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Three new *Psammothidium* species from lakes of Olympic and Cascade Mountains in Washington State, USA

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Abstract

Populations of several *Psammothidium* species were found in core sediments from nine remote, high elevation, ultraoligotrophic and oligotrophic, Olympic and Cascade Mountain lakes. Three of these species, *P. lacustre, P. alpinum*, and *P. nivale*, are described here as new. The morphology of the silica frustules of these species was documented using light and scanning electron microscopy. We discuss the similarities and differences with previously described *Psammothidium* species.

Key words: diatom, lake sediments, alpine lakes, LM, SEM

Introduction

Diatom assemblages were analyzed in core sediments from nine Washington Cascade Mountains lakes. These lakes were selected by the U.S. Geological Survey (USGS) for a paleolimnological investigation of the impact of inorganic nitrogen atmospheric deposition on lake biogeochemistry. However, these lakes are still generally considered oligotrophic systems.

Species of *Psammothidium* Bukhtiyarova & Round (1996: 3) are very abundant (up to 28% relative abundance) and diverse in study lakes and they were investigated with both light (LM) and scanning electron microscopy (SEM). *Psammothidium* was separated from *Achnanthidium* Kützing (1844: 75) by Bukhtiyarova & Round (1996) to designate adnate taxa commonly attached to sand grains by the raphe valve face, as opposed to *Achnanthidium* species, which are more common on stable surfaces such as rocks and plants and found attached via short mucilaginous stalks. Bukhtiyarova & Round (1996) pointed out that the major morphological differences between *Psammothidium* and *Achnanthidium* are (1) the shape of the valve, oval-elliptic as opposed to linear/linear-capitate; (2) the convex flexure of the raphe valve as opposed to concave in *Achnanthidium;* (3) striae reaching the sternum as opposed to marginal in *Achnanthidium*; and (4) raphe fissures located in a central channel (or near the valve center) as opposed to non-channeled with pin-hole central endings in *Achnanthidium* (Bukhtiyarova & Round 1996).

Among *Psammothidium* species occurring in the study lakes, based on silica frustule morphological characteristics, three species are new to science. Here we describe those new species using light (LM) and scanning electron microscopy (SEM) observations.

Material and Methods

Sediment cores were collected by USGS crews in the summer of 2009 from nine lakes in Olympic, North Cascades, and Mt. Rainier national parks, located at a minimum elevation of 1335 m. A summary of lake physical characteristics is given in Table 1. Cores were collected by boat from the deepest portion of each lake using a Uwitec gravity corer (http://www.uwitec.at/).

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TABLE 1: Physical properties of study lakes. OLYM—Olympic National Park, NOCA—North Cascades National Park, MORA—Mt. Rainier National Park.

Study Lake	*Park*	*Elevation (m)*	*Area (ha)*	*Max. Depth (m)*
Eunice Lake	MORA	1640	5.32	10.05
Snow Lake	MORA	1438	2.39	9.75
Copper Lake	NOCA	1600	5.14	21.03
Hidden Lake	NOCA	1835	24.98	61.56
Stiletto Lake	NOCA	2066	0.03	25.9
Thornton Lake	NOCA	1384	22.65	31.69
Heather Lake	OLYM	1589	0.40	7.01
Hoh Lake	OLYM	1335	7.40	14.93
Milk Lake	OLYM	1435	1.10	11.88

Diatom slides were prepared using strong acid digestions in a microwave (CEM model MDS-21 00) with enclosed vessels, and temperature and pressure monitoring and control systems. Diatom-coated coverslips were mounted on glass slides using Naphrax[R] mounting medium (http://diatom.ansp.org/nawqa/protocols.asp). Diatom species were investigated in core top (0–0.5-cm depth) and bottom sediments, and in an additional interval from Snow Lake (2–2.5 cm).

A Zeiss Axio Imager microscope equipped with AxioCam MRm digital camera was used for LM. For SEM, cleaned material was dried on aluminum stubs, which were sputter-coated with Pt-Pd and observed with a Zeiss Supra 50 VP FE SEM (Carl Zeiss, Jena, Germany) at an accelerating voltage of 10 kV. Terminology used to describe valve structure follows Anonymous (1975), Bukhtiyarova & Round (1996) and Round et al. (1990).

Water samples for nutrient analysis were collected and processed according to methods outlined in the USGS National Field Manual (US Geological Survey, 2006; Wilde et al., 2004). Water chemistry measurements indicated that the study lakes are ultraoligotrophic or oligotrophic (Table 2).

TABLE 2: Water chemistry of study lakes from surface water collections, 2009. *Sample date also represents sediment core collection date.

LAKE NAME	*Sample Date**	*Total-N* (mgL^{-1})	*Total-P* (mgL^{-1})	*PO4-P* (mgL^{-1})	*NO3-N+NO2-N* (mgL^{-1})	*NH3-N* (mgL^{-1})
Snow Lake	10/7/2009	<0.01	<0.003	<0.003	<0.003	<0.01
Eunice Lake	11/4/2009	0.25	0.012	<0.003	0.002	<0.01
Hoh Lake	9/8/2009	0.16	0.007	<0.003	<0.003	<0.01
Heather Lake	9/10/2009	0.06	0.005	<0.003	0.007	<0.01
Milk Lake	9/11/2009	0.04	0.004	<0.003	<0.003	<0.01
Stiletto Lake	8/26/2009	0.27	0.007	<0.003	0.006	<0.01
Copper Lake	9/22/2009	0.07	0.004	<0.003	<0.003	<0.01
Lower Thornton Lake	9/23/2009	0.10	0.003	<0.003	0.004	<0.01
Hidden Lake NOCA	9/24/2009	0.05	0.002	<0.003	<0.003	<0.01

Results and Discussion

The following *Psammothidium* species were identified in core sediments from study lakes: *P. marginulatum* (Grunow in Cleve & Grunow) Bukhtiyarova et Round (1996: 5), *P. helveticum* (Hustedt) Bukhtiyarova et Round (1996: 8), *P. subatomoides* (Hustedt in Schmidt et al.) Bukhtiyarova et Round (1996: 13–14), *P. scoticum* (Flower et Jones) Bukhtiyarova et Round (1996: 22), *P. curtissimum* (Carter) Aboal in Aboal, Alvarez-Cobelas, Cambra & Ector (2003:171), *P. altaicum* Bukhtiyarova in Bukhtiyarova et Round (1996: 5), *P. lacustre* sp. nov., *P. alpinum* sp. nov., and *P. nivale* sp. nov.

The number of *Psammothidium* species in core top sediments was higher than in bottom sediments. For example, *P. marginulatum* was observed in bottom sediments from four lakes versus top sediments from seven lakes, while *P. helveticum* was observed in bottom sediments from five lakes versus top sediments from seven lakes (Table 3). The most abundant species across all study sites were *P. curtissimum* (maximum relative abundance 28%) and *P. scoticum* (maximum relative abundance 22%). The new species (*P. lacustre* sp. nov., *P. alpinum* sp. nov., and *P. nivale* sp. nov) were found in core material from six lakes: Hidden Lake NOCA, Eunice, Snow, Stiletto, Hoh, and Copper lakes.

TABLE 3: Relative abundance (%) of *Psammothidium* species identified in lake sediment samples from the Olympic and Cascade Mountains. *P. cur = P. curtissimum*; *P. hel = P. helveticum*; *P. mar = P. marginulatum*; *P. scot = P. scoticum*; *P. sub = P. subatomoides*; *P. lac = P. lacustre sp. nov.*; *P. alp = P. alpinum sp. nov.*; *P. niv = P. nivale sp. nov.*

	ANSP Code	*Interval (cm)*	*P. cur*	*P. hel*	*P. mar*	*P. scot*	*P. sub*	*P. lac*	*P. alp*	*P. niv*
Snow L.	WACA001	0–0.5	5.4	0.6	0.8	1	2.2	0.4	0	0
Snow L.	WACA002	28–29	4.8	2	0	15	1	1	0	0
Snow L.	WACA019	2–2.5	11.4	1	2.2	2.6	3.6	1.2	0.1	0.1
Eunice L.	WACA003	0–0.5	7.2	0.6	0.8	11.6	5	1	0	0
Eunice L.	WACA004	39–40	2.4	0	0.4	8.4	2	2.2	0	0
Hoh L.	WACA005	0–0.5	0	0	0.4	0	0	0	0	0
Hoh L.	WACA006	37–38	0	0	0	0	0	0	0	0
Heather L.	WACA007	0–0.5	0	0	0	0	0	0	0	0
Heather L.	WACA008	17–18.5	0	0	0	0	0	0	0	0
Milk L.	WACA009	0–0.5	0	0.2	0	0	0	0	0	0
Milk L.	WACA010	37–38	0	0	0	0	0	0	0	0
Stiletto L.	WACA011	0–0.5	11.8	0.2	0.8	2.6	2.4	0.6	0	0
Stiletto L.	WACA012	30–31	28.2	1	0.2	4	0.8	0	0	0
Copper L.	WACA013	0–0.5	4.4	6	0.4	6.6	9	0	0	0
Copper L.	WACA014	38–39	5.8	2	1.2	8.8	3.6	0.8	0	0
Lower Thornton L.	WACA015	0–0.5	0.6	5.8	0.8	7.8	1.4	0	0	0
Lower Thornton L.	WACA016	15–16	0.8	5.6	0	9.4	0.8	0	0	0
Hidden L.	WACA017	0–0.5	11.6	6.4	4.4	9.8	4.6	0	1.2	0.1
Hidden L.	WACA018	34–35	6.8	13.6	4.8	21.8	7.2	2	2.8	0

New species description

Division **Bacillariophyta**

Class **Bacillariophyceae** Haeckel 1878 *emend*. D.G. Mann in Round *et al*. 1990

Order **Achnanthales** Silva 1962

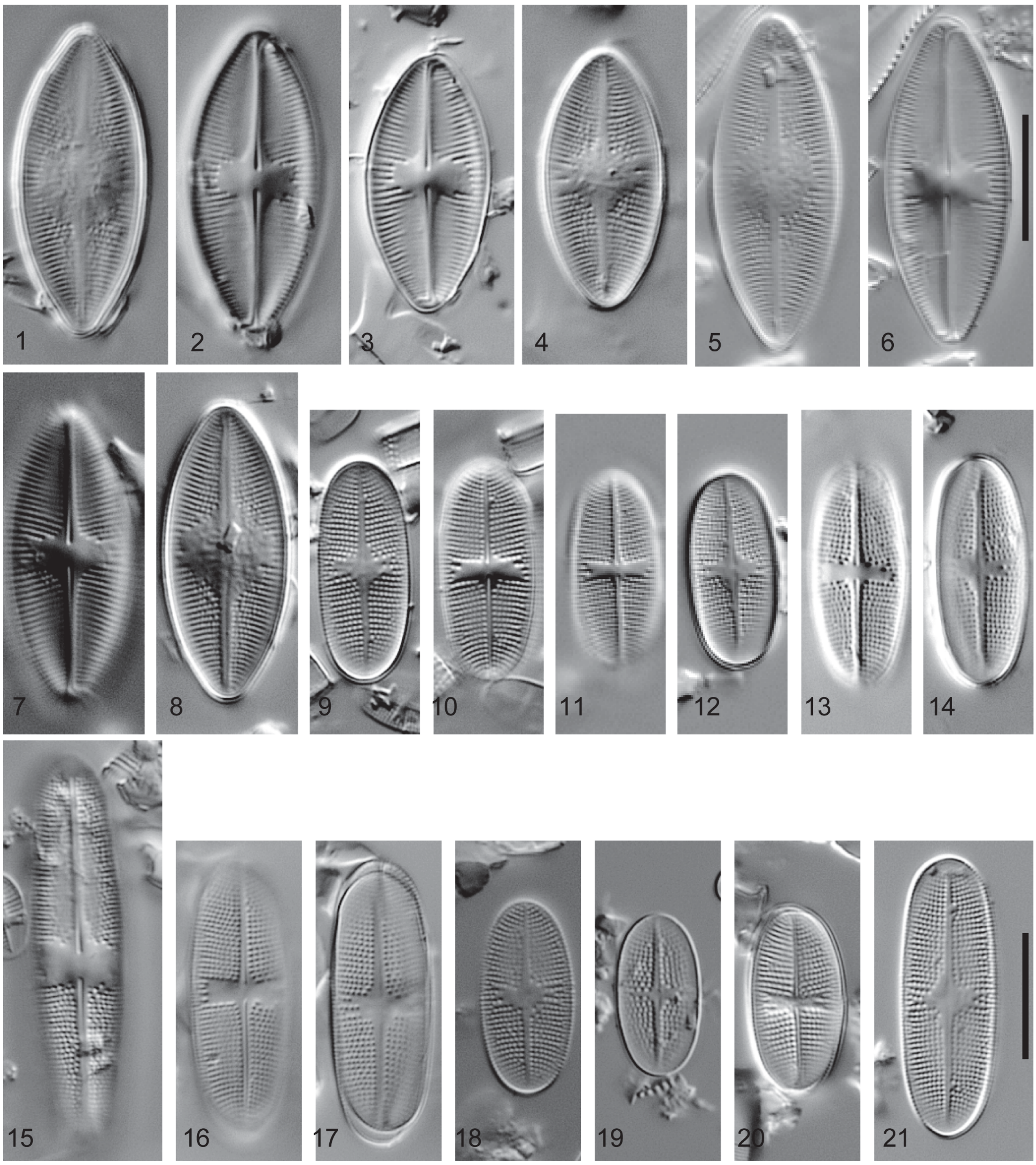
Genus ***Psammothidium*** Bukhtiyarova & Round (1996: 3)

Psammothidium lacustre Enache & Potapova *sp. nov*. (Figs 1–8, 35–39)

Valvae lanceolate, 8.4–11.2 µm latae, 16.7–28 µm longae. Raphovalva cum area axialis linearis versus centro expanso, area centralis rectangularis, ½ vel 2/3 valvae lata. Raphe recta poris centralibus simples cum cristae et sulci, interne paulo curvatae. Fissuris terminalibus simplex, longae, ad versum curvatae. Araphovalva cum area axialis angusta, area centralis circulare, 2/3 valvae lata. Striae transapicales radiatae 22–26 in 10 µm; areolae cum foramina externae circulares, foramina internae transapicalem elongatae, 40–60 in 10 µm.

Valves lanceolate, 8.4–11.2 µm wide, 16.7–28 µm long. Raphe valve with linear axial area, rectangular central area, occupying ½ to 2/3 of valve breadth (Figs 2, 3, 6, 7). Proximal raphe endings slightly deflected internally (Fig. 36). Terminal raphe fissures simple, long, deflected in opposite directions (Figs 2, 3, 6). Rapheless valve with narrow axial area, round central area occupying 2/3 of the valve breadth (Figs 1, 4, 5, 8). Striae radiate, 22–26 in 10 µm; areolae 40–60 in 10 µm, with round external foramina, and internal openings elongated transapically on both valves (Figs 35–39).

Type:—USA. Washington: Cascade Mountains, Snow Lake, 46.7576° N, 121.6982468° W, lake sediment (2–2.5-cm depth core interval; lake maximum depth 9.75 m), collected 07 October 2009, collection *WACA019*, (Circled specimen (Figs 1, 2) on slide *GC64860*, *accession #GC64860* (ANSP!), **holotype, designated here**; circled specimens on slide *GC64861* (ANSP!) and slide *84222* (CANA!), **isotypes, designated here**).



FIGURES 1–21: LM micrographs of *Psammothidium* species from Snow Lake, Washington Cascades. Figs 1–8. *Psammothidium lacustre* sp. nov. Figs 1–2. Holotype specimen, slide ANSP GC64860. Figs 9–21. *Psammothidium alpinum* sp. nov. Figs 9–10. Holotype specimen, slide ANSP GC64862. Scale bar = 10 µm.

Etymology:—specific epithet refers to the occurrence of the new species in a lacustrine environment.

The species with the closest morphological features to *Psammothidium lacustre* is *P. helveticum* (Figs 32–34). *Psammothidium lacustre* has much larger size, with valve width greather than 8 µm, and more acute valve ends compared to *P. helveticum*. SEM investigations (Figs 35–39) revealed that the proximal raphe endings are internally slightly deflected in opposite directions (Fig. 36), similarly to *P. helveticum* (see Bukhtiyarova & Round 1996, figure 23). The striae extend uninterrupted onto the mantle, and short striae are present near the central area and occasionally in other parts of valve margin (Fig. 38). Areolae have round external foramina and transapically elongated internal openings (Figs 37, 39).

The shape of the axial and central areas on rapheless valves is similar in *Psammothidium lacustre* and *P. helveticum*, except that the central area is narrower in *P. helveticum*. Internally, the shape of areolae is also different: elongated with rims and grooves in *P. lacustre* versus round or rectangular in *P. helveticum* (see Bukhtiyarova & Round 1996, figure 23). In LM, *P. lacustre* is relatively easy to separate from *P. helveticum*; *P. lacustre* is larger, has more lanceolate shape, and coarser striae (22–24 versus 23–28) and areolae. *Psammothidium lacustre* was rare in study lakes (<1% relative abundance).

Psammothidium alpinum Potapova & Enache *sp. nov*. (Figs 9–21, 40–44)

Valvae linearae ellipticae, ovalis, 6.3–9.1 µm latae, 14.6–30 µm longae. Raphovalva cum area axialis angusta linearis versus, area centralis rectagularis. Raphe recta poris centralibus unciformis, termini distale punctiformis. Araphovalva cum area axialis angusta, area centralis circularis, irregularis, 1/2 valvae lata. Striae transapicales paulo radiatae, 22–25 in 10 µm cum striae breviores intercalares. Areolae rotundae, grossae, 25 in 10 µm.

Valves linear-elliptic to oval, 6.3–9.1 µm wide, 14.6–30 µm long. Raphe valve with narrow linear axial area and rectangular-irregular central area. Raphe straight with hook-like proximal pores, drop-shaped distal endings (Figs 10, 11, 13, 15, 16, 20). Rapheless valve with narrow axial area, irregular, rounded central area, occupying about ½ of valve breadth (Figs 9, 12, 14, 17–19, 21). Transapical striae slightly radiate, 22–25 in 10 µm, with shortened marginal striae present (figs 41, 43). Areolae coarse, round, 25 in 10 µm (Fig. 44).

Type:—USA. Washington: Cascade Mountains, Snow Lake, 46.7576° N, 121.6982468° W, lake sediment (2–2.5-cm depth core interval; lake maximum depth 9.75 m), collected 07 October 2009, collection *WACA019*, (Circled specimen (Figs 9, 10) on slide *GC64862, accession #GC64862* (ANSP!), **holotype, designated here**; circled specimens on slide *GC64863* (ANSP!) and slide *84223* (CANA!), **isotypes, designated here**).

Etymology:—specific epithet refers to the occurrence of the new species in alpine, high elevation lakes.

Psammothidium alpinum is similar to *P. bioretii* (Germain 1957: 85) Bukhtiyarova et Round (1996: 9) in valve size and possession of relatively coarse areolae. It differs from *P. bioretii* by having more elongate, linear-elliptical valve shape with nearly parallel sides, and a straight central sternum (versus diagonal in *P. bioretii*). In SEM (Figs 40–44), *P. alpinum* displays a characteristic doublet of smaller areolae at the valve/mantle junction and around the mantle (Figs 40, 42). On raphe valves, the central area nearly reaches the valve margin and is bounded by 4–8 short striae. The raphe has characteristic hook-like widely spaced proximal endings (Fig 40) that deflect in the same direction externally (Fig. 40) and opposite internally (Fig. 41). While *P. bioretii* has terminal raphe fissures curved to opposite sides, *P. alpinum* lacks terminal fissures, and distal external raphe endings are drop-shaped and do not expand beyond the last stria (Fig. 41). Similar to *P. bioretii*, *P. alpinum* has coarse, round areolae visible in LM; a row of coarser areolae border the sternum and central area in LM (Figs 10, 11) and a double row of finer areolae bordering the valve face-mantle junction is visible in SEM (Figs 40, 42). Short intercalary striae are present along the valve margin and expand on the mantle (Figs 41, 43). *Psammothidium alpinum* has similar valve shape as *P. chlidanos* (Hohn et Hellerman 1963: 273) Lange-Bertalot (1999: 285) but it can be easily distinguished by its coarser striae and areolae visible in LM.

P. alpinum was present in Hidden Lake NOCA (bottom sample, 2% relative abundance) and very rare in top samples from Snow and Stiletto lakes.

Psammothidium nivale Potapova & Enache *sp. nov*. (Figs 22–31, 45–48)

Valvae ellipticae, 5.6–6 µm latae, 11.7–15 µm longae. Raphovalva cum area axialis linearis, area centralis transapicalem rectagularis vel ovalis, 2/3 valvae lata. Raphe recta cum poris proximales paene expansae, in sulci, cum terminis distales simplices. Araphovalva cum area axialis ad apicem angusta, versus centro expansa, rhombico-lanceolata, 2/3 valvae lata. Striae transapicales paulo radiatae, 26–28 in 10 µm in araphovalva, 30–32 in 10 µm in raphovalva.

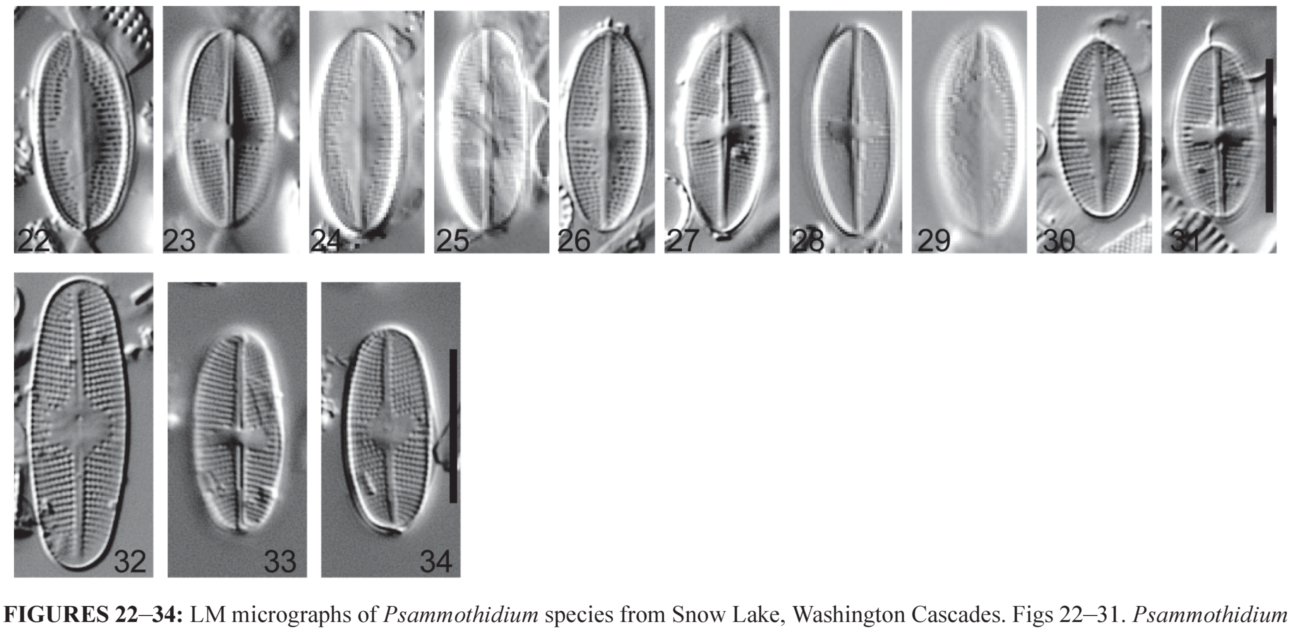
Valves elliptical, 5.6–6 µm wide and 11.7–15 µm long. Raphe valve with linear axial area, central area transapically rectangular to oval occupying 2/3 of the valve breadth. Raphe with proximal pores slightly expanded, located in grooves, and with simple distal endings (Figs 23, 25, 27, 28). Rapheless valve with axial area narrow at apices and widening towards valve centre in a rhombic-lanceolate shape, occupying 2/3 of the valve breadth (Figs 22, 24, 26, 29, 30). Striae slightly radiate, 26–28 in 10 µm on rapheless valve, 30–32 on raphe valve.

Type:— USA. Washington: Cascade Mountains, Snow Lake, 46.7576° N, 121.6982468° W, lake sediment (2–2.5-cm depth core interval; lake maximum depth 9.75 m), collected 07 October 2009, collection *WACA019*, (Circled specimen (Figs 22, 23) on slide *GC64864*, *accession #GC64864* (ANSP!), **holotype, designated here**; circled specimens on slide *GC64865* (ANSP!) and slide *84224* (CANA!), **isotypes, designated here**).

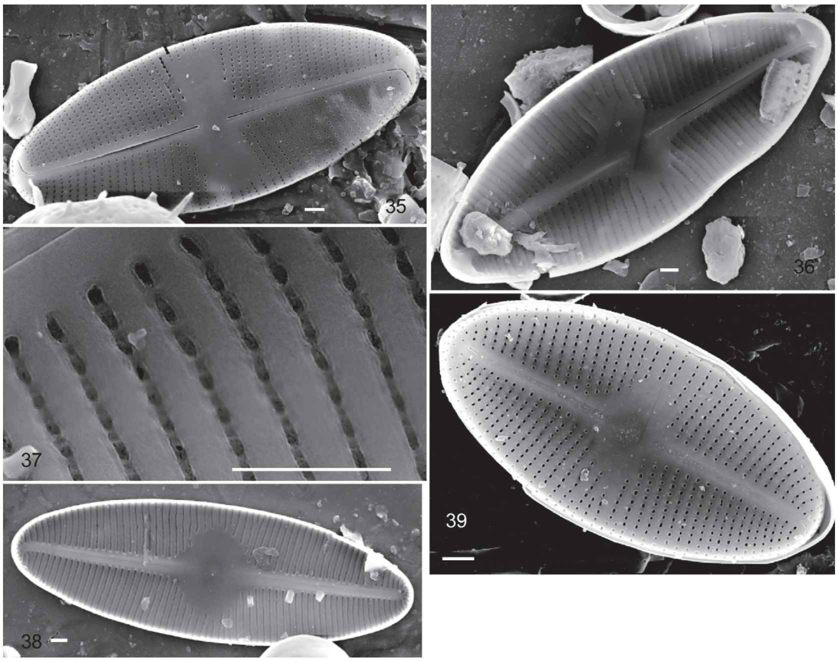
Etymology:—specific epithet refers to the name of the lake (Snow Lake) from where the species was found and described.

Psammothidium nivale is distinguished from other *Psammothidium* species by characteristic coarser striae and areolae on rapheless valve compared to raphe valve. The linear axial area on raphe valves widens slightly near the valve center, and the transapically rectangular to oval central area is bounded by 4–5 shortened striae. External proximal raphe endings are located in grooves, which gradually widen toward valve center. External distal raphe endings are drop-shaped and do not extend beyond the last stria (Fig. 45). Characteristic coarse areolae on rapheless valves have the appearance of transapically elongated slits in SEM (Figs 47–48).

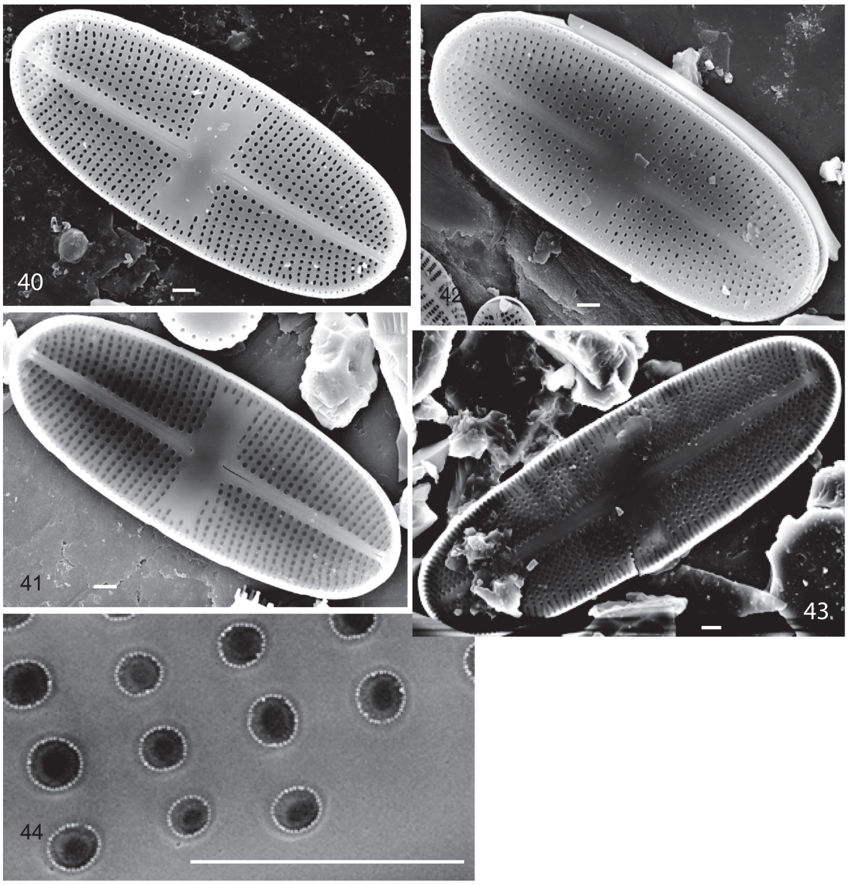
In the SEM, the shape of the areolae—transapically elongate—resemble those of *P. subatomoides* in SEM. However, *P. nivale* is distinguished from the latter species by all other valve characteristics: more elongated shape of the valve and larger size, shape of central area on both raphe and rapheless valve and clear dimorphism of raphe and rapheless valve in striae density and areolae size. *P. nivale* was found in Snow Lake and Hidden Lake NOCA in very low abundance (< 0.25% relative abundance).



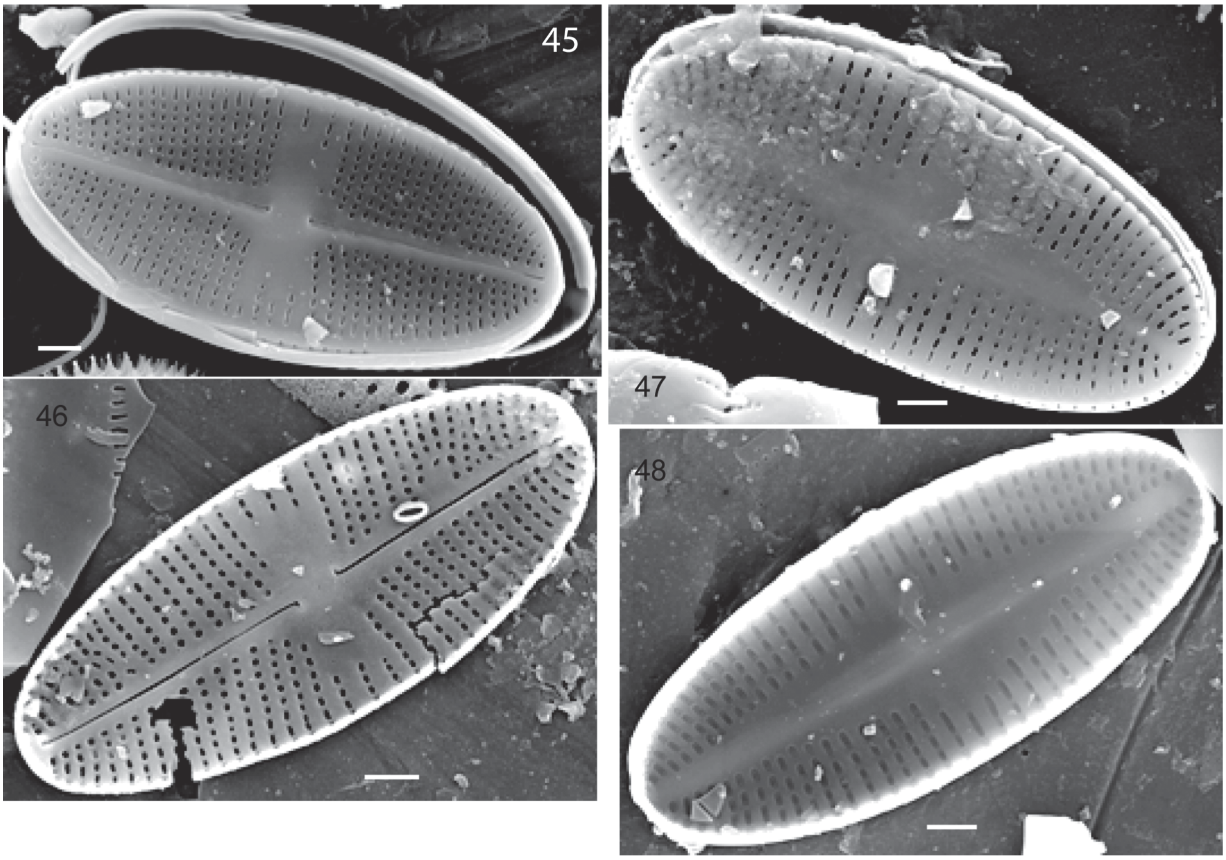
FIGURES 22–34: LM micrographs of *Psammothidium* species from Snow Lake, Washington Cascades. Figs 22–31. *Psammothidium nivale* sp. nov. Figs 22–23. Holotype specimen, slide ANSP GC64684. Figs 32–34. *Psammothidium helveticum.* Scale bar = 10 µm.



FIGURES 35–39: *Psammothidium lacustre* sp. nov., SEM. Fig. 35. External view of raphe valve, Hidden Lake NOCA, sample ANSP WACA017. Fig. 36. Internal view of raphe valve, type material, sample ANSP WACA019. Fig. 37. Areolae on internal surface of raphe valve, type material, sample ANSP WACA019. Fig. 38. Internal view of rapheless valve, Hidden Lake NOCA, sample ANSP WACA017. Fig. 39. External view of rapheless valve, type material, sample ANSP WACA019. Scale bars = 1 µm



FIGURES 40–44: *Psammothidium alpinum* sp. nov., SEM. Figs 40–42. Type material, Snow Lake, sample ANSP WACA019 Fig. 40. External view of raphe valve. Fig. 41. Internal view of raphe valve. Fig. 42. External view of rapheless valve. Fig. 43. Internal view of rapheless valve, Hidden Lake NOCA, sample ANSP WACA018. Fig. 44. Areolae on the internal surface of the valve, Snow Lake, sample ANSP WACA001. Scale bars = 1 µm.



FIGURES 45–48: *Psammothidium nivale* sp. nov., SEM. Figs 45–47. Type material, sample ANSP WACA019, Snow Lake. Fig. 45. External view of raphe valve. Fig. 46. Internal view of raphe valve. Fig. 47. External view of rapheless valve. Fig. 48. External view of rapheless valve, Hidden Lake NOCA, sample ANSP WACA018. Scale bars = 1 µm.

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