Miguel Island (Guest et al. 1999), has at least 12 thermal waters (Cruz et al. 1999). Two types of thermal waters are found in Furnas caldera: hydrothermal fluids derive from shallow aquifers at temperatures about 160 °C overlying a residual heat source close to the surface (Moore 1990, Cruz et al. 1999), and carbon dioxide rich waters heated during ascent to the surface (Cruz et al. 1999).

In the past, Azorean thermal waters where regarded with interest mainly because of assumed therapeutic benefits (Cruz *et al.* 1999), but also for biodiversity research (O'Meara 1874, Quintela *et al.* 2013). However, the biodiversity of Azorean thermal systems remains poorly known. With the aim to continue the taxonomic and ecological studies on Azorean thermal systems a survey of benthic diatoms in thermal springs of São Miguel Island took place. Here we present a detailed morphological description of *Platichthys furnensis* sp. nov., using light (LM) and scanning electron microscopy (SEM) and provide information on its ecology.

#### Materials and methods

Sample collection. Water and diatom samples were collected, in September 2015, in six thermal springs with temperatures between 37.9-43.3 °C in São Miguel Island (Figure 1): Poça da Dona Beija (37.9 °C), Ribeira Quente (38.2 °C), Caldeira Velha (39.0 °C), Ribeira Quente 1 (41.7 °C), Caldeiras (42.4 °C) and Poça da Tia Silvina (43.3 °C).

Water samples were collected simultaneously, stored at 4 °C in darkness, and transported to the laboratory for posterior analysis. At each sampling site environmental parameters such as water temperature (°C), pH, dissolved oxygen (mg L<sup>-1</sup>), oxygen saturation (% O<sub>2</sub>) and electric conductivity ( $\mu$ S cm<sup>-1</sup>, at 25 °C) were measured with a Multi parametric Probe model Horiba U-52G (Horiba Instruments, U.K.). The biofilm was removed from stones by scraping their upper surface with a toothbrush. Samples were finally preserved with stock solution formalin (37 %) and diluted to 10 % final concentration.

*Laboratory treatment.* Aliquots of the samples (*ca.* 3 mL) were cleaned with 4-6 mL of nitric acid (65 % v/v) and potassium dichromate ( $K_2Cr_2O_7$ ) at room temperature for 24 hours. Afterwards, samples were repeatedly centrifuged (1,500 rpm) and rinsed with distilled water at least three times to remove oxidation by-products.

Standard methods for water chemical analysis were carried out by following Eaton *et al.* (1995). Anions such as chlorides (mg Cl<sup>-</sup> L<sup>-1</sup>), nitrates (mg N-NO<sub>3</sub><sup>-</sup> L<sup>-1</sup>), nitrites (mg N-NO<sub>2</sub><sup>-</sup> L<sup>-1</sup>), sulphates (mg SO<sub>4</sub><sup>2-</sup> L<sup>-1</sup>) and phosphates (mg P-PO<sub>4</sub><sup>3-</sup> L<sup>-1</sup>) were determined using ionic chromatography (Dionex ICS-3000; Dionex Corp., USA); ammonium (mg N-NH<sub>4</sub><sup>+</sup> L<sup>-1</sup>) and silicon dioxide (mg SiO<sub>2</sub> L<sup>-1</sup>) were quantified using an auto-analyser (Auto-Analyzer AA3, Bran + Luebbe, Germany). Cations, such as iron (mg Fe<sup>2+</sup> L<sup>-1</sup>), aluminium (mg Al<sup>3+</sup> L<sup>-1</sup>), calcium (mg Ca<sup>2+</sup> L<sup>-1</sup>), magnesium (mg Mg<sup>2+</sup> L<sup>-1</sup>), potassium (mg K<sup>+</sup> L<sup>-1</sup>), sodium (mg Na<sup>+</sup> L<sup>-1</sup>) and arsenic (mg As<sup>3+</sup> L<sup>-1</sup>) were analysed by Inductively Coupled Plasma-Mass Spectometry (ICP-MS Agilent 7700x; Agilent Technologies, USA) and the meq de HCO<sub>3</sub><sup>-</sup> L<sup>-1</sup> with a base acid titration.

Observations under light and scanning electron microscope (LM and SEM). Permanent slides were mounted with Naphrax<sup>®</sup> (Brunel Microscopes LTD) for LM analysis. Light images were taken using an Olympus DP70 camera (Olympus Corporation, Japan) attached to a Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Germany) with differential interference contrast (DIC) using a 100 × immersion objective (NA 1.40).



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Figure 1. Map with the location of Poça de Dona Beija in Sao Miguel Island (Acores Islands, Portugal).

Diatoms were identified to the lowest taxonomic level according to taxonomic literature, *e.g.*, <u>Cantonati *et al.*</u> (2017), <u>Krammer (2000, 2002, 2003)</u>, <u>Krammer & Lange-Bertalot (1986-1991)</u>, <u>Lange-Bertalot (2001)</u>, <u>Lange-Bertalot *et al.* (2003, 2011), <u>Levkov (2009)</u> <u>Werum & Lange-Bertalot (2004)</u>, <u>Trobajo *et al.* (2013) and <u>Witkowski *et al.* (2015)</u>.</u></u>

For SEM studies, a Hitachi SU-70 electron microscope (Hitachi, Japan) operating at 7 kV and 10 mm working distance was used for image acquisition. Aliquots of organic free samples were air-dried on a metal stub, which was coated with a gold-palladium mixture (Polaron equipment limited SEM coating unit E5000; Polaron PLC, UK) before SEM observations.

Measurements of valve length and width were taken from 20-30 valves on the light microscope, while striae density was measured on SEM images. Ultrastructural features were examined in SEM micrographs. Micrographs and plates containing LM and SEM pictures were prepared using CorelDraw X5<sup>®</sup>. Diatom terminology follows <u>Anonymous (1975)</u> and <u>Round *et al.* (1990)</u>.

#### Results

*Classification.* Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska (Medlin & Kaczmarska 2004), Subclass Bacillariophycidae Round (Round et al. 1990), Order Surirellales D.G. Mann (Round et al. 1990), Family Entomoneidaceae Reimer (Patrick & Reimer 1975), Genus *Platichthys* Lange-Bertalot, Kulikovskiy, Witkowski, Seddon & Kociolek (<u>Lange-Bertalot et al. 2015</u>), *Platichthys furnensis* C. Delgado, V. Gonçalves & S.F.P. Almeida sp. nov. (Figures 2 A-J, LM, <u>3 A-D</u>, <u>4 A-E</u> SEM).

*Type.* PORTUGAL, São Miguel Island, Azores Archipelago, Poça da Dona Beija, 37° 46.246' N, 25° 18.432' W, September 11, 2015, pond epilithon. Poça de Dona Beija\_1 sampled by *Cristina Delgado & Vitor Gonçalves* (Holotype: UK; BM 101-858. The valve representing the holotype is illustrated in Figure 2A; Isotype: Hustedt Collection at the AWI, Bremerhaven, Germany; ZU10/76.

Diagnosis. This new taxon is the narrowest of the three species of this genus (2.7-3.8  $\mu$ m wide) followed by *P. krammeri* with 3.5-4.0  $\mu$ m, and the widest is *P. darwiniana* with 4.0-5.5  $\mu$ m. Striae of *P. furnensis* are clearly visible under the LM. A hymen on the outer valve face occludes the areolae in *P. furnensis*, while the two previously described species don't show occlusions in the areolae. *Platichthys furnensis* also differs in the number of fibulae, having 4 to 6 in 10  $\mu$ m, while *P. krammeri* and *P. darwiniana* have 5.5 to 7.5 in 10  $\mu$ m (Table 4). *P. furnensis* showed irregular length of fibulae while the other two species showed regular fibulae. Particularly, *P. furnensis* shows small circular openings (portulae) between fibulae.



Figure 2. LM micrographs of *Platichthys furnensis* C. Delgado, V. Gonçalves and S.F.P. Almeida sp. nov. from Poça da Dona Beija (São Miguel, Azores Archipelago), 11/09/2015 (type population). A) - holotype; A-D) complete frustules; 2E-J) single valves.

*Description*. LM observations.- Frustules 30.5-37.5  $\mu$ m long and in girdle view 7.0-10.0  $\mu$ m broad (Figure 2A-D). Valves in girdle view 2.7-3.8  $\mu$ m broad (Figure 2E-J). Frustules lying in girdle view are linear-elliptical to linear with broadly rounded ends. The dorsal valve margins are slightly concave in the middle, becoming convex towards the apices. The ventral valve margin is slightly convex with slightly capitate ends (Figure 2E-J). Fibulae are large, prominent and apically arranged at some distance below the frustule margin, 4-6 in 10  $\mu$ m (Figure 2A-D), and are positioned at some distance from the raphe. The transapical striae are visible in the light microscope (Figure 2A-J), 22-23 in 10  $\mu$ m.

SEM observations.- The fibulae are large, apically elongated and irregular in length (Figure 3 A), occurring internally close to the axial area. The valve face is compressed laterally with a narrow keel in the axial area, so the valve has a steep V shape (Figure 2 A, C). The two raphe branches are separated by a central nodule (Figure 3D), whereas at the apices the raphe terminates in a small helictoglossa (Figure 4A, C; white arrows). The girdle is composed of a variable number of open, non-perforated bands (Figure 4B, 8 girdle bands). The valvocopula is perforated in this species with one or two lines of occluded pores (Figure 4D, E). External apical endings are strongly hooked (Figure 3B) and the valve mantle is a narrow band (Figures 3A; black arrow; 4D, E). Between the fibulae there are circular openings (portulae) corresponding to the end of 1-2 streae (Figures 3A, 4C). The number of striae converging to each fibula is variable (from 2 to 7, Figure 3A).

The striae are composed of straight, uniseriate rows of areolae, *ca.* 55-66 in 10  $\mu$ m (Figures 3A, 4C). The areolae are simple poroids in the internal valve face (Figure 4C). Areolar occlusions are present in the external valve face (Figure 4B, E). The external raphe fissure is visible in SEM, positioned in the narrow axial area (Figure 3B, D).

*Etymology.* The specific epithet *furnensis* refers to Furnas, the name of the type locality (São Miguel, Azores Archipelago).

Distribution and ecology. Platichtys furnensis was only found in the samples from the epilithon of Poça da Dona Beija, with an abundance of 48 %, in September 2015. Poça da Dona Beija is a thermal spring with water temperature between 37-39 °C. The type locality of *Platichthys* furnensis has low to medium conductivity values (363.0  $\mu$ S cm<sup>-1</sup>), low dissolved oxygen levels (0.6 mg L<sup>-1</sup>) corresponding to 9 % of saturation, and is acidic (pH = 5.3), poor in nutrients but rich in other chemical elements which are characteristic of volcanic areas (Table 1). Associated flora. Detailed microhabitat observations took place at the type locality in Poça da Dona Beija where the new species was found. The algal community in the type locality (September 11, 2015) was dominated by filamentous Cyanobacteria belonging to Pseudanabaena Lauterborn, Leptolyngbya Anagnostidis & Komárek and Oscillatoria Vaucher ex Gomont. Among the filamentous Cyanobacteria, the cyanobacterium Aphanocapsa and Chlorella-like green algae were also present. The other diatoms co-occuring with this new taxon were: Eunotia sp. (15.4 %), Planothidium lanceolatum (Brèbisson ex Kützing) Lange-Bertalot (14.7 %), Eolimna minima (Grunow in Van Heurck) Lange-Bertalot (7.9 %), Achnanthidium sp. (1.9 %), Pinnularia sp. (1.9 %), Pseudostaurosira sp. (1.2 %) and Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot (1.2 %).

Comparison of Platichthys with other genera. Platichthys is generally smaller and narrower than the genera Tryblionella W. Smith, Hantzschia Grunow and Entomoneis Ehrenberg (Round et al. 1990). Concerning size, Nitzschia is an extremely diverse genus, with many smaller species than Platichthys (e.g., N. inconspicua Grunow, N. microcephala Grunow, N. soratensis E.A. Morales & M.L.Vis and as many larger ones (e.g., N. recta Hantzsch ex Rabenhorst, N. acicularis (Kützing) W. Smith, N. sigmoidea (Nitzsch) W. Smith). The valve face is strongly compressed laterally in Plathicthys and Entomoneis but not in the other genera, nevertheless Entomoneis is arched forming a thin bilobate wing or keel (Table 2).

The two *Platichthys* species previously described (Lange-Bertalot et al. 2015) have a high density of stria and areolae in comparison to other diatoms bearing a canal raphe. The presently described species, on the other hand, has low density in striae. As *Nitzschia* is a very diverse taxon the number of striae is variable attaining high density in some species (e.g., N. gadmannii, N. schefterae) from this genus. Concerning striation, *Entomoneis* usually has biseriate or multiseriate striae (Round et al. 1990) not uniseriate as *Platichthys*. The other genera may have uniseriate but also biseriate or multiseriate striae, so it cannot be used to differentiate these genera (<u>Table 2</u>).

Girdle bands or copulae in these genera are variable in number and ornamentation (Table 2) and cannot be used to distinguish between them. Copulae in *Nitzschia* species normally have one row of poroids, but Mann (1986) refers the presence of two or more rows. Another example is given for the type species of the genus *Nitzschia*, *N. sigmoidea* that has two rows of poroids in the copulae (Knattrup *et al.* 2007).

Other characters separate *Nitzschia* from *Platichthys* and consolidate the position of *P. furnensis* sp. nov. in the latter genus and not in *Nitzschia* the morphologically closest



**Figure 3.** SEM micrographs of *Platichthys furnensis* sp. nov. from Poça da Dona Beija, (São Miguel, Açores Archipelago), 11/09/2015 (type population). A) General view of a broken valve showing the position of the fibulae and the striae composed of fine areolae, opened internally and occluded on the external side. The valve mantle is a narrow structure band (black arrow). B) External view of valve apice showing external raphe fissure (white arrow). C) Broken valve showing steep V shape with details of the internal and external view. D) External view of the central part of a valve showing the central nodule (white arrow). Scale bar is 10 µm in A; 3 µm in B-C; 2 µm in D.



**Figure 4.** SEM images of *Platichthys furnensis* sp. nov. from Poça da Dona Beija, (São Miguel, Açores Archipelago), 11/09/2015 (type population). A) Apical part of the internal valve view showing the raphe end and the helictoglossa (white arrow). B) Apical part of the frustule showing strongly bent raphe end (white arrow) and several copulae. C) Internal view of one valve showing the circular openings that separate the fibulae after tilting the metal stub and showing the raphe end and the helictoglossa (white arrow). D) Apical part of the frustule showing two valves, valvocopula (VC) with pores (black arrow) and several copulae (C1, C2, C3). E) Apical part of the valve showing the valvocopula (VC) with pores. Scale bar is 1 $\mu$ m in A; 5  $\mu$ m in B; 3  $\mu$ m C-E.

genus to the new taxon presently described. *P. furnensis* sp. nov. presents differentiation between valve face and mantle, regular cross-section and conopeum covering the external raphe slit such as the other two species of the genus *Platichthys* (Lange-Bertalot *et al.* 2015). Despite the clear differences between *Nitzschia* and *Platichthys* we noticed that some *Nitzschia* species can show similar frustule outline in LM identification *e.g.*, *N. bilobata* (50-150 µm; 5-14 µm), *N. bremensis* (60-90 µm; 6-9 µm) and *N. dippelii* (30-110 µm; 5-10 µm) (Krammer & Lange-Bertalot 1986-1991). Nevertheless, *P. furnensis* sp. nov. (30.5-37.5 µm; 2.7-3.8 µm) is smaller than these taxa both in length and in width.

**Table 1.** Physical and chemical variables corresponding to the ecological data in the type locality Poça da Dona Beija where *Platichthys furnensis* sp. nov. was collected.

	Poça da Dona Beija (11/09/2015)
Water temperature (°C)	37.0-39.0
рН	5.30
Conductivity (µS cm <sup>-1</sup> )	363.00
Dissolved oxygen (%)	9.00
Dissolved oxygen (mg L-1)	0.60
Sílica (mg SiO <sub>2</sub> L <sup>-1</sup> )	61.74
Chlorides (mg Cl L-1)	120.18
Na <sup>+</sup> (mg L <sup>-1</sup> )	70.25
Fe <sup>2+</sup> (mg L <sup>-1</sup> )	11.88
$K^{+}(mg L^{-1})$	14.83
Ca <sup>2+</sup> (mg L <sup>-1</sup> )	11.32
<b>Mg</b> <sup>2+</sup> ( <b>mg L</b> <sup>-1</sup> )	5.12
<b>Al<sup>3+</sup>(mg L<sup>-1</sup>)</b>	0.01
As <sup>3+</sup> (mg L <sup>-1</sup> )	0.04
N-NH4⁺(mg N L⁻¹)	0.22
<b>N-NO</b> <sub>3</sub> (mg L <sup>-1</sup> )	0.00
<b>P-PO</b> <sub>4</sub> <sup>3-</sup> ( <b>mg PO</b> <sub>4</sub> <sup>3-</sup> L <sup>-1</sup> )	0.05
Meq HCO <sub>3</sub> L <sup>-1</sup>	0.30

Comparison of Platichthys furnensis with the other Platichthys species. At the ultrastructural level, *P. furnensis* shows a coarser structure than the other two species. Stria density is much lower in *P. furnensis* (22-23 in 10  $\mu$ m) than in *P. darwiniana* and *P. krammeri* (Table 3). Striae of *P. furnensis* are clearly visible under the LM. A hymen on the outer valve face occludes the areolae in *P. furnensis*, while the two previously described species show no occlusions in the areolae.

The number of girdle bands (copulae) in P. furnensis is higher than in the other two species previously described (Lange-Bertalot et al. 2015). The girdle band adjacent to each valve is often more complex than the other bands and are named valvocopulae (Round et al. 1990). The ornamentation of these valvocopulae is not a descriptive character of Platichthys, particularly, P. darwininiana presents perforated valvocopulae with multiple rows of fine puncta (arrow in Figure 19; Lange-Bertalot et al. 2015). This character was not described in P. krammeri, but in the images of the holotype specimen (Figures 51, 52, Lange-Bertalot et al. 2015) we cannot observe multiple rows of puncta, similar to what happens in P. furnensis in which valvocopulae have one or two lines of pores (Figure 3D, E). We were able to distinguish up to 8 copulae while the other two species have fewer copulae; in published SEM photos up to 4-5 copulae can be counted.

*Conservation status.* After evaluation of the conservation status of this new species in accordance with the categories and criteria of the IUCN Red List <u>https://www.iucn</u>redlist.org/ the status is "unknown".

#### Discussion

The principal characteristics that led us to consider this taxon in the genus *Platichthys* included general shape, placement of the fibulae at the same level in both valves, and steep valve face compressed laterally to form a narrow keel, broadening the valves below the keel (Lange-Bertalot *et al.* 2015). *Platichthys furnensis* sp. nov. also shared with the generitype other features the apical external raphe endings bent in the same direction, uniseriate transapical striae, two raphe branches separated by a narrow central nodule and ending in a small helictoglossa at the apices (Lange-Bertalot *et al.* 2015).

The portulae seen in *P. furnensis* are relatively small compared to the internal columns formed by the fibulae (Figures 3A, 4C). Lange-Bertalot *et al.* (2015) also mention the dense striation as a distinctive feature of the genus, although the use of such quantitative characters for the delimitation of diatom genera has been criticized in the past (*see*<u>Round 1996</u>). As previously shown, quantitative characters may vary within species of a genus, therefore we believe that the differences in striae density between *P. furnensis* and the two other *Platichthys* species only reports variability within this genus.

The shape of the valves (steep V shaped) of the newly described species is very different from *Nitzschia*, *Hantzschia* (flat valves), *Tryblionella* (longitudinally undulate) and *Entomoneis* (laterally compressed but highly arched to form a thin bilobate wing, or keel twisted along the apical axis) as well as the placement of the fibulae (in

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Table 2. Comparison of the genus Platichthys with other genera. Retrieved information from Lange-Bertalot et al. (2015	<u>), Round <i>et a</i></u>	<u>l. (1990)</u>
and <u>Bahls (2011)</u> .		

Characterization	<i>Platichthys</i> Lange- Bertalot, Kulikovskiy, Witkowski, Seddon & Kociolek	<i>Entomoneis</i> C.G. Ehrenberg	<i>Hantzschia</i> Grunow	Nitzschia Hassall	<i>Tryblionella</i> W. Smith
Frustules	Cells solitary. Lying exclusively in girdle view	Cells solitary, twisted about the apical axis. Usually lying in girdle view and then appearing bilobate.	Cells solitary. Usually lying in girdle view	Cells solitary or forming chain-like or stellate colonies or living in mucilage tubes. Lying in valve or girdle view	Cells solitary. Lying in valve view
Frustule outline	Linear-elliptical to linear with broadly rounded ends	Present a great variety of aspects due to the torsion of the cell	Straight or sigmoid, markedly dorsiventral	Usually straight and needle-like, sometimes sigmoid	Diagonally symmetrical about the median valvar plane
Valve outline	Straight lightly convex or slightly concave proximally	Lanceolate or linear often strongly compressed laterally	Asymmetrical with respect to the apical plane or sigmoid	Valves straight or sigmoid, narrow; linear, lanceolate or elliptical, sometimes expanded centrally	Valves robust, broad; elliptical, linear or panduriform
Valve face	Steep and compressed laterally to form a narrow keel (V shaped)	Bearing a high narrow keel, which becomes lower in the centre of the valve, and also decreases in height towards the poles	Usually flat	Usually flat	Often bearing warts or ridges, externally undulate. Bounded on one side by the keeled raphe system.
Striation	Simple transapical areolae arranged in straight uniseriate rows, very spaced. Areolae simple.	Usually biseriate or multiseriate, containgin small round poroids occluded by hymenes	Uniseriate or biseriate rows of round or reniform poroids containing hymenes	Usually uniseriate not interrupted by lateral sterna, containing small round poroids occluded by hymens and sometimes by cribra as well.	Uniseriate to multiseriate, usually interrrupted by one or more sterna and containing small round poroids occluded by hymens.
Valve mantle	Very narrow, structureless rim	Discrete mantle usually absent	Usually ornamented, and shallow	Usually ornamented, and shallow	Shallow
Valve poles	Slightly rostrate in an oblique position	Acute poles	Often rostrate or subrostrate	Often rostrate or capitate	Bluntly rounded or apiculate poles
Girdle bands	Numerous open, porous copulae	Numerous, open and porous	Girdle complex, containing open or closed bands, of which at least some bear two or more rows of poroids.	Open, very variable in number, usually with one to several transverse rows of poroids apiece	Girdle narrow, containing plain or sparsely pourous open bands
Raphe	Raphe canal centrally positioned in the valve in a very narrow, steep axial area (the angle of the V shaped valve)	Raphe system fibulate, or the sides of the keel fused beneath the raphe so that the subraphe canal is connected to the rest of the cell lumen only near the central and polar raphe endings.	Canal raphe is eccentrically placed on the valve; both raphes are on the same, usually less convex, ventral side of the cell	Canal raphe system lightly to strongly eccentic, closer to the proximal margin. Raphe system of the two valves on the same (hantzschioid symmetry) but generally on opposite sides (nitzschiod symmetry)	Canal raphe strongly eccentric, keeled, fibulate

Characterization	<i>Platichthys</i> Lange- Bertalot, Kulikovskiy, Witkowski, Seddon & Kociolek	<i>Entomoneis</i> C.G. Ehrenberg	<i>Hantzschia</i> Grunow	<i>Nitzschia</i> Hassall	<i>Tryblionella</i> W. Smith
Fibulae	Positioned at some distance from the raphe. Internally are column shaped, short and arched. Few striae merge in one fibula	Fibulae may occur at many levels eneath the raphe, especially where the keel is high. Fibulae are short, bar- like struts and are borne on the transapical costae	Raphe subtended internally by fibulae which are massive, or slender and rib- like	Very diverse sometimes extended across the valve	Fibulae squat, often as broad or broader apically than transapically
Central raphe endings	The raphe is interrupted at a narrow central nodule	Straight, and not or only slightly expanded	Raphe continuous from pole to pole or interrupted centrally, often biarcuate	Central raphe endings present in some species and raphe continuous from pole to pole in others	Central external raphe endings close together, slightly expanded or deflected, ocassionally absent
Terminal raphe fissures	Strongly hooked	Very closed to each pole	Polar raphe endings simple or hooked towards the dorsal side	Terminal fissures usually present, turned or hooked towards the proximal or distal side	Short, deflected
Helictoglossa	Internal apical raphe endings terminate in a small helictoglossa	-	-	Sometimes a double helictoglossa in central intenal raphe endings	Internal raphe fissures ending centrally in a double helictoglossa

the angle of the V). Under the LM, the presently described taxon as well as the two *Platichtys* species so far described only show one focal point where the canal raphe on both valves can be seen, this does not happen either in *Nitzschia*, nor in *Tryblionella* or in *Hantzschia*. As Lange-Bertalot *et al.* (2015) stated, "in straight view the fibulae lie on the same level in both valves. Consequently, a "nitzschioid" or "hantzschioid" symmetry classification (i.e. raphe on the same or opposite sides, respectively) becomes obsolete". In the case of this new taxon this also happens.

*Entomoneis* and *Platichthys* differ in few morphological features (Liu *et al.* 2018); species in *Platichthys* do not have twisted panduriform frustules, and only possess uniseriate striae, and they do not have a sigmoid keel (Lange-Bertalot *et al.* 2015), such as in *Platichtys furnensis*. The highlighted similarities between the two described *Platichtys* species and the new species presently described, concerning valve structure and position of the canal raphe place the new taxon in *Platichtys* and separates it from the other genera.

Until now, only two species had been described in the genus *Platichthys* (Lange-Bertalot *et al.* 2015) in the

Southern Hemisphere. *Platichthys darwiniana* was described from the Galapagos Islands in the Ecuador area (Pacific Ocean) in a fossil record spanning the last ~2600 years, found in a coastal lagoon with salinity levels varying from marine to brackish (Seddon *et al.* 2014). *Platichthys krammeri* was described from a sample collected in 1940 in the efflux of a thermal freshwater spring in Chile (Lange-Bertalot *et al.* 2015), a similar habitat to that of the new species here described. So far those species have been found only in the type locations. Despite the dissimilarity between *P. furnensis* and the other two species, we believe that these differences are representative of the overall variability of the genus and will be more fully circumscribed with the description of new species.

The description of *Platichthys furnensis* is interesting because it is the first species within this genus to be described in the Northern Hemisphere, occurring also in a thermal spring. The geographical distance between the type localities of the species described in the genus would indicate a wide original distribution area for the genus, but with narrow ecological preferences.

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Characterization	erization Platichthys furnensis sp. nov. Platichthys darwiniana Seddon & Witkowski		<i>Platichthys krammeri</i> Lange- Bertalot & Kulikovskiy	
Length (µm)	30.5 - 37.5	23 - 33	27 - 35	
Width (µm)	2.7 - 3.8	4.0 - 5.5	3.5 - 4.0	
Fibulae (10 µm )	4 -6	5.5 - 7.5	5.5 - 7.5	
Striae (10 µm)	22 - 23	48 - 61	60 - 70	
Puncta (10 µm )	55 - 56	56 to 72	80	
Valve outline	Linear-elliptical to linear. Dorsal valve margins are slightly concave in the middle, becoming convex towards the apices	Dorsal valve margins are slightly concave in the middle, becoming convex towards the apices	Straight lightly convex or slightly concave proximally	
Raphe	Positioned in a very narrow axial area	Positioned in a very narrow axial area	Positioned in a very narrow axial area	
Ends	Broadly rounded	Apical endings strongly bent in one direction	Strongly hooked	
Central raphe	The raphe is interrupted at a central nodule	The raphe is interrupted at a narrow central nodule	The raphe is interrupted at a narrow central nodule	
Girdle bands	Several (up to 8) open, non-perforated bands	Few (4-5) open, perforated bands	Few (4-5) open, perforated bands	
Portulae	Very simple, circular	Very simple, formed as a result of a slight broadening of the base of the fibulae	-	
Fibulae	Large, apically elongated and irregular in length are positioned at some distance from the raphe	Massive and large are positioned at some distance from the raphe	Prominent fibulae, approximately developed as circular structures are apically arranged at some distance below the frustule margins	
Helictoglossa	Internal apical raphe endings terminate in a small helictoglossa	Internal apical raphe endings terminate in a small helictoglossa	Internal apical raphe endings terminate in a small helictoglossa	
Striation	The striae are composed of straight, uniseriate rows of areolae	Straight, uniseriate rows of areolae	Areolae are arranged in straight uniseriate rows. Few striae merge in one fibula	

Table 3.	Comparison	of three	species	of the	genus	Platichthys
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