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Planothidium sheathii, a new monoraphid diatom species from rivers in California, USA

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Abstract

Planothidium sheathii sp. nov., a new species from rivers in California, USA, is described based on light and scanning electron microscopy. *P. sheathii* has broadly lanceolate to elliptic valves with obtusely rounded apices and a unique combination of morphological features, as follows: 1) cavum with broad hood aperture that flares out at the valve margin with open borders fused with the neighboring virgae on SV, 2) multiseriate striae, composed of five to six rows of same sized areolae on both valves, interrupted at valve mantle junction, and 3) areolae groups on valve mantle, with rounded areolae on RV, and rounded and elongated areolae on SV. Valve morphology of *P. sheathii* is discussed in comparison with similar freshwater taxa, from which the new species is clearly distinct.

Keywords: *Planothidium*, Bacillariophyta, rivers, California, new species, electron microscopy

Introduction

In the last decade, the extensive stream bioassessment sampling of the California Water Resource Control Board Surface Water Ambient Monitoring Program (SWAMP) provided data for a description of new-to-science species belonging to the diatom genera *Amphora* Ehrenberg ex Kützing (1844: 107), *Halamphora* (Cleve) Levkov (2009: 165) (Stepanek & Kociolek 2013), *Rhoicosphenia* Grunow (1860: 511) (Thomas & Kociolek 2015), *Gomphonema* Ehrenbrg (1832: 87), *Gomphoneis* Cleve (1894: 73) (Stancheva *et al.* 2016), *Fallacia* Stickle & D.G. Mann (1990: 554) (Stancheva & Manoylov 2018), *Cocconeis* Ehrenberg (1836: 173) (Stancheva in press) and to the green algal genera *Zygnema* C.A. Agardh (1817: 98) (Stancheva *et al*. 2012), *Spirogyra* Link (in Nees 1820: 5) (Stancheva *et al*. 2013), and *Ochlochaete* Thwaites (1849: Pl. 226) (Hall *et al.* 2018).

Another common component of the diatom stream benthos in the studied data set in California is the monoraphid genus *Planothidium* Round & Bukhtiyarova (1996: 351). *Planothidium* cells are usually solitary, living on aquatic plants, algae, or inorganic substratum. *Planothidium* is characterized by a convex rapheless sternum valve (SV), and a slightly concave raphe valve (RV). The genus was separated from *Achnanthes* Bory (1822: 79) based on thick bi- to multiseriate striae interrupted unilaterally by a "horseshoe" depression on the SV (Round & Bukhtiyarova 1996). Recent molecular analysis of freshwater *Planothidium* species showed that the shape of the depression on the SV (i.e., a single sinus vs a double cavum with a hood) and the striae ultrastructure are important taxonomic features (Jahn *et al.* 2017). Indeed, scanning electron microscope (SEM) is needed to observe these highly variable structures, particularly striae. Currently, there are 98 taxonomically accepted *Planothidium* species and infraspecific names (Guiry & Guiry 2019), 24 of which are listed and 11 pictured in The Diatoms of North America (Spaulding *et al.* 2008). The challenging taxonomy of the genus has been significantly improved lately by reinvestigating type materials with SEM and a more precise defining of new species from freshwaters worldwide (i.e., Novis *et al.* 2012, Potapova 2012, Alvarez-Blanco & Blanco 2013, Blanco *et al.* 2013, Van de Vijver *et al.* 2013, 2018, Wetzel *et al.* 2013, 2014, Bąk & Lange-Bertalot 2014, Jahn *et al.* 2017).

The aim of the present report is to describe a new species of *Planothidium* collected from two rivers in California, and to document size diminution and valve morphology using light and scanning electron microscopy. The newly described species is compared with morphologically related *Planothidium* taxa.

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Materials and methods

The diatom samples for this study were obtained from two non-wadeable rivers in California sampled in 2018 as part of the United States Environmental Protection Agency`s National Rivers and Streams Assessment Program (NRSA). Quantitative composite benthic diatom samples were collected from whatever substrata were present (e.g., cobble, silt/sand, gravel, bedrock, wood) at 11 objectively selected locations, but not deeper than 0.5 m, along with hydrological parameters (USEPA 2017). The attached periphyton was collected from the removable substratum by brushing with a stiff-bristled toothbrush for 30 seconds. For light microscopy (LM) and SEM observations, the preserved diatom samples were oxidized with 30% hydrogen peroxide, and heated on a hot plate for three hours to remove the organic matter (Stancheva *et al.* 2015). Afterwards, the diatoms were rinsed repeatedly with distilled water to neutrality and mounted in Naphrax®. *Planothidium* specimens were surveyed by observing at least 25 SV and 25 RV valves. Light microscopic analysis and imaging of the specimens were performed using an Olympus® BX41 Photomicroscope (Olympus America Inc., Center Valley, Pennsylvania) with differential interference contrast optics and an Olympus® SC30 Digital Camera. Scanning electron microscopy was done on cleaned valves air dried onto cover glasses, attached to aluminum stubs, sputter-coated with 20 nm of iridium. Diatom valves were examined in high vacuum mode using a Zeiss SIGMA 500 (Carl Zeiss Microscopy, Thornwood, NY, USA) at the Nano3 Facility at the University of California, San Diego (La Jolla, CA, USA). The main taxonomic references used were Patrick & Reimer 1966, Lange-Bertalot & Krammer 1989, Krammer & Lange-Bertalot 1991, Cantonati *et al.* 2017, Jahn *et al.* 2017.

Results

Planothidium sheathii Stancheva, *sp. nov.* (Figs 1–29)
Http://phycobank.org/100928

Description

Valve outline and dimensions: Valves broadly lanceolate to elliptic with obtusely rounded, never protracted apices, 6.3–8.8 µm wide, 14–29 µm long (50 valves were measured). Striae 11–13 in 10 µm on SV, 11–12 in 10 µm on RV measured at the central part of the valve face between valve sternum and margins, on SV measured at the valve part opposite to the unilateral expansion.

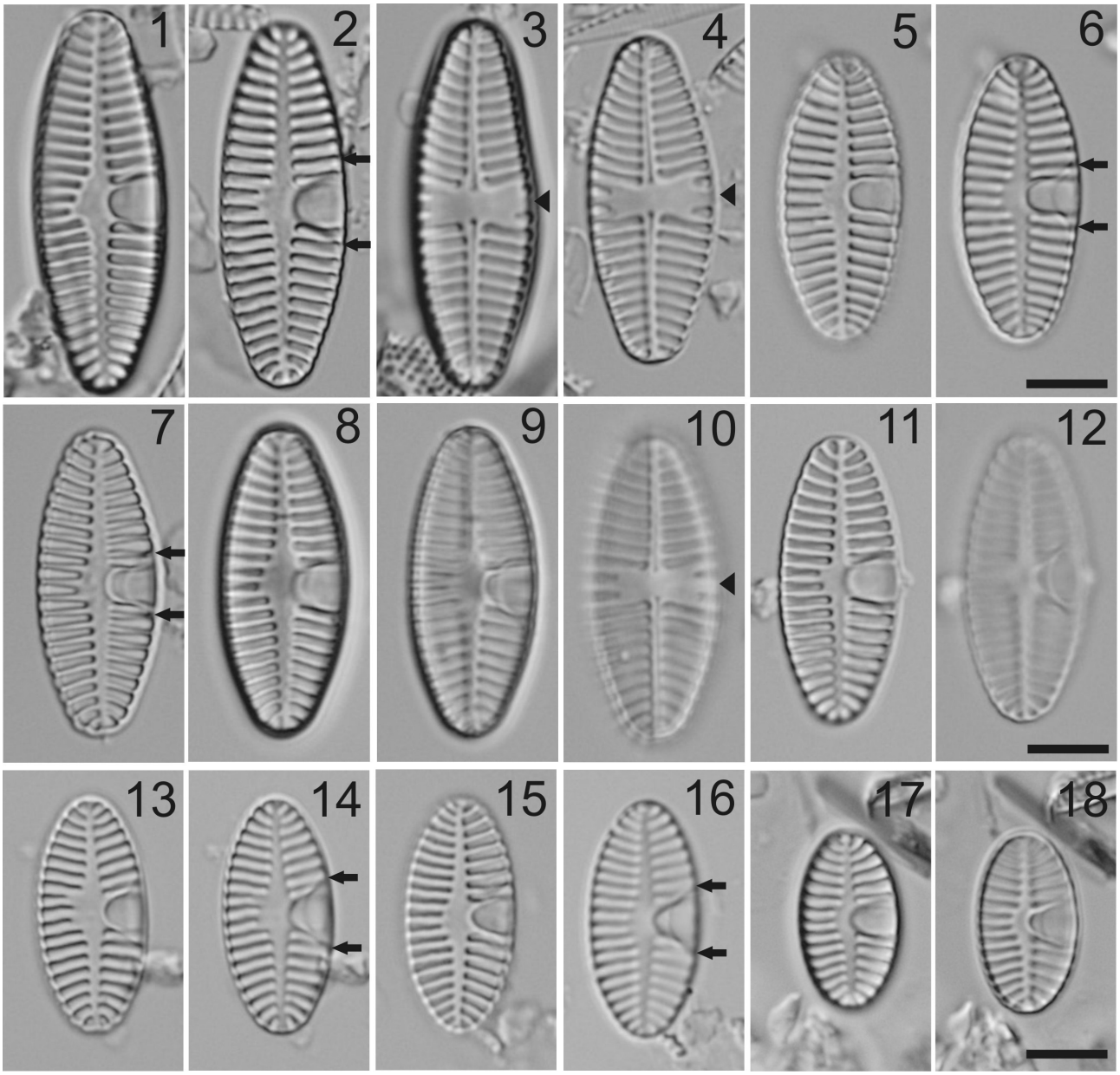
LM of SV (Figs 1, 2, 5–9, 11–18): Axial area narrow lanceolate, slightly expanded in the middle. Central area with a prominent cavum, which is oblong with parallel sides (Figs 5, 7, 11) to slightly V-shaped (Figs 2, 8, 13, 15, 17) when viewed at valve surface focal plane. Internally, the cavum is covered by a broad hood. At a deeper focal plane, the cavum hood aperture is visible as curved line (Figs 6, 7, 9, 12), which crosses the cavum and neighboring striae, and endings near the mantle at virgae (Figs 6, 7, 14, 16). Striae parallel at valve middle to weakly radiate towards apices.

LM of RV (Figs 3, 4, 10): Axial area very narrow linear. Central area transapically rectangular, bordered by two shortened striae on both sides (rarely three on one side). The short central striae more distant on one side. Striae parallel at valve middle to radiate towards apices. Raphe branches straight with expanded, drop-like proximal raphe endings.

SEM of SV (Figs 19–21, 26–28): Externally, striae multiseriate, composed of five to six rows of small rounded same sized areolae, interrupted at juncture of valve and valve mantle (Figs 19, 20, 28). Valve mantle shallow, with regular groups of 8–13 rounded areolae offset from the valve striae, each bordered by two elongated areolae in oblique position to each areola group (Fig. 28). Axial and central areas are irregular depressions on valve surface (Figs 19, 20). Internally, the large cavum is covered with broad hood (Figs 21, 26, 27). The hood aperture flares out at the valve margin with open borders fuse with the neighboring virgae (Figs 26, 27). Striae sunken between raised virgae (Fig. 21).

SEM of RV (Figs 22–25, 29): Externally, striae multiseriate, composed of four to six rows of small rounded same sized areolae on valve face (Figs 22, 23), with groups of seven to nine rounded areolae on mantle offset from the valve striae (Fig. 29). Two or three central striae shortened, parallel and more distant on secondary side of the valve (Figs 22, 23). Proximal raphe endings expanded, distal raphe endings curved terminating on valve face by a short fissure extending just beyond apical striae, unilaterally deflected (Figs 22, 23). Internally, proximal raphe ends not widened, end directly opposite each other on inner valve surface (Fig. 24). Helictoglossae small (Fig. 24).

Type locality USA, California, San Joaquin River, 36.7790352, -120.233343, collector *Gary Ichikawa,* July 09, 2018.



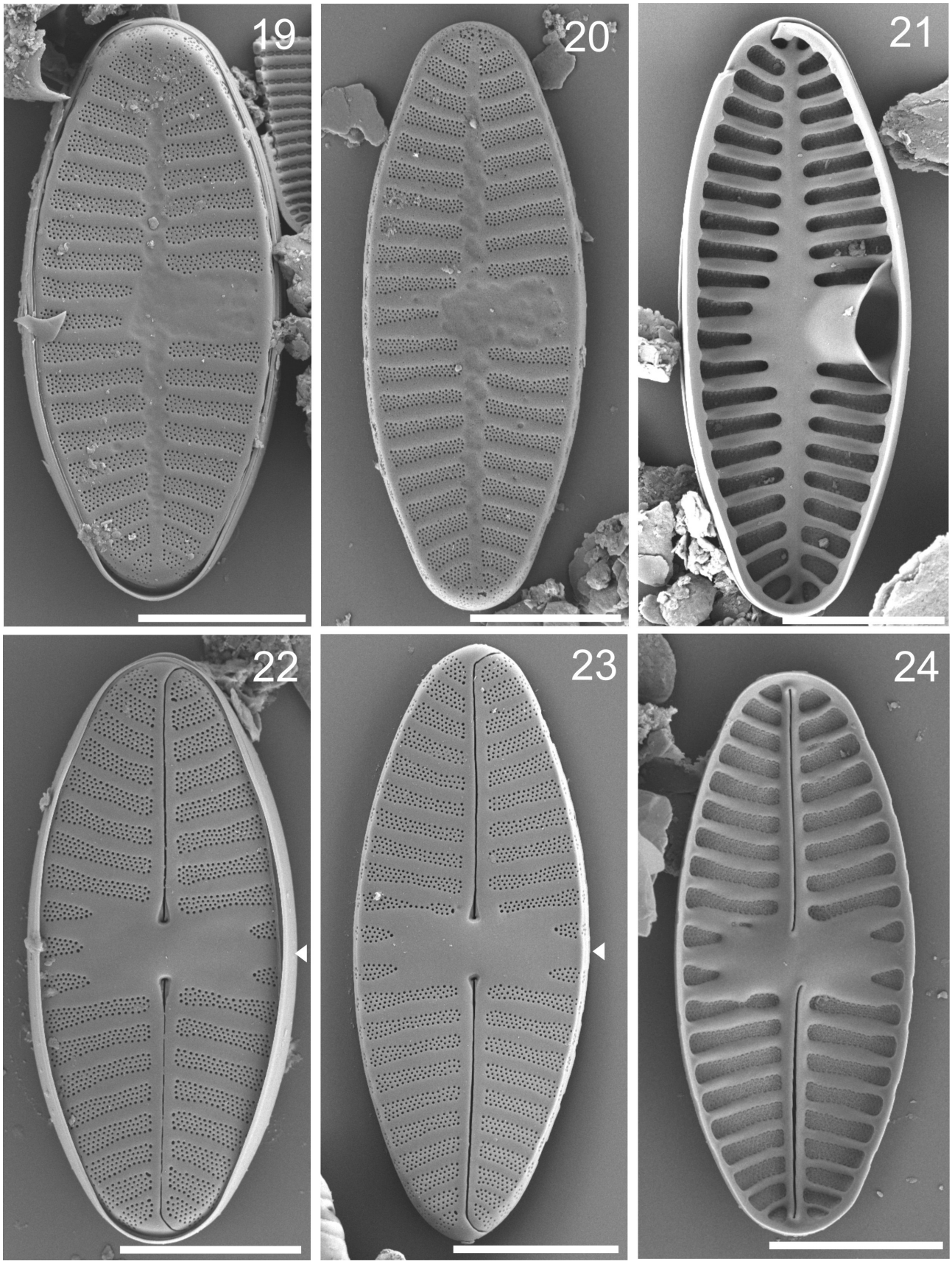
FIGURES 1–18. *Planothidium sheathii* LM views. Figs 1, 2, 5–9, 11–18 are SV. Figs 3, 4, 10 are RV. The same valve is presented at different focal plane to show the cavum hood aperture on figures 5 and 6, 11 and 12, 13 and 14, 15 and 16, 17 and 18. Figs 8, 9 and 10 are the same frustule. Arrows show cavum hood aperture borders fusion with neighboring virga on SV, arrowheads show more distant central shortened striae on RV. Figs 1–16 from the type locality San Joaquin River. Fig. 7 represents the holotype. Figs 17, 18 from Merced River, California, USA. All scale bars: 5 µm.

Holotype: Slide GC 65325 at The Academy of Natural Sciences of Drexel University Diatom Herbarium, Philadelphia, USA. Holotype specimen is illustrated on Fig. 7.

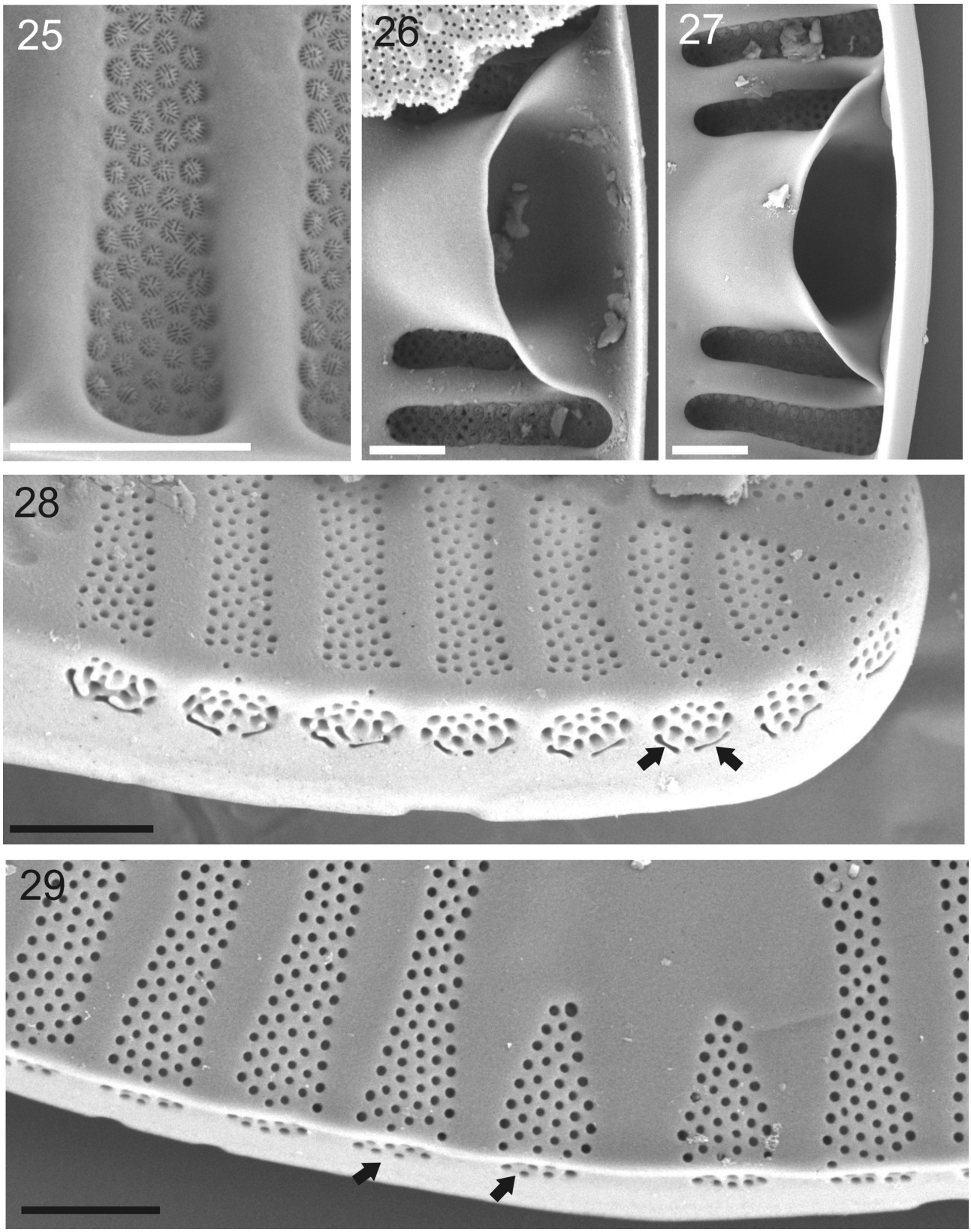
Isotype: Slide RS 056 and cleaned material RS 057, CSUSM, USA.

Etymology: The species is named in honor of Dr. Robert G. Sheath in recognition of his contributions to the stream algal bioassessment in California.

Distribution and ecological notes: Recorded in the type locality and in Merced River, California (site CA10030, 37.39213, -120.7909, July 16, 2018) with low relative abundance and within pH range of 7.5–7.7, specific conductivity 38–78 µS cm^{-1}, and dissolved oxygen 88.2–97.9 mg L^{-1}.



FIGURES 19–24. *Planothidium sheathii* SEM views. Figs 19, 20. External SV view. Fig. 21. Internal SV view with cavum. Figs 22, 23. External RV view. Note the more distant central shortened striae on secondary side (arrowheads). Fig. 24. Internal RV view. All figures from the type locality San Joaquin River, California, USA. All scale bars: 5 µm.



FIGURES 25–29. *Planothidium sheathii* SEM views. Fig. 25. Internal view of RV areolae. Figs 26, 27. Detail of cavum aperture. Note the boarders of the aperture attached to neighbor virgae. Fig. 28. External view of SV periphery and mantle. Striae 5–6 seriate, interrupted at valve mantle junction. Note the separated groups of 8–13 rounded areolae, bordered by 2 elongated areolae on mantle in oblique position to each areola group (arrows). Fig. 29. External view of RV periphery and mantle. Striae 5–6 seriate, interrupted at valve mantle junction. Note the separated groups of 7 rounded areolae on mantle (arrows). All figures from the type locality San Joaquin River, California, USA. All scale bars: 1 μm.

TABLE 1. Comparison of *Planothidum sheathii* (50 valves were measured) with morphologically related species with cavum, covered by a broad hood that flares out at the valve margin.

Planothidium taxon	Valve outline	Apices	Width (µm)	Length (µm)	Striae/10 µm	Areola rows/ stria	Areola groups on mantle	Axial area	Striae/ 10 µm	Areola rows/ stria	Areola groups on mantle	Central shortened striae	Central area	Reference
	SV/RV						SV				RV			
sheathii	broadly lanceolate to elliptic	obtusely rounded, never protracted	6.3–8.8	14–29	11–13	5–6 same size	8–13 rounded 2 elongated	narrow lanceolate, slightly expanded in the middle	11–12	4–6	7–9 rounded	2–3 parallel	transversely rectangular	This study
bagualensis	broadly lanceolate to elliptic	obtusely rounded, never protracted	6.7–9.5	15.5–32.5	11–13	3–4 middle smaller	4–7 rounded	very narrow linear	11–13	3–6	0–3 rounded	3–6 slightly radiate	round to rhombic	Wetzel & Ector 2014
biporomum	lanceolate	capitate to subcapitate	6–7	13–26	13–15	2–3 middle smaller	1 rounded 2 elongated	narrow linear, slightly expanded in the middle	13–15	3–4	1–3 rounded	2–3 parallel	rectangular to rounded	Wetzel *et al.* 2013
incuriatum	lanceolate to elliptic-lanceolate	rostrate, protracted	6.4–7	18–25.4	13–15	2–3 middle smaller	1 rounded 2 elongated	narrow linear	13–15	3–4	2 rounded	2–3 parallel	rectangular to slightly rounded	Wetzel *et al.* 2013
infrequens	elliptic-lanceolate	protracted to subrostrate	6–7	14–18	11–13	NA	NA	very narrow linear	11–13	4	NA	2–3 parallel	transversely elliptical	Rumrich *et al.* 2000
comperei	elliptic-lanceolate	subrostrate, slightly protracted	7.7–9.0	17–23	11–13	3–4 middle smaller	3–5 rounded	narrow linear	10–11	5–7	2–4 rounded	2–3 radiate	irregularly rounded	N'Guessan *et al.* 2014
abbreviatum	elliptic-lanceolate	Subrostrate	6.1–8.5	11–18	11–12	5–6 same size	NA	wide rhombic	11–12	5–6	NA	2 slightly radiate	transversely rectangular to elliptic	Potapova 2011, 2012
joursacense	elliptic to elliptic-lanceolate	broadly rounded, never protracted	5.5–7.3	9.6–15.6	17–19	NA	NA	Rhombic	17–19	NA	NA	1–2 slightly radiate	small rounded	Bishop & Potapova 2017

Discussion

Recent phylogenetic analysis of *Planothidium* taxa from freshwater habitats worldwide demonstrated the taxonomic importance of the presence of a sinus or cavum, its form and size, the number of striae areola rows and their size on both the SV and RV, and merged and/or offset areolae on the valve mantle (Jahn *et al.* 2017). *Planothidium sheathii* is characterized by unique combination of valve features, as follows: 1) cavum with broad hood aperture that flares out at the valve margin with open borders fused with the neighboring virgae on SV; 2) multiseriate striae, composed of five to six rows same sized areolae on both valves, interrupted at valve mantle junction, and 3) areolae groups on valve mantle, with rounded areolae on RV, and rounded and elongated areolae on SV.

A characteristic and consistent feature of *P. sheathii* is the SV with horseshoe-shaped central area that is composed of a cavum covered internally by a broad hood that flares out at the valve margin (Figs 21, 26, 27). This feature can be easily seen with LM at proper focal plane (Figs 6, 7, 9, 12, 14, 16) and can be used to distinguish the SV of *P. sheathii* from *P. lanceolatum* (Brébisson ex Kützing) Lange-Bertalot (1999: 287) (which bears a sinus) and *P. frequentissimum* (Lange-Bertalot) Lange-Bertalot (1999: 282) (which bears a cavum with narrow hood). Regardless the considerable morphological variability of the cavum structure within *P. frequentissimum* species complex, a broad hood aperture that fuse with neighboring virgae had not been documented, neither in var. *magnum* (F. Straub) Lange-Bertalot (Straub 1985) nor in newly separated species by Jahn *et al.* (2017). Furthermore, taxa of the *P. frequentissimum sensu lato* clade (Jahn *et al.* 2017) have multiseriate striae with three to four rows of similar sized areolae on both valves, merged areolae on the SV mantle, and none on RV mantle. Indeed, the RVs of *P. sheathii* are hard to distinguish in LM from some RVs of *P. lanceolatum* and *P. frequentissimum*, based only on slight difference in striae thickness.

Specimens similar to *P. sheathii* in terms of gross valve morphology were illustrated with LM images by Krammer & Lange-Bertalot (1991) as *Achnathes lanceolata* ssp. *frequentissima* Lange-Bertalot from Firehole River, USA (Tafel 44: Figs 15–23) and Euphrat, Syrien (Tafel 44: Figs 32, 33), and by Peeters & Ector (2018) from rivers in France as *Planothiduim* sp. 1. However, the lack of SEM observations of valve ultrastructure precludes sufficient comparison with the newly described species from California.

Several *Planothidum* taxa with broad cavum hood aperture extending to neighboring virga, which share some morphological features with *P. sheathii* are discussed below (Table 1). *Planothidium sheathii* should be compared with two morphologically similar species, described from the Savannah River, Georgia, USA (i.e. *P. abbreviatum* (Reimer) Potapova (2012: 40) and *P. biporomum* (M. H. Hohn & Hellernam) Lange-Bertalot (1999: 275). The former species has valves with different elliptic-lanceolate outline, subrostrate apices, wider rhomboidal axial area and narrower hood aperture on SV (Potapova 2011, 2012), and the latter has lanceolate valve with capitate to subcapitate apices, and two to three areola rows on SV with smaller middle areolae (Potapova 2010, Wetzel *et al.* 2013). *P. incuriatum* C.E. Wetzel, Van de Vijver & Ector (2013: 45), a common freshwater species in Europe and South America (Wetzel *el al*. 2013), is very similar to *P. biporomum* in terms of valve outline and striae with 2–3 areola rows, and its lanceolate valves with rostrate, protracted apices are easily distinguished from *P. sheathii* in LM. An interesting ultrastructural feature, common for *P. sheathii, P. biporomum* and *P. incuriatum* is the presence of two elongated areolae in oblique position to each areola group offset on the SV mantle (Wetzel *el al*. 2013, Figs 44, 60, 61 and Fig. 28 this study).

Planothidium infrequens Lange-Bertalot & Rumrich (2000: 212), described from acidic freshwaters in Chile (Rumrich *et al.* 200), and *P. comperei* C.E. Wetzel, K.R. N'Guessan & J. Tison-Rosebery (2014: 456) known from West African rivers (N'Guessan *et al.* 2014), both have elliptic-lanceolate valves with protracted to subrostrate apices. Furthermore, *P. comperei* differs by SV striae, composed of three to four areola rows with smaller middle areolae and RV with larger rounded central area, bordered by two to three shortened radiate striae with conical shape. *Planothidium joursacense* (Héribaud) Lange-Bertalot (1999: 277), which has been recorded in rivers and lakes the USA (Bishop & Potapova 2017) and *P. bagualensis* C.E. Wetzel & Ector (2014: 203), described from southern Brazilian rivers (Wetzel & Ector 2014), have elliptical or elliptic-lanceolate valves with rounded apices, but the former has smaller central area on RV bordered by only a slightly shortened single central stria on both sides (Bishop & Potapova 2017), and the latter has larger round to rhombic central area on RV, bordered by three shortened slightly radiate striae (Wetzel & Ector 2014). In addition, the striae density is higher in *P. joursacense,* compared to *P. sheathii.* None of these species fit the morphological description of this new species from California.

Planothidium sheathii is recorded as rare in two rivers in California. It was not previously observed in samples collected from wadeable streams across the state by SWAMP. So far, little is known about the ecological preferences of *P. sheathii*. The examination of the preserved uncleaned sample from the type locality of *P. sheathii* showed filamentous green algae *Oedogonium* Link ex Hirn (1900: 1), *Spirogyra*, *Mougeotia* C. Agardh (1824: 83), and N_2-

fixing cyanobacteria *Calothrix* C.Agardh ex Bornet & Flahault (1886: 345), *Anabaena* Bory ex Bornet & Flahault (1886: 233), and *Cylindrospermum* Kützing ex Bornet & Flahault (1886: 249). However, *P. sheathii* cells were associated with inorganic particles rather than with filamentous algae. It is worth mentioning that *P. sheathii* coexists with other diatoms, described from North America, such as *Gomphonema caperatum* Ponader & Potapova (2017: 60) and *Pseudostaurosiropsis connecticutensis* Morales (2001: 117), as well as a few other hitherto undescribed fragilarioid and naviculoid diatoms.

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