

A new *Psammothidium* species (Bacillariophyta, Achnanthesiaceae) from Cimera Lake (Gredos mountain range), Central Spain

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Abstract – A population of an unknown *Psammothidium* species (Bacillariophyta, Achnanthesiaceae) was found in core sediments collected from Cimera Lake, an oligotrophic, undisturbed mountain lake in Central Spain (Gredos mountain range). The morphology and ultrastructure of this taxon is hereby documented in detail by means of light (LM) and scanning electron (SEM) micrographs. Morphologically, the closest taxon is *P. levanderi*, and the type of this species is analyzed here to provide a differential diagnosis. A comparison with other similar small *Psammothidium* species with an elliptic outline show that the combination of features exhibited by this taxon is unique and it is thus described here as *Psammothidium toroi* sp. nov.

Achnantheid / diatoms / lake sediments / new taxon / paleolimnology / type material

INTRODUCTION

The genus *Psammothidium* Bukht. & Round was erected in 1996 to encompass monoraphid diatom taxa sharing four distinctive features, namely i) an oval/elliptic valve outline, sometimes slightly capitate, ii) a convex raphe valve, iii) striae reaching the sternum, and iv) the presence of raphe fissures lying in a channel (especially near the center) (Bukhtiyarova & Round, 1996). However, in their critical revision of the genus, Monnier *et al.* (2007) observed that the only observable difference between *Psammothidium* and other Achnantheid taxa such as *Achnantheidium* Kütz. is the presence of ridges accompanying the raphe slit, and a

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well-marked vestigial raphe on the rapheless valve, but the structure of the raphe endings was found insufficient to distinguish these taxa at the generic level since this feature is dependent on the stage of completeness of frustule silicification. Moreover, many *Psammothidium* species appear rather flat in girdle view, being only slightly concave at the center and slightly convex towards the apices. Thus, these authors rejected the new genus because it cannot be unambiguously defined on the basis of morphological criteria and has no ontogenetic or phylogenetic support, although this proposal has been not followed in the subsequent literature (Hofmann *et al.*, 2011; Potapova, 2012; Kulikovskiy *et al.*, 2016).

Additional morphological features found in most *Psammothidium* species include: i) regularly-spaced areolae forming uniseriate striae in the raphe valve (sometimes biseriate in the rapheless valve), ii) simple central raphe endings, iii) a transapically expanded central area, iv) areolae internally closed by a cribrum, and v) a cingulum constituted by plain bands. *Psammothidium* shares several diagnostic features with other comparable genera: the recently described *Pseudachnanthidium* Riaux-Gob. presents also small, elliptic frustules, but lacks the distinctive row of pores in the mantle and presents regularly-spaced macroareolae in the rapheless valve (*vs* simple areolae in *Psammothidium*) (Riaux-Gobin & Witkowski, 2015). The terminal raphe fissures in *Madinithidium* Witkowski, Desrosiers & Riaux-Gob. in Desrosiers *et al.* 2014 are hooked in the same direction, while these fissures in *Psammothidium* are curved to opposite sides. *Rossithidium* Round & Bukht. is best distinguished by the presence of concave raphe valves. Contrary to those in *Psammothidium*, the raphe fissures in *Platessa* Lange-Bert. are not hooked, and the raphe valve is slightly concave in the latter.

Psammothidium is frequently observed in both lentic and lotic habitats, and it is commonly regarded as an indicator of oligotrophic conditions. Most species of the genus prefer acid waters, other species also form an important component of diatom associations in circumneutral and more alkaline waters (Wojtal, 2004). They are very abundant in periphytic communities. *Psammothidium* cells appear usually attached to sand grains by the raphe valve face (Enache *et al.*, 2013), but they have been also observed growing attached to rocks (epilithic) and other substrata (Rudd *et al.*, 2016). Some studies (e.g. Van de Vijver *et al.*, 2004) indicate their preference for dry habitats. To date, no marine or brackish *Psammothidium* taxa are known. Species of this genus are commonly recorded in fossil or subfossil deposits (e.g. Stager *et al.*, 2013; Leira *et al.*, 2015; Peszek *et al.*, 2015), especially at high latitudes (Biskaborn *et al.*, 2012; Sterken *et al.*, 2012; Finkelstein *et al.*, 2014). In paleoecological studies, increases in the relative abundance of *Psammothidium* cells can be related to the decrease in the proportion of planktonic taxa in the diatom assemblages (Saulnier-Talbot *et al.*, 2015). In general, large abundances of epipsammic diatoms are indicative of low turbidity waters (Buczko *et al.*, 2015).

During a paleolimnological investigation of surface sediments collected in Cimera Lake (Gredos mountains, Central Spain), an unknown *Psammothidium* species was recorded as a subdominant taxon. This population is described hereby as a new species and its morphology, ultrastructure and ecology are compared with those of similar taxa. For comparative purposes, the type population of *P. levanderi* (Hust.) Bukht. & Round is also analyzed under SEM.

MATERIALS AND METHODS

Cimera Lake ($40^{\circ} 15' 47''$ N, $5^{\circ} 18' 20''$ W) is a small (4.49 ha), shallow (9.4 m maximum depth) and the highest (2140 m a.s.l.) of a chain of 5 alpine lakes that developed in a glacial cirque on a granite catchment in the Massif Central of Sierra de Gredos (Central Range, Spain, Fig. 1). It is a discontinuous, cold, polymictic lake that typically freezes between November and December, becoming ice-free in May-June (mean ice period length 194 days). The substrate is mainly silty, with a relatively low organic matter content (*ca* 16% LOI) below 5-7 m but sand dominates in the littoral. A large number of rocky blocks of various sizes can be found irregularly distributed all over the lake bottom. Unlike other glacial lakes in this mountain range, Cimera remains almost undisturbed (Granados & Toro, 2000; Sánchez-Hernández *et al.*, 2015; Sánchez-López *et al.*, 2015). The only alteration in the lake is the presence of a stable population of an exotic fish, the brook trout (*Salvelinus fontinalis* Mitchell), introduced at the middle of 20th century (Toro & Granados, 2001; Toro *et al.*, 2006). The lake was formed sometime between 13 and 10 ka when the glacier had disappeared (Palacios *et al.*, 2012).

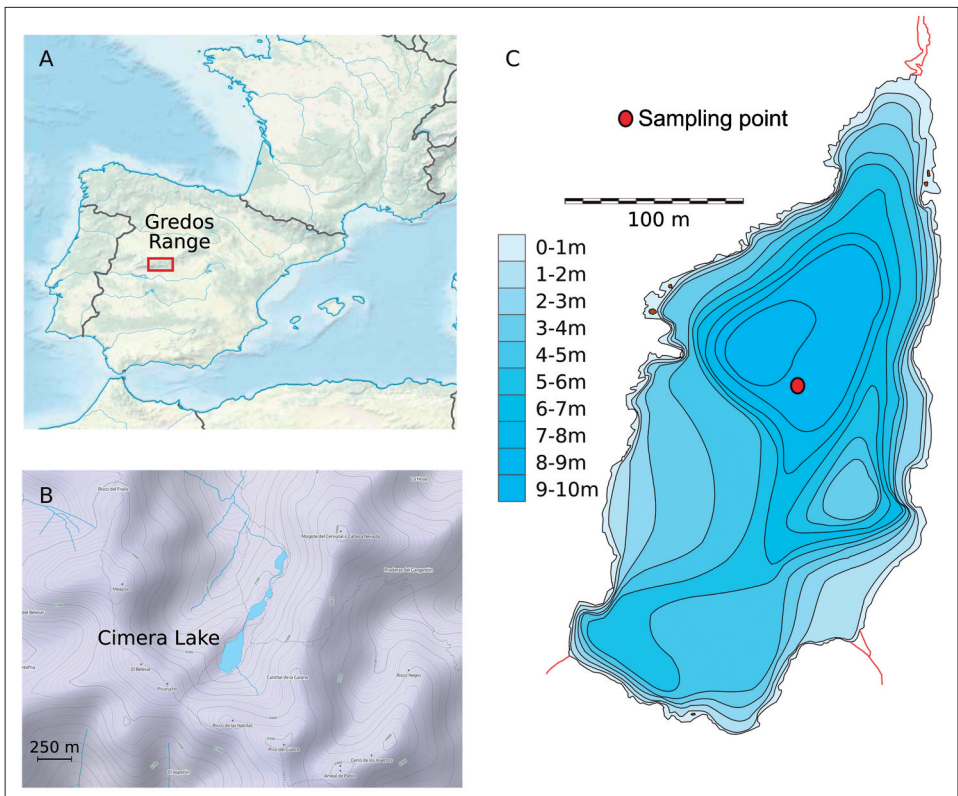


Fig. 1. Map of the Gredos Range in Spain (A) and Cimera Lake (B). Cimera Lake bathymetry (C), with the sampling point near the deepest area of the lake. (A) modified from Wikimedia Commons and (B) modified from Open Cycle Maps.

In winter 2012, CIM12-04A sediment core was sampled near the deepest zone in the lake using the UWITEC[®] gravity corer (UWITEC devices, Mondsee, Austria). The sediment core was sealed in site and then transported to the laboratory where was stored in a dark cool room at + 4°C until subsampling (Sánchez-López *et al.*, 2016).

Additionally, the original gathering of *A. levanderi* Hustedt [Hustedt collection, Bremerhaven (BRM)] corresponding to the samples E2884, E2885 and E1318 [Hustedt collection, Bremerhaven], were prepared for SEM analysis.

Clean frustule suspensions were obtained oxidizing organic matter with hot hydrogen peroxide 30% v/v. Carbonate inclusions were removed adding a few drops of hydrochloric acid. Permanent microscopic slides were mounted using a refractive resin (Naphrax[®]). Diatoms were identified under 1000× light microscopy (LM) with a 100X /1.4 Zeiss Pan-Apochromat objective mounted in a Zeiss scope-A1 microscope equipped with Differential Interference Contrast (Nomarski) optics and double oil immersion. LM photographs were taken with an Invenio 5SII camera. Samples selected for scanning electron microscopy analysis were filtrated through polycarbonate membrane filters with a pore diameter of 1 or 3 µm, mounted on stubs, sputtered with gold (40 nm) with a Modular High Vacuum Coating System (BALZERS SCD 004) and studied with ultra-high-resolution analytical field emission (FE) scanning electron microscope, Hitachi SU-70 (Hitachi High-Technologies Corporation, Japan), operated at 5 kV and 10 mm distance. SEM images were taken using the lower (SE-L) detector signal. The morphological terminology follows Bukhtiyarova & Round (1996) and Riaux-Gobin & Witkowski (2015).

OBSERVATIONS

Division Bacillariophyta
 Class Bacillariophyceae
 Subclass Bacillariophycidae
 Order Achnanthes
 Family Achnanthesiaceae
 Genus *Psammothidium*

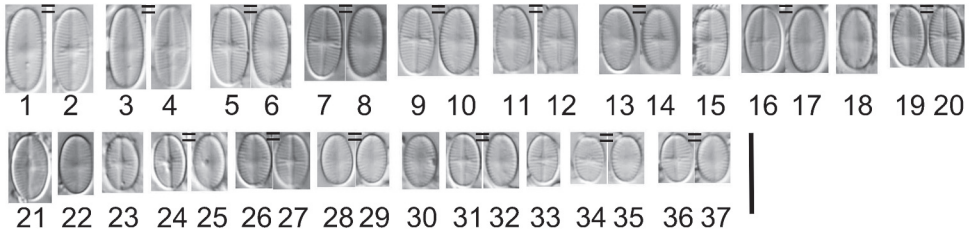
Psammothidium toroi sp. nov.

Figs 2-41

Type: Cimera Lake, Spain, 2 mm core surface sediment sample, leg. S. Pla-Rabès, coll. date March 21th 2012 (holotype BR-4472, Botanic Garden Meise, Belgium, here illustrated as Figs 2-41).

Etymology: Named after Dr. Manuel Toro Velasco, Spanish limnologist, for his outstanding contribution to the study of the Gredos lakes.

Diagnosis: *Valvae lineares-ellipticae ad late ellipticae apicibus non protractis late rotundatis, 5.3-10.1 (7.2 ± 1.4) µm longae, 3.4-4.3 (4.0 ± 0.2) µm latae, ratio longitudinis et latitudinis 1.6-2.3. Raphovalva: Raphe filiformis fere recta, externe extremis terminalibus fere rectis vel proximalibus externe modice latera opposita declinatis, pori centrales et fissurae terminales inconspicuae. Area centralis transverse oblonga. Striae transapicales radiantes omnio, 22-26 (24.1 ± 1.4) in 10 µm. Areovalva: area centralis valde ampla, late elliptica. Striae transapicales valde abbreviatae omnio, radiatae, 22-28 (25.2 ± 1.6) in 10 µm. Areolae poroides, parvae, ca 40 in 10 µm. 37 valvae mensae.*



Figs 2-37. *Psammothidium toroi* sp. nov., LM, valve view. Type material from Cimera Lake, Spain. Size diminution series of whole frustules photographed at different foci to show the raphe and the rapheless valves (the images of the same frustule are paired with a “=” symbol). Scale bar = 10 μm .

Description: Valves linear-elliptic in larger individuals to broadly elliptic in smaller individuals, with non-protracted, broadly rounded apices, 5.3-10.1 (7.2 ± 1.4) μm long, 3.4-4.3 (4.0 ± 0.2) μm wide, length-to-width ratio 1.6-2.3 (Figs 2-38) (Figs 2-37). Raphe valve: Valve face slightly convex (Fig. 38). Axial area narrow, somewhat wider near the central area, which is irregularly oblong elliptical or diamond-shaped, bordered by several shorter striae (Fig. 38). Striae radiate throughout the whole valve, not becoming distinctly denser towards the apices, 22-26 (24.1 ± 1.4) in 10 μm (Figs 2-37). Areolae foramina internally and externally irregularly squared (Figs 38, 39), *ca* 40 in 10 μm . Raphe straight, filiform, with straight proximal and terminal fissures as seen externally (Fig. 38), internally the central ends curve slightly toward opposite sides (Fig. 39). Helictoglossae not seen. Mantle striae are reduced to a single row of slit-like areolae (Fig. 38) with relatively higher density than the valve face striae (on both valves). Rapheless valve: axial area fused with a wide (up to 2/3 of the valve) central area, transapically expanded near the center, and surrounded by more or less regularly shortened striae (Fig. 41). Areolae foramina internally squared (Fig. 40), externally oblong elliptic (Fig. 41). The areolae delimiting the axial area are generally roundish (Fig. 41). Striae radiate throughout the whole valve, not becoming distinctly denser towards the apices, 22-28 (25.2 ± 1.6) in 10 μm (Figs 2-37). 37 valves measured.

Distribution: So far only known from the type locality.

Ecology: *Psammothidium toroi* was a co-dominant species (abundance up to 12%) in the surface sediment collected in Cimera Lake at 8.5 m depth near the deepest point (9.4 m). The samples were dominated by other *Psammothidium* spp. (*ca* 41%), the dominant taxon being *Achnanthes helvetica* var. *minor* Flower & V.J. Jones (15%). In addition, *Eolimna* spp. also reached higher abundances (up to 10%). Cimera Lake is an oligotrophic (mean annual total phosphorus: 6 $\mu\text{g P-PO}_4\cdot\text{L}^{-1}$; mean annual Chl *a*: 2.1 $\mu\text{g}\cdot\text{L}^{-1}$, maximum annual Chl *a*: 11.5 $\mu\text{g}\cdot\text{L}^{-1}$) lake (following criteria by OCDE 1982), slightly acidic (mean pH = 6.2-6.8) and very low-mineralized (mean conductivity < 10 $\mu\text{S}\cdot\text{cm}^{-1}$ at 25°C). In fact, as far as we know, it reaches on time to time one of the lowest conductivities recorded in lakes (1.34 $\mu\text{S}\cdot\text{cm}^{-1}$ at 25°C; Toro *et al.*, 2006). It has also very low alkalinity values, usually below 50 $\mu\text{eq}\cdot\text{L}^{-1}$. The mean annual water retention time in the lake is 62 days (Toro *et al.*, 2006), but in the thaw period or during rainy autumns it could be only a few days. Enough light reaches the bottom of the lake (mean annual Secchi depth > 5 m) with benthic algae and very sparse mosses (*Drepanocladus exannulatus*) growing on the sediment surface. Consequently, together with the large abundance of *P. toroi* on the surface sediment sample, the dominance of psammic habitats in the littoral suggest that growth on epipsammon of *P. toroi* occurs

under optimal or sub-optimal conditions. To properly define the species' autecology, samples from different habitats should be taken in Cimera Lake and from other Spanish Central Range. To date, *P. toroi* has been not found in the extensive surveys in the Pyrenees (Catalan *et al.*, 2009).

***Psammothidium levanderi* (Hust.) Bukht. & Round 1996**

Figs 42-45

Basionym: *Achnanthes levanderi* Hustedt 1933, *Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete*, p. 404, fig. 856.

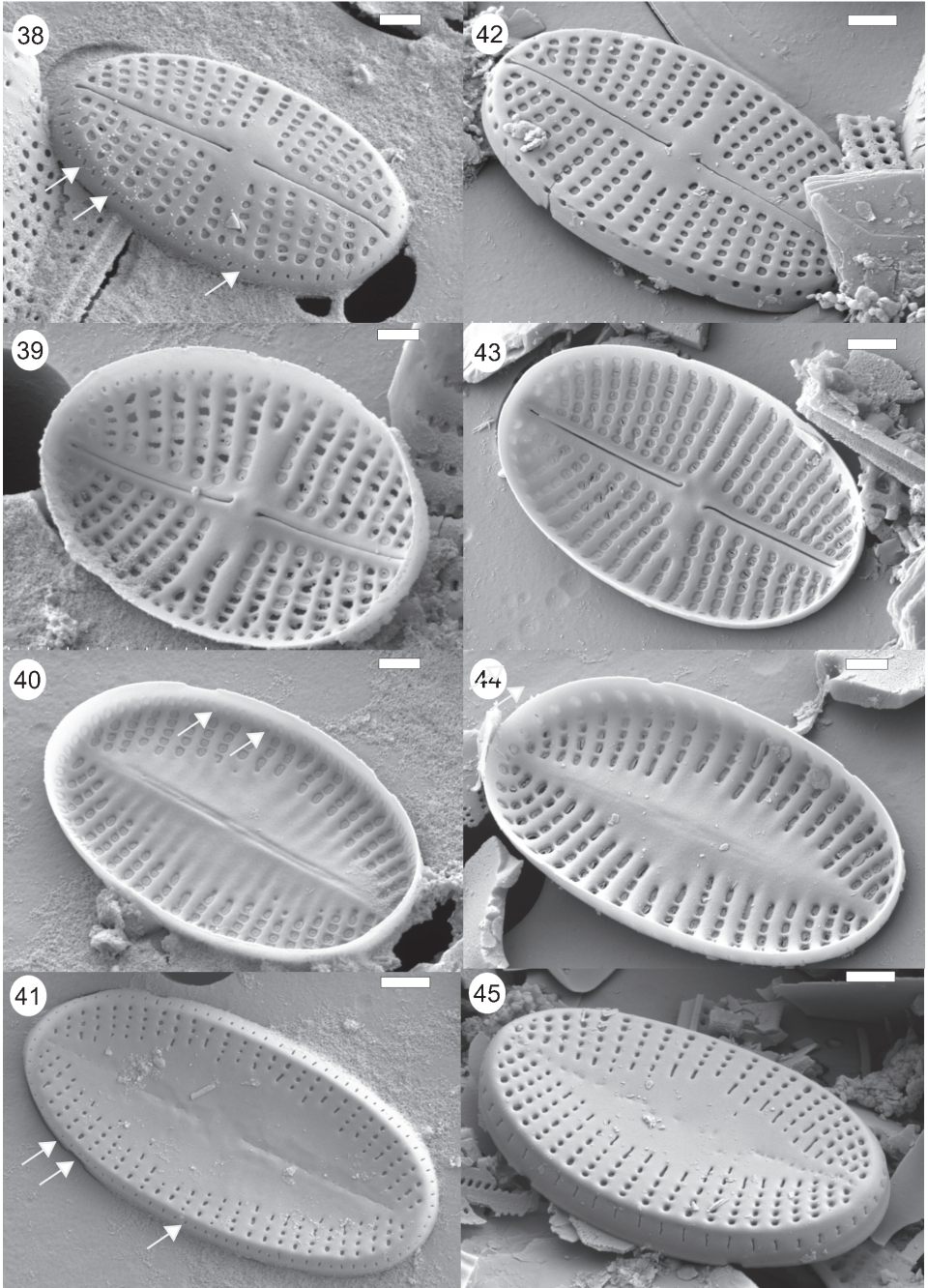
Synonyms: *Achnanthes levanderi* var. *helvetica* Hustedt 1933, *Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete*, p. 404, fig. 856B; *Achnantheidium levanderi* (Hust.) Czarn. in Czarnecki & Edlund 1995, *Diatom research* 10 (1), p. 208; *Psammothidium levanderi* (Hust.) Bukht. 2008, *International journal on algae* 10 (1), p. 62 [isonym].

Type: Pond at east foot of Wuokatti, Helsingfors, Finland. BRM (coll. Hustedt N12/84), fide Simonsen (1987, *Atlas Cat. Diat. Types Fr. Hustedt*, slide N12/84, finder 403.2, pl. 221, figs 9-12).

Description: Valves linear-elliptic in larger individuals to broadly elliptic in smaller individuals, with broadly rounded apices, 6-9 µm long, 4-5 µm wide according to the protologue (6-11 µm long as found in the literature). Raphe valve: Valve face flat (Fig. 42). Linear axial area that widens into a small, irregular, clearly asymmetric central area. Raphe straight, filiform, with straight ends (Fig. 42). Internally, the central raphe ends are curved toward opposite sides (Fig. 43). Striae radiate throughout the whole valve, 21-23 in 10 µm according to the protologue. Areolae foramina internally and externally more or less squared (Figs 42, 43). Rhapheless valve: axial area fused with a wide (up to 1/2 of the valve¹) central area, transapically expanded near the center, and surrounded by more or less regularly shortened striae (Fig. 45). Areolae foramina internally and externally transapically oblong elliptic (Figs 44, 45). The areolae delimiting the axial area are slit-like (Fig. 45). A vestigial raphe is generally well developed (Fig. 45). Striae are slightly radiate in the center, becoming more strongly radiate at the apices, 22-28 in 10 µm (up to 30 in 10 µm as found in the literature). Areolae are often visible in LM. Mantle striae are reduced to a single row of round (raphe valve, fig. 42) or slit-like (rapheless valve, fig. 45) areolae.

Figs 38-45. *Psammothidium toroi* sp. nov., compared with *P. levanderi*, SEM, valve views. **38-41.** *Psammothidium toroi* sp. nov. Type material from Cimera Lake, Spain. **38.** Raphe valve, external view. Note the slit-like external foramina of the mantle areolae. **39.** Raphe valve, internal view. **40.** Rapheless valve, external view. Note the transapically expanded central area fused with the axial area and the vestigial raphe. **41.** Rapheless valve, internal view. Note the roundish areolae delimiting the central area. In all four illustrated valves "extra" areolae are present (more than one areola per striae, white arrows). **42-45.** *Psammothidium levanderi*. Type material from a pond at east foot of Wuokatti, Finland. **42.** Raphe valve, external view. Note the roundish external foramina of the mantle areolae. **43.** Raphe valve, internal view. **44.** Rapheless valve, external view. Note the much narrower central area, occupying 1/2 of valve breadth. **45.** Rapheless valve, internal view. Note the slit-like areolae delimiting the central area. Each areola on the valve mantle has a corresponding valve face stria. Scale bars: 1 µm.

1. While this central area in some populations illustrated in the literature (including Hustedt's original drawings) can be wider, it is clearly narrower in the type population here examined, in accordance with the specimen selected by Simonsen (1987, pl. 221, figs 9-12, reproduced in Krammer & Lange-Bertalot, 1991, pl. 15, figs 8-10).



Literature: Hustedt (1933), Lange-Bertalot & Krammer (1989), Krammer & Lange-Bertalot (1991), Bukhtiyarova & Round (1996), Heudre *et al.* (2017).

Distribution: Holarctic region, Antarctica and Antarctic islands, numerous fossil and recent occurrences documented specially in high-latitude sites. Unconfirmed reports from South America, Africa and Indonesia.

Ecology: This taxon inhabits pristine freshwaters, occurring in mountainous rivers with oligotrophic, oligosaprobic waters and low electrolyte contents. Circumneutral or slightly acidophilic.

DISCUSSION

Psammothidium toroi exhibits all the distinctive features of the genus *Psammothidium* as defined in Bukhtiyarova & Round (1996) with regards to valve shape and striation. The presence of ridges accompanying externally the raphe slit, especially near the central nodule, is only evident in few *Psammothidium* taxa, e.g. *P. therezienii* (Le Cohu & R.Maillard) Van de Vijver (Van de Vijver *et al.*, 2002, pl. 28, fig. 3) or *P. lauenburgianum* (Hust.) Bukht. & Round (Bukhtiyarova & Round, 1996, fig. 62); while it is apparently absent in many others such as *P. lacustre* Enache & Potapova (Enache *et al.*, 2013, fig. 35), *P. hainanii* Kociolek & Y.Liu (Liu *et al.*, 2014, fig. 17) or *P. toroi* (Fig. 39), which presents simple proximal raphe endings which appear as conspicuous central pores under LM.

Psammothidium taxa show varying degrees of heterovalvy. In general, both valves differ in the shape and size of the axial area, which, in the raphe valve, develops transapically only in the central part, forming a stauros-like fascia in many taxa, while in the rapheless valve is more or less evidently fused with the central area, forming a more or less widely lanceolate sternum. The shape and density of the areolae also differs frequently, with usually larger (and, thus, less densely spaced) areolae forming the striae of the rapheless valve. However, as in the case of *P. toroi*, the orientation and density of striae is similar in both valves (Figs 2-38). The degree of heterovalvy is maximal in *P. oblongellum* (Østrup) Van de Vijver, with near-parallel striae in the rapheless valve, formed by macroareolae, and delimiting a narrow, fusiform axial area. These characteristics led to its transference to the more suitable genus *Karayevia* Round & Bukht. by Aboal (Aboal *et al.*, 2003).

Within the Achnantheid taxa with comparable valve outlines, similar species (Table 1) include *P. daonense* (Lange-Bert.) Lange-Bert., *P. pseudoinvestians* Flower, and *Achnanthes helvetica* var. *minor* Flower & V.J.Jones. However, *P. toroi* presents smaller valves (up to 4.3 µm wide and not > 5 µm). *Psammothidium marginulatum* (Grun.) Bukht. & Round and *P. acidoclinatum* (Lange-Bert.) Lange-Bert. are wider (> 5 µm). The size range of *P. scoticum* (Flower & V.J.Jones) Bukht. & Round and *P. microscopicum* (Cholnoky) S.Blanco (2016) overlap with that of the new species here described, although the raphe valves show a denser striation (27-30 striae in 10 µm vs. 22-26 in *P. toroi*). Moreover, *P. microscopicum* presents a much coarser areolation (ca 10 areolae in 10 µm in both valves). The central area of the rapheless valve in *P. grischunum* (Wuthrich) Bukht. & Round is comparatively reduced, occupying at most ½ of valve width. *Psammothidium subatomoides* (Hust.) Bukht. & Round differs mainly in valve breadth (always above 6 µm), but the stria density, the shape of the central areas and the areolae foramina are also dissimilar. *Psammothidium bristolium* Bukht. and *P. metakryophilum* (Lange-Bert. & Rol.

Table 1. Main diagnostic features of *P. toroi* and related taxa. Data from Flower & Jones (1989), Bukhtiyarova & Round (1996), Manoylov (2007), and Genkal & Khartitov (2012)

	<i>L</i> (μm)	<i>W</i> (μm)	<i>L/W</i>	<i>Outline</i>	<i>S</i>	<i>Striae</i> (in 10 μm)	<i>Central area, RLV</i>	<i>Central area, RV</i>	<i>Raphe features</i>
<i>P. acidoclinatum</i>	8-14 (16)	5-7	2.0-2.3	Broad elliptical	28-32	Shortened throughout	Wide, elliptic	Rhombic-lanceolate	Straight, filiform
<i>P. alpinum</i>	14.6-30	6.3-9.1	2.3-3.3	Elliptical/linear elliptical	22-25	Slightly radiate	Round, 1/2 of valve width	Rectangular-irregular	Straight with hook-like proximal pores, drop-shaped distal endings
<i>P. bristolicum</i>	12-30	5-11	2.4-2.7	Linear-elliptical	25-30	Radiate	Elliptic, 1/2 of valve width	Elliptical, 1/2 of valve width	Central pores distant, ridges between grooves, terminal fissures simple, long, curved in opposite directions
<i>P. confisum</i> var. <i>atomoides</i>	9-11	3-4	2.7-3.0	Linear	28-34	Slightly radiate	Absent	Stauros-like	Straight, filiform
<i>P. daonense</i>	10-26	5-8	2.0-3.2	Elliptical/linear elliptical	27-32	Radiate	Rhomboid	Stauros-like	Straight, filiform
<i>P. grischunum</i>	6-18	4-5.5	1.5-3.3	Elliptical/linear elliptical	18-27	Radiate	Elliptic-rhombic, 1/2 of valve width	Broad elliptic, occupying from 1/2 to 2/3 of the valve	Terminal pores extend to the mantle
<i>A. helvetica</i> var. <i>minor</i>	8-11	5-6	1.6-1.8	Elliptical	22-26	Radiate	Round, 1/2 of valve width	Round, 1/2 of valve width	Hooked distal ends turning in opposite directions
<i>P. helveticum</i>	7-28	5-7.5	1.4-3.7	Elliptical/linear elliptical	23-28	Radiate	Round, 1/2 of valve width	Broad fascia	Proximal ends expanded, terminal ends straight
<i>Achnanthes helvetica</i> var. <i>minor</i>	8-11	5-6	1.6-1.8	Elliptical	22-26	Radiate	Round, 1/2 of valve width	Round, 1/2 of valve width	Hooked distal ends turning in opposite directions
<i>P. lacus-vulcani</i>	8-10	4-4.5	2-2.2	Elliptical	27	Slightly radiate	Absent	Narrow rhombic	Straight, filiform
<i>P. levandieri</i>	6-11	4-5	1.5-2.2	Elliptical	22-28	Slightly radiate	Wide lanceolate, depressed	Small, asymmetric	Straight, filiform

Table 1. Main diagnostic features of *P. toroi* and related taxa. Data from Flower & Jones (1989), Bukhtiyarova & Round (1996), Manoylov (2007), and Genkal & Kharitonov (2012) (continued)

	<i>L</i> (μm)	<i>W</i> (μm)	<i>L/W</i>	<i>Outline</i>	<i>S</i>	<i>Striae</i> (in 10 μm)	<i>Central area, RLV</i>	<i>Central area, RV</i>	<i>Raphe features</i>
<i>P. marginulatum</i>	6-21	5-6	1.5-3.5	Lanceolate-elliptical	27-30	Radiate	Wide lanceolate, depressed	Semifascia	Straight, filiform
<i>P. metakryophilum</i>	11-18	4.3-5.2	2.6-3.7	Lanceolate-elliptical	23-28	Radiate	Rhombic	Rectangular-elliptic	Central pores non-coaxial
<i>P. microscopium</i>	5.5-7	3.5	1.6-2	Elliptical	26-30	Radiate	Wide lanceolate	Narrow	Straight, filiform
<i>P. nivale</i>	11.7-15	5.6-6	2.1-2.5	Elliptical	26-32	Slightly radiate	Rhombic-lanceolate, 2/3 valve width	Rectangular-elliptic, 2/3 valve width	Central pores slightly expanded, in grooves
<i>P. pennsylvanicum</i>	9-13	5-5.4	1.8-2.4	Elliptical	25-29	Slightly radiate	Asymmetric	Rectangular-elliptic	Slightly expanded, almost straight or very slightly deflected external distal and proximal pores
<i>P. pseudoinvestians</i>	8-12.5	5-5.5	1.6-2.3	Sub-round to lanceolate	34	Radiate	Asymmetric	Asymmetric	Terminal end turned in opposite directions
<i>P. saccutum</i>	7-11	3.8-4.5	1.8-2.4	Elliptical/linear elliptical	30	Radiate	Narrow linear	Asymmetric	Straight, filiform
<i>P. scoticum</i>	7-13	3.5-5	2-2.6	Elliptical	25-28	Radiate	Wide lanceolate	Wide lanceolate	Straight, filiform
<i>P. subatomoides</i>	6-15	3.5-6.5	1.7-2.3	Elliptical	28-40	Radiate	Rectangular, 1/4 of valve width	Rectangular, 1/4 of valve width	Proximal ends expanded, terminal ends straight
<i>P. toroi</i> sp. nov.	5.3-10.1	3.4-4.3	1.6-2.3	Elliptical/linear elliptical	22-28	Radiate	Wide, 2/3 of valve width	Irregularly oblong elliptical or diamond-shaped	Straight, filiform

Schmidt) Sabbe are also similar in valve outline and striation, but present consistently larger valves (12-30 μm long, 5-11 μm wide and 11-18 μm long, 4.3-5.2 μm wide, respectively). Some raphe valves of *P. confusum* var. *atomoides* (Manguin) Van de Vijver (Van de Vijver *et al.* 2004, pl. 31, figs 27-30, but not figs 23-26) resemble those of *P. toroi*, although the ultrastructure of the rapheless valve is completely different (cf. l.c. pl. 32, fig. 4). The central area in the raphe valve of *Psammothidium lacus-vulcani* (Lange-Bertalot & Krammer) Bukht. is much smaller and often reduced to a single shortened central stria. Contrary to *P. toroi*, *P. sacculum* (J.R.Carter) Bukht. and *P. pennsylvanicum* Potapova show an asymmetric central area in the raphe and the rapheless valve, respectively. Finally, the recently described *Psammothidium* taxa from core sediments in Washington State, USA (Enache *et al.*, 2013) *P. alpinum* Enache & Potapova and *P. nivale* Enache & Potapova share a similar outline, but the valves are larger, with no morphometric overlap with *P. toroi*.

Within the described *Psammothidium* taxa, *P. levanderi* seems to be morphologically the closest taxon to *P. toroi*. Both share comparable morphometric ranges (Table 1), valve outlines, ecological preferences and many ultrastructural features. However, a number of differences lead us to reject the conspecificity of these two taxa, namely: i) *P. toroi* has an average valve width of 4.0 μm , with only 20% of individuals with valve widths > 4.1 μm in the type population, whereas the literature refers 4-5 as the width range for *P. levanderi*; ii) the axial/central area in the rapheless valve is consistently much wider in *P. toroi*, usually occupying > 2/3 of valve width (Fig. 41), so that the central striae are often reduced to 1-2 areolae; iii) in external view, the areolae delimiting the central area in the rapheless valve of *P. levanderi* are always slit-like (Fig. 45), while the equivalent areolae in *P. toroi* are round to oblong (Fig. 41); iv) conversely, the external openings of the mantle areolae in the raphe valve are roundish in *P. levanderi* (Fig. 42), being slit-like in *P. toroi* (Fig. 38), and v) in the raphe valve, the central area is more conspicuously asymmetric and the valve surface is consistently flatter in *P. levanderi* (Fig. 42). Moreover, the number of areolae in the mantle is higher than the striae density in *P. toroi*, while in *P. levanderi* the row of areolae on the valve mantle correspond to the exactly number of valve face striae; in *P. toroi*, extra areolae will be displayed irregularly in the interstriae (virgae) (Figs 38-41, white arrows).

Additional CIM12-04A samples were analyzed up to 20 cm depth (*ca* 1400 A.D., core description and date model can be found in Sánchez-López *et al.*, 2016). *Psammothidium toroi* showed a clear trend to increase from 5 cm depth (*ca* 1960 A.D.) that parallels a rapid increase in organic matter in the sediment record (Sánchez-López *et al.*, 2016), which could be related to recent warming in Cimera Lake air temperature (Granados & Toro, 2000). *Eolimna* cf. *raederiae* (Lange-Bert.) Lange-Bert. & Kulikovskiy showed similar trends. *Psammothidium toroi* showed always an abundance below 3% before 5 cm sediment. An inverse trend in abundance was observed for *Pinnularia microstauron* var. *nonfasciata* Krammer, which decreases from *ca* 9% to 1% abundance (core top sample).

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