



## New Freshwater Gomphonemoid Diatoms from Streams in the Sierra Nevada Mountains, California, USA

ROSALINA STANCHEVA<sup>1\*</sup>, ROBERT G. SHEATH<sup>1</sup> & J. PATRICK KOCIOLEK<sup>2,3</sup>

<sup>1</sup>Department of Biological Sciences, California State University San Marcos, San Marcos, California 92096, USA.

<sup>2</sup>Museum of Natural History and Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309

<sup>3</sup>University of Michigan Biological Station, 9009 Biological Road, Pellston, MI 49769

\*Corresponding author (E-mail: [rhrstov@csusm.edu](mailto:rhrstov@csusm.edu))

### Abstract

Light and scanning electron microscopic observations of three new species of freshwater gomphonemoid diatoms are presented from mountain streams in California: *Gomphonema californicum* sp. nov., *Gomphonema sierrianum* sp. nov., and *Gomphoneis oreophila* sp. nov. Two of the species have morphological novelties related to the structure of the areolae, which are discussed in comparison with similar taxa. *Gomphonema californicum* shows a variety of areolar morphologies (i.e. lineola-like, c-shaped, and circular) within a single uniseriate stria. Unique to *Gomphoneis oreophila*, a member of the *Herculeana* subgroup, are the uniform areolae with circular openings without apparent occlusions. All of these species are distributed in remote streams of the Sierra Nevada Mountains of California, suggesting there are still new species to be found in this biodiversity-rich region.

**Keywords:** *Gomphonema*, *Gomphoneis*, Bacillariophyta, diatom, endemic, streams, California, new species, electron microscopy

### Introduction

The state of California is known as a biodiversity hotspot (Myers *et al.* 1999), in large part due to its unique higher plant flora (the California Floristic Province, Burge *et al.* 2016), but reflected in other biodiversity elements as well (Calsbeek *et al.* 2003). The taxonomic composition of the freshwater algae in California, particularly from running waters, is relatively unknown. Patrick & Reimer (1966, 1975) considered species distributed in California as part of their work on the diatom flora of the USA. 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), *Halumphora* (Cleve) Levkov (2009: 165) and *Rhoicosphenia* Grunow (1860: 511) (Stepanek & Kociolek 2013, Thomas & Kociolek 2015) and to the green algal genera *Spirogyra* Link (in Nees 1820: 5) and *Zygnema* C.A. Agardh (1817: 98) (Stancheva *et al.* 2012, 2013). Kociolek (2012a) created an identification guide to 450 freshwater diatom taxa from the southern California Bight, and Kociolek (2005) lists 13 species described from California. A master checklist of diatoms recognized from water quality studies in California (Kociolek 2012b) lists a total of 1275 taxa in 117 genera, of which 78 taxa are from *Gomphonema* Ehrenberg (1832: 87) and 24 are of *Gomphoneis* Cleve (1894: 73).

According to Kociolek & Kingston (1999), little attention has been paid to the endemism and regionalism in the U.S. diatom flora, which is apparent among some gomphonemoid diatoms restricted to the western states. It is the case that some endemic *Gomphonema* species are known only from the Sierra Nevada Mountains (Kociolek & Kingston 1999 and references therein) and *Gomphoneis mammilla* (Ehrenb.) Cleve (1894: 73) has been found only in California and Oregon (Kociolek & Rosen 1984). Other endemic *Gomphoneis* species have been recorded by Kociolek and Stoermer (1986, 1988).

After relatively modest attention in the mid/late 19<sup>th</sup> and early 20<sup>th</sup> centuries (Ehrenberg 1849, Grunow in Van Heurck 1880, Cleve 1894), and two papers by Sovereign (1958, 1963), the freshwater diatom flora of the western United States has begun to receive significant attention. Bahls and co-workers (Bahls 2011a, b; 2012a, b; 2013; 2014a, b; Bahls & Potapova 2015; Bahls *et al.* 2013) have published a number of taxonomic treatments on the diatoms of the west, particularly from Montana and the Pacific Northwest. Kociolek and colleagues have documented species from across the west (Spaulding *et al.* 2002; Thomas & Kociolek 2008; Kociolek *et al.* 2014, 2015; Ress *et al.* 2016).

**TABLE 1.** A summary of the environmental conditions in the stream localities of the new to science *Gomphonema* and *Gomphonopsis* species. Abbreviations: (\*) type locality of diatom species described here, (N/A) not applied, (BDL) below detection limits.

Station code	507DCABSF*	507SPIUBL	521BTC303	518RCNAPC*	533SPILSA*	536SPILJW	514PCASMR
New <i>Gomphonema</i> species	<i>G. californicum</i>	<i>G. californicum</i>	<i>G. californicum</i>	<i>G. californicum</i>	<i>G. sierruanum</i>	<i>G. sierritanum</i>	<i>G. sierritanum</i>
New <i>Gomphonopsis</i> species	N/A	N/A	N/A	<i>G. oreophila</i>	N/A	N/A	N/A
Sampling date	31 Aug 2015	1 Sep 2015	1 June 2015	1 Jul 2015	9 Oct 2015	9 Sept 2015	3 Sept 2015
Creek name	Digger Creek*	Bailey Creek	Butte Creek	Rice Creek*	San Antonio Creek*	Jawbone Creek	Pilot Creek
Geographic coordinates	40.44284°N 121.72501°W	40.498°N 121.638°W	40.10343°N 121.46482°W	40.3999°N 121.44109°W	38.2758°N 120.3361°W	37.95265°N 119.99081°W	38.89600°N 120.54980°W
Elevation (m a.s.l.)	1133	1523	1661	1902	1226	1388	1400
AFDM (g m <sup>2</sup> )	6.03	2.45	4.94	N/A	6.70	4.80	6.95
Alkalinity as CaCO <sub>3</sub> (mg L <sup>-1</sup> )	35	32	76	62	30	25	15
Ammonia as N (mg L <sup>-1</sup> )	0.0076	0.0046	BDL	0.0188	BDL	0.111	0.0108
Chloride (mg L <sup>-1</sup> )	0.36	0.18	0.36	0.17	1.16	0.62	2.66
Chlorophyll a (mg m <sup>2</sup> )	25.1	25.5	37.3	20.8	19.4	7.47	17.8
Dissolved Organic Carbon (mg L <sup>-1</sup> )	1.54	1.60	N/A	N/A	1.50	1.22	1.30
Hardness as CaCO <sub>3</sub> (mg L <sup>-1</sup> )	16.2	25.1	48.4	26.6	22.5	21.0	14.5
Nitrate + Nitrite as N (mg L <sup>-1</sup> )	0.0078	0.0233	0.0153	N/A	0.0385	0.143	0.0375
Nitrogen, Total (mg L <sup>-1</sup> )	0.105	0.104	N/A	0.0421	0.116	0.104	0.0780
OrthoPhosphate as P (mg L <sup>-1</sup> )	0.0683	0.0418	0.0418	0.068	0.0248	0.0169	0.0093
Oxygen Dissolved (mg L <sup>-1</sup> )	8.71	9.7	11.11	9.2	8.25	7.75	8.82
pH	8.3	8.1	8.5	8.3	8.5	8.3	7.5
Phosphorus as P (mg L <sup>-1</sup> )	0.0112	0.0203	0.0218	0.051	0.0151	BDL	BDL
Silica as SiO <sub>2</sub> (mg L <sup>-1</sup> )	133	36.5	28.5	48.8	18.5	19.5	12.9
Specific Conductivity (µS cm <sup>-1</sup> )	26	78.1	107.6	74.8	53.3	52.2	24.1
Sulfate (mg L <sup>-1</sup> )	0.28	14.2	0.39	0.88	1.19	0.91	6.46
Temperature (C°)	11.6	12.3	8.4	11.4	11.7	15.5	12.1
Total Suspended Solids (mg L <sup>-1</sup> )	0.8	1.0	0.3	0.5	0.3	0.8	0.4
Turbidity (NTU)	0.36	0.56	93.3	0.49	0.38	0.97	0.16

The purpose of the present report is to describe two new species of *Gomphonema* and one new species of *Gomphoneis*, collected during water quality analyses of streams in the Sierra Nevada Mountains in California. We document size diminution and valve morphology using light and scanning electron microscopy, compare our species with other, similar taxa and discuss the systematic affinities of these three new species.

## Material and methods

The new species described below were found in seven samples from streams in the Sierra Nevada Mountain, California, provided by SWAMP. The diatom samples and environmental variables were collected quantitatively in 2015 using the multihabitat sampling protocol (Fetscher *et al.* 2009). A summary of the stream locations and environmental variables is provided in Table 1.

For LM and SEM observations, the preserved diatom samples were cleaned by the hydrogen peroxide method, mounted in Naphrax®, and 600 diatom valves were identified to species and counted (for details see Stancheva *et al.* 2015). LM analysis and imaging of the specimens was performed using an Olympus® BX41 Photomicroscope (Olympus America Inc., Center Valley, Pennsylvania) with differential interference contrast optics and Olympus® SC30 Digital Camera attached. SEM was performed with cleaned specimens air dried onto cover glasses, attached to aluminum stubs, sputter-coated with 5 nm of iridium or platinum and examined in high vacuum mode using a Zeiss SIGMA 500 (Carl Zeiss Microscopy, Thornwood, NY, USA), FEI Quanta 600 FEG SEM (FEI; North America NanoPort, Hillsboro, OR, USA) and by Hitachi S-2700 SEM (Hitachi High Technologies America Inc., Pleasanton, CA, USA) with an accelerating voltage of 5 k. SEM was performed at the Nano3 Facility at the University of California, San Diego, and the San Diego State University Electron Microscopy Facility. Holotype slides and material are deposited at the University Herbarium at University of California, Berkeley, USA and isotype slides and type material are housed by the author Rosalina Stancheva at the California State University San Marcos (CSUSM).

## Results

### *Gomphonema californicum* Stancheva & Kocielek, *sp. nov.* (Figs 1–27)

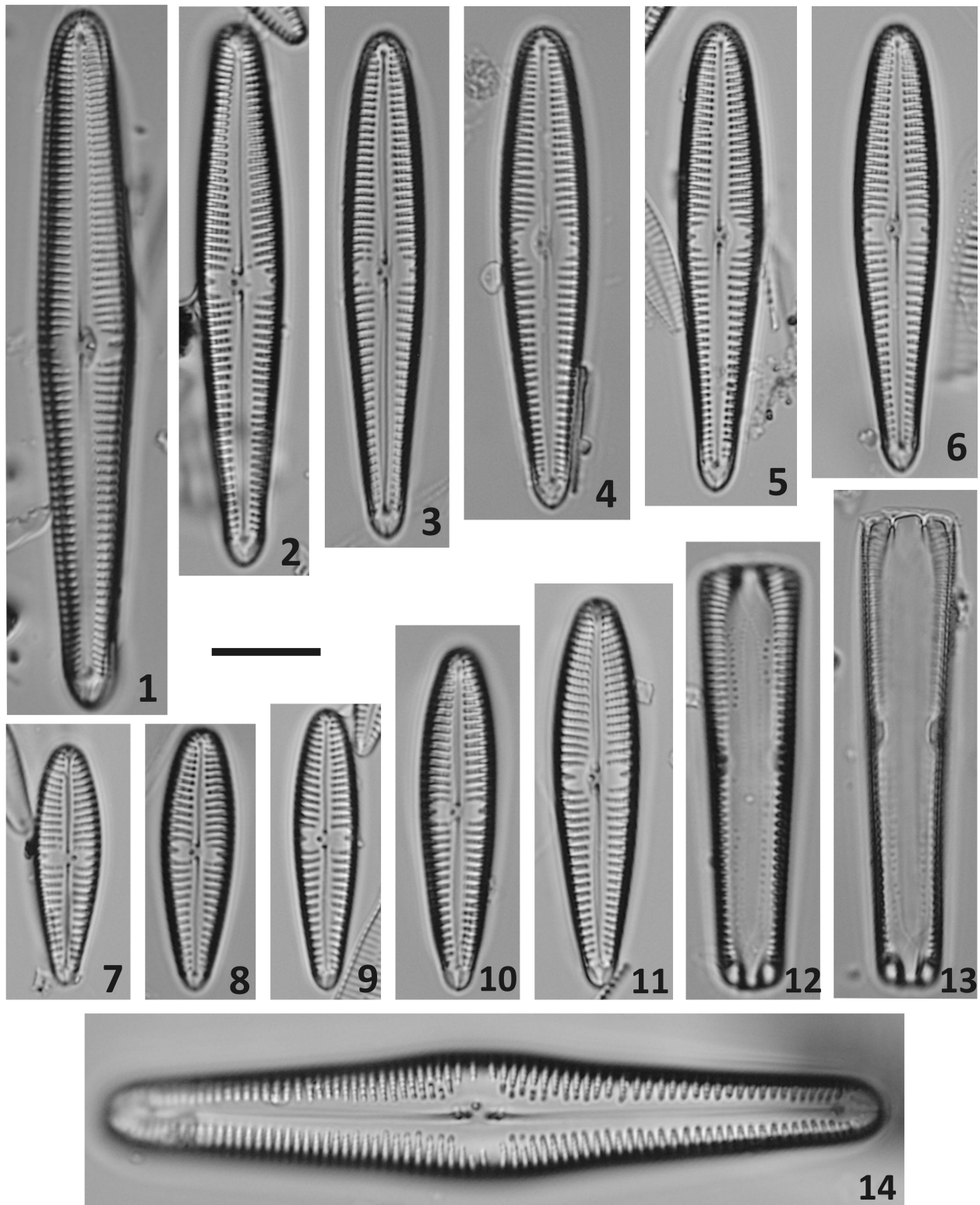
#### LM observations

Valves are lanceolate-clavate with rounded head pole and narrower foot pole. Cells are 5.6–8.3 µm wide, 22–67 µm long (Figs 1–13); initial cell 10 µm wide, 71 µm long (Fig. 14). Striae are slightly radiate at the center of the valve 10–13 in 10 µm, becoming denser up to 15 in 10 µm near both apices and strongly radiate toward the foot pole. Striae composed of areolae, which are coarser and distinguishable near the head pole, 28–32 in 10 µm. Striae sometimes are interrupted, particularly around the central area (Figs 2 and 3). Axial area narrow, lanceolate. One, occasionally two median striae on both sides of the central area are shorter than the others (Figs 4–6). Central area is rectangular transversely extended, delimited by two or three curved striae (Figs 2, 6, 8, 10), rarely elliptical (Fig. 4). One isolated stigma is positioned close to the proximal raphe ends. Sometimes one or a few small indistinct depressions are present in the central area, not easily resolvable with LM (Fig. 2). The raphe is lateral, slightly undulate. Frustules are slightly cuneate in girdle view with single or double irregular rows of puncta on each mantle, in some specimens interrupted near the middle (Figs 12 and 13).

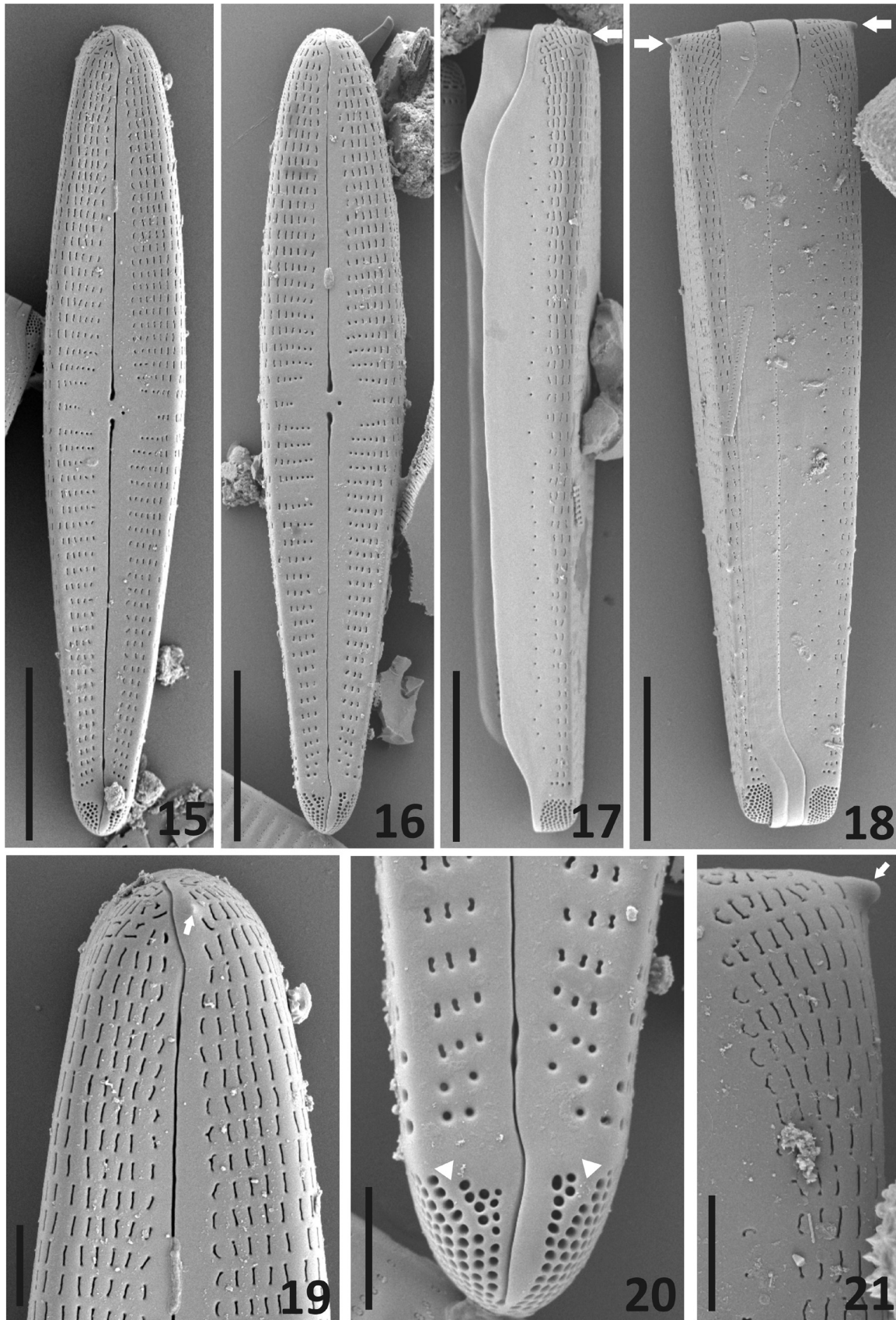
#### SEM observations

Externally, striae are uniseriate, composed of slit-like (lineola-like) areolae, which extend onto the mantle (Fig. 15–18). Areolae near the head pole (Fig. 19) are coarser and longer than the areolae close to the foot pole (Fig. 20). Areolae adjacent to the axial area are shorter and slightly undulate or c-shaped (Figs 15, 16, 19). Small areolae with circular external openings form: (1) the tips of the median short striae and the adjacent two or three striae (Figs 22, 23), and (2) the entire basal stria at the foot pole (Figs. 17, 18, 20). Apical pore field with porelli, separated from striae by a hyaline area is present at the foot pole (Figs 15–18, 20). The porelli are either regularly ordered within the apical pore fields (Fig. 15) or split by a narrow, unornamented area (Fig. 20). Distal raphe ends are deflected before the apices and extend into the mantle (Figs 19, 20). A small single apical spine is present at the head pole (Fig. 19), better observed in girdle view (Figs 17, 18, 21). In some specimens the spine may be very small or missing (Fig. 16), but in other

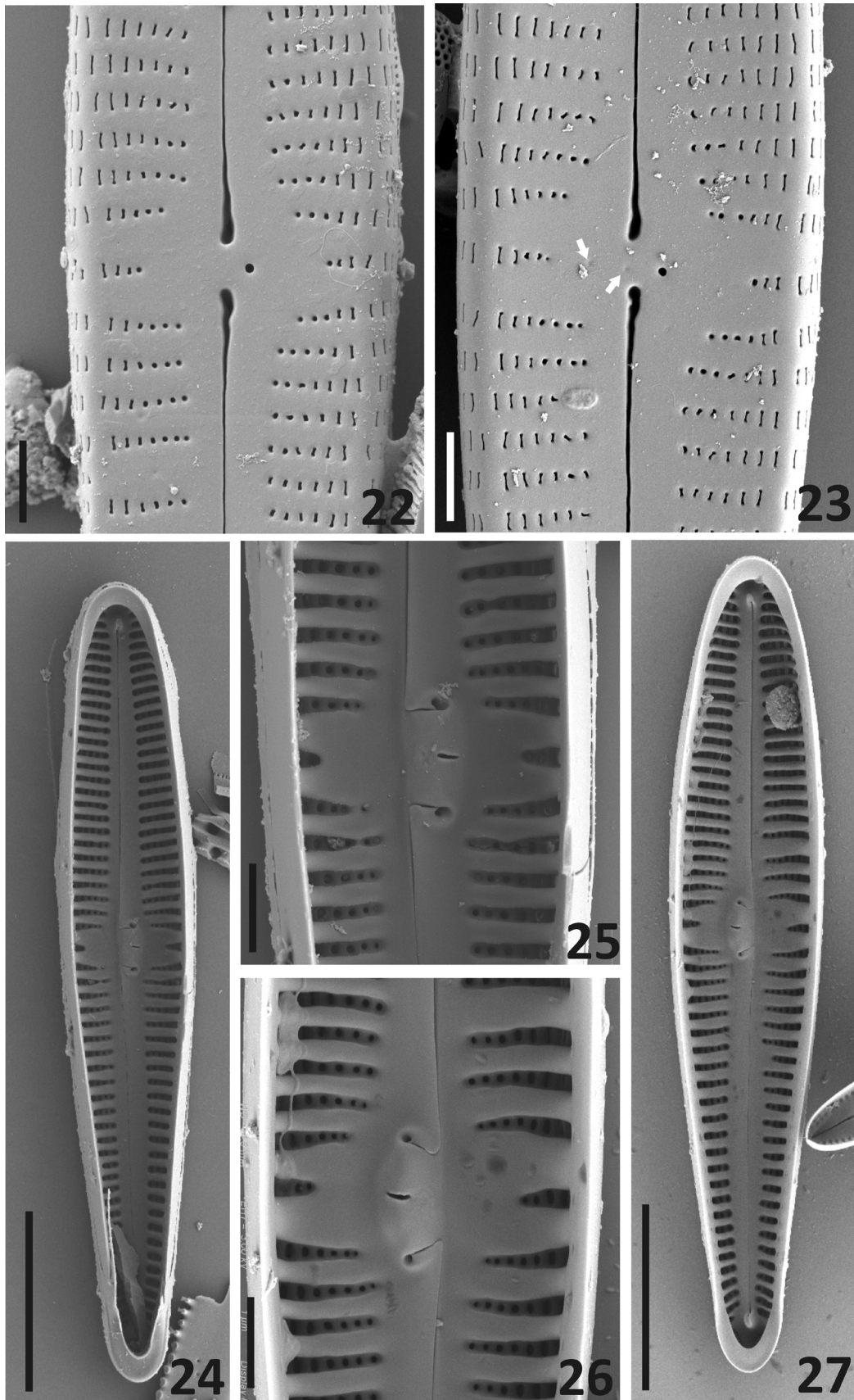
specimens is pronounced and visible with LM (Fig. 13) In the central area, one isolated stigma with a circular opening is positioned close to the dilated and drop-shaped proximal raphe ends (Figs 22, 23). Sometimes, one or two small indistinct depressions are visible near the stigma in the central area (Fig. 23). These do not extend through the valve and are not visible on the internal side of the valve. Each mantle with pores in an irregular single row, becoming double near the head pole, and more widely spaced near the middle (Figs 17, 18). Internally, the helictoglossa and pseudosepta are visible at both poles (Figs 24, 27). The central nodule is slightly raised, bearing a slit-like stigma opening and dilated and recurved proximal raphe ends (Figs 25, 26).



**FIGURES 1–14.** *Gomphonema californicum* Stancheva & Kociolek, *sp. nov.*, LM images. Figs 1, 4, 5, 6 (type material, site 507DCABSF). Figs 3, 10, 12, 13, 14 (site 518RCNAPC). Figs 2, 7, 8, 9, 11 (site 521BTC303). Figs 1–11 valve view. Figs 12 and 13 girdle view; note that the focus on Fig. 13 is on the apical spines. Fig. 14 initial valve. Scale bar: 10  $\mu$ m.



**FIGURES 15–21.** *Gomphonema californicum* Stancheva & Kociolek, *sp. nov.* type material from Digger Creek, California, USA (site 507DCABSF). SEM images. Figs 15, 16, 19, 20 external valve view. Figs 17, 18, 21 external girdle view. Figs 17, 18, 19, 21 show an apical spine at the head pole (white arrows). Fig. 20 illustrates narrow, unornamented area within the apical pore field at the foot pole (white arrowheads). Figs showing the same specimen are: 15 and 19; 16 and 20; 18 and 21. Scale bars: Figs 15–18 = 10 μm; Figs 19–21 = 2 μm.



**FIGURES 22–27.** *Gomphonema californicum* Stancheva & Kociolek, *sp. nov.* type material from Digger Creek, California, USA (site 507DCABSF). SEM images. Figs 22 and 23 show external valve view of the central area with isolated stigma and small depressions (white arrows), surrounded by short striae composed of areolae with variable shape. Figs 24–27 internal valve view. Figs 25 and 26 illustrate the interval view of the central nodule, bearing a slit-like stigma opening and dilated and recurved proximal raphe ends. Figs showing the same specimen are: 24 and 25; 26 and 27. Scale bars: Figs 24, 27 = 10  $\mu\text{m}$ ; Figs 22, 23, 25, 26 = 2  $\mu\text{m}$ .

**Type:**—USA. California: Digger Creek, Sierra Nevada Mts, 40.44284° N, 121.72501° W, *Nathan Mack, August 31, 2015* (holotype UC2050494 circled specimen on slide; isotype RS! 007, circled specimen on slide and material, CSUSM, USA).

**Etymology:**—The epithet refers to the USA state of California, where the species was first observed.

**Distribution and ecological notes:**—Found in four sites in the Sierra Nevada Mts, CA (elevation 1133 to 1902 m a.s.l.). Habitats are generally characterized by low nutrients (total nitrogen (TN) <0.1 mg L<sup>-1</sup>, total phosphorus (TP) <0.05 mg L<sup>-1</sup>), low conductivity (<107.6 μS cm<sup>-1</sup>), slightly alkaline (35–76 mg L<sup>-1</sup> CaCO<sub>3</sub>), and pH 8.1–8.5 (see Table 1).

**Comments:**—In terms of outline, and overall dimensions, this species resembles *G. dichotomum* Kützing (1833: 569) as illustrated by Reichardt & Lange-Bertalot (1991) and *G. grasmueckii* Lange-Bertalot & Reichardt (Lange-Bertalot 1993). Striae densities of both diatoms, however, are lower compare to the California specimens having striae that are 10–13 in 10 μm, and rarely up to 15 in 10 μm (Table 2). Furthermore, the shape of central area differs among three species, being significantly smaller in *G. grasmueckii*. Perhaps more importantly, ultrastructurally the three species are quite different. Reichardt & Lange-Bertalot (1991) show *G. dichotomum* to have large c-shaped areolae uniform throughout the entire valve, while in *G. californicum* the areolae are variable in shape. In *G. grasmueckii* areolae are lineola-lake and circular, but the striae are only partially uniseriate, becoming biseriate toward the mantle (Lange-Bertalot 1993). The presence of an apical spine at the head pole is an unique feature of *G. californicum*.

**TABLE 2.** Morphological characters of three new *Gomphonema* and *Gomphoneis* species from Californian streams and similar taxa.

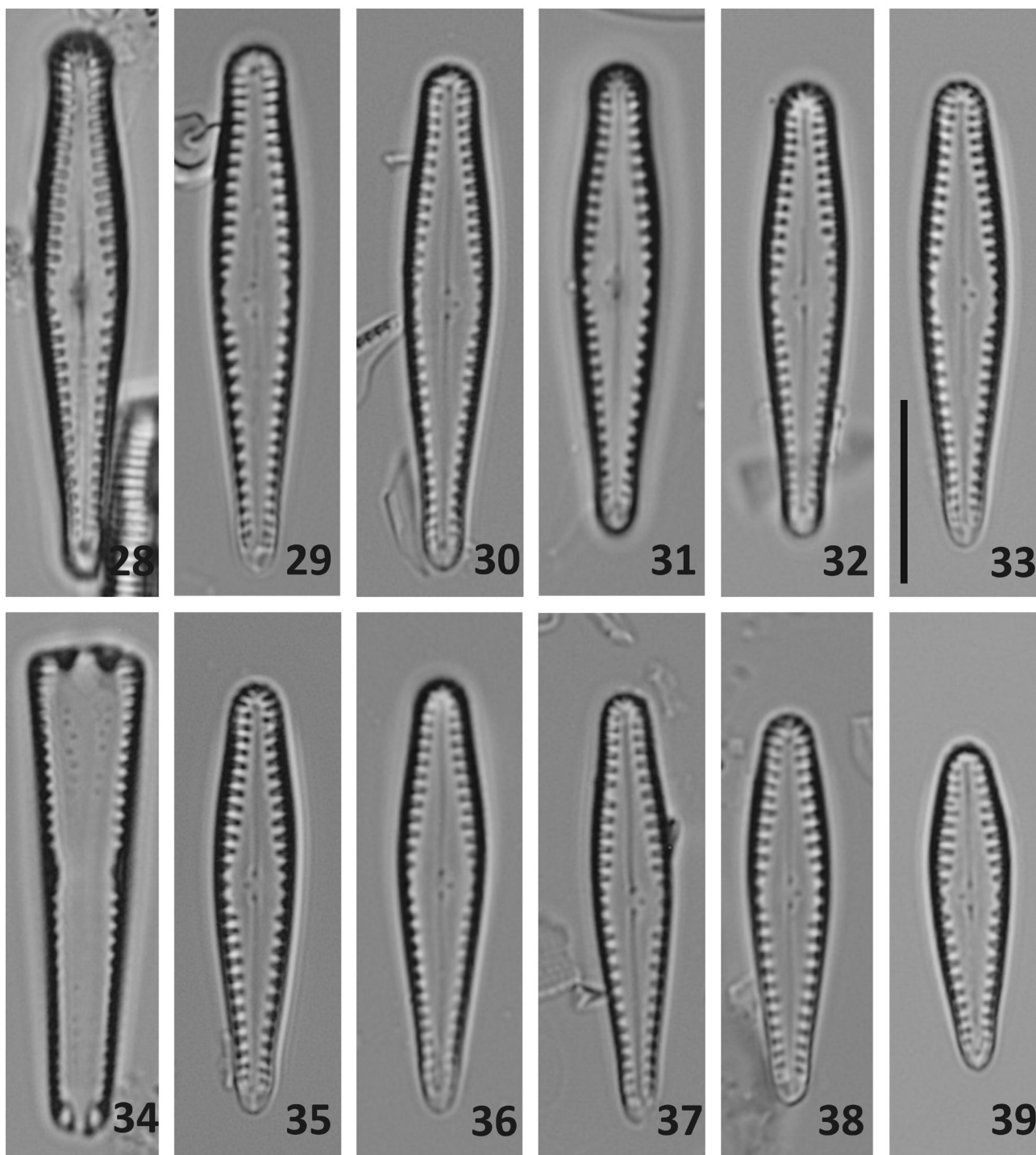
Taxon name	Valve width (μm)	Valve length (μm)	Striae number in 10 μm	Striae orientation	Areolae shape	Striae	Reference
<i>Gomphonema californicum</i> Stancheva & Kociolek	5.6–8.3	22–67	10–15	slightly radiate in the center; strongly radiate toward the foot pole	lineola-lake; c-shaped; circular	uniseriate	This study
<i>G. dichotomum</i> Kützing	6–8.3	29–66	9–11	radiate in the center; strongly radiate toward the foot pole	c-shaped	uniseriate	Reichardt & Lange-Bertalot 1991
<i>Gomphonema grasmueckii</i> Lange-Bertalot & Reichardt	5–7.5	20–50	9–12	radiate in the center; strongly radiate towards the poles	lineola-lake; circular	uniseriate becoming biseriate	Lange-Bertalot 1993
<i>Gomphonema sierrianum</i> Stancheva & Kociolek	4–5.2	17–31	11–14	slightly radiate in the center; strongly radiate toward the poles	c-shaped occluded	uniseriate	This study
<i>Gomphonema chinense</i> Liu et Kociolek	4.6–5.1	24.4–31.8	10–13	almost parallel in the center, slightly radiate towards the poles	c-shaped unoccluded	uniseriate	Liu et al. 2013
<i>Gomphoneis oreophila</i> Stancheva & Kociolek	10.3–16	51–105	10–12	nearly parallel to slightly radiate in the center; strongly radiate towards the poles	circular unoccluded	biseriate	This study
<i>Gomphoneis linearis</i> Kociolek & Stoermer	12–14	65–90	9–11	Slightly radiate	unknown	biseriate	Kociolek & Stoermer 1986

*Gomphonema sierrianum* Stancheva & Kociolek, *sp. nov.* (Figs 28–45)

**LM observations**

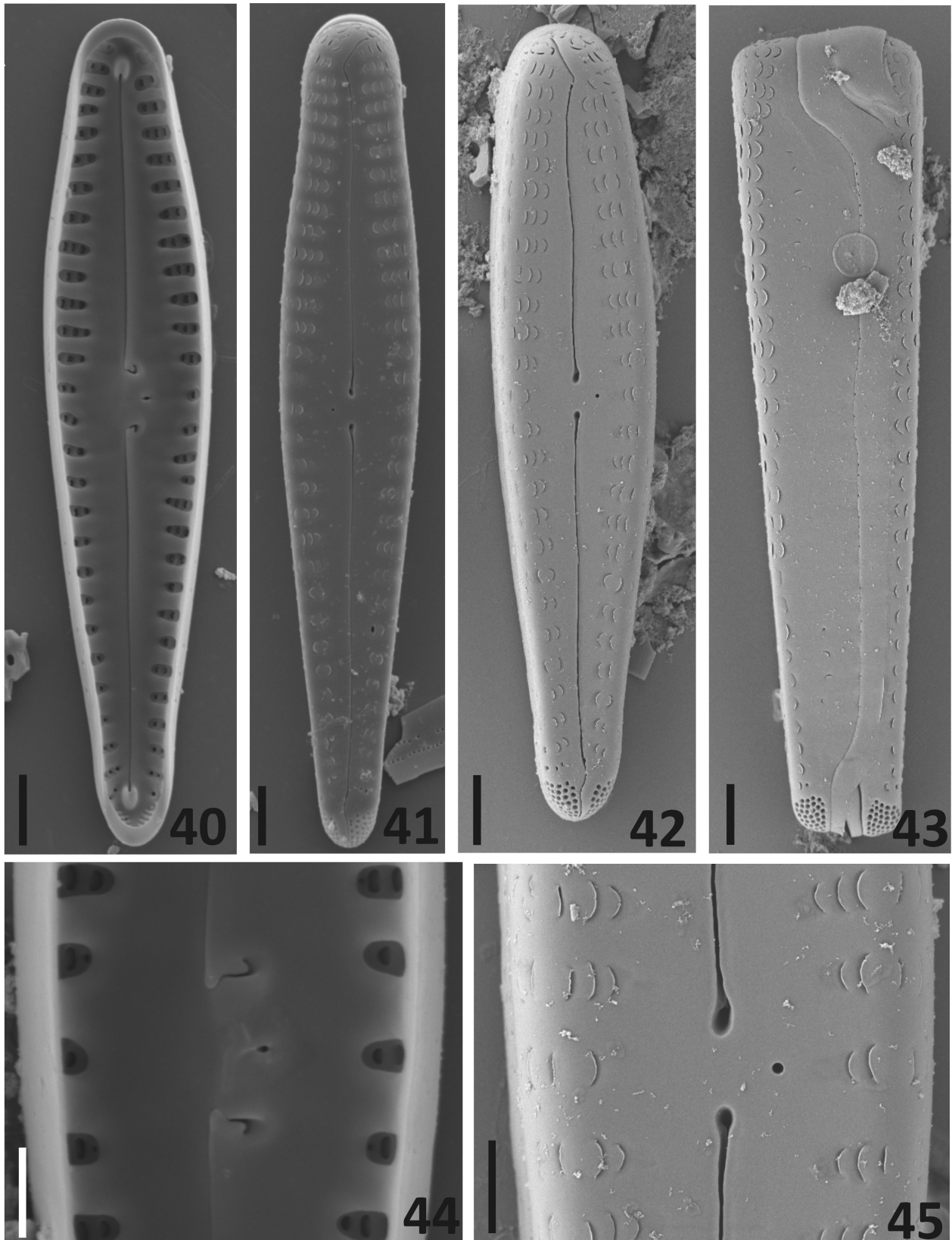
Valves are lanceolate-clavate with produced broadly rostrate to slightly capitate head pole and narrower rounded foot pole (Figs 28–39). Cells are 4–5.2  $\mu\text{m}$  wide, 17–31  $\mu\text{m}$  long. The axial area is wide, lanceolate bordered by short striae. Striae at the center are slightly radiate and shortened, becoming strongly radiate toward the poles, 11–14 in 10  $\mu\text{m}$ . Areolae not distinguishable in LM.

The raphe is filiform, with weakly expanded proximal ends that are bent slightly to one side. A single, small stigma is present at the central area. Frustules are cuneate in girdle view with single irregular row of puncta on each mantle interrupted in the central part (Fig. 34).



**FIGURES 28–39.** *Gomphonema sierrianum* Stancheva & Kociolek, *sp. nov.* type material from San Antonio Creek, California, USA (site 533SPILSA). LM images. Figs 28–33, 35–39 valve view. Fig. 34 girdle view. Scale bar: 10  $\mu\text{m}$ .





**FIGURES 40–45.** *Gomphonema sierrianum* Stancheva & Kociolek, *sp. nov.* type material from San Antonio Creek, California, USA (site 533SPILSA). SEM images. Figs 40, 44 internal valve view. Figs 41, 42, 45 external valve view. Fig. 43 girdle view showing each mantle with pores near both poles. Fig. 44 illustrates the interval view of the central nodule, bearing a circular, slightly transversely elongated stigma opening and dilated and recurved proximal raphe ends. Fig 45 shows external valve view of the central area with isolated stigma. Figs 42 and 45 are the same specimen. Scale bars: Figs 40–43 = 2  $\mu$ m; Figs 44, 45 = 1  $\mu$ m.

### SEM observations

Externally, striae are uniseriate, composed of c-shaped areolae, occluded by siliceous flaps (volae), which extend onto the mantle (Figs 41–43, 45). Striae near the head pole are longer, consisting of 3, rarely 2 or 4 areolae, while the shortened central striae and the striae near the foot pole contain 1 or 2 areolae (Figs 42 and 45). The raphe is slightly undulate, with distal raphe ends deflected before the apices and extended into the mantle (Fig. 42). In the central area, one isolated stigma with circular opening is positioned close to the dilated and drop-shaped proximal raphe ends (Fig. 45). Mantels with pores in an irregular single row near the poles (Fig. 43). Internally, pseudosepta and helictoglossa are visible at both poles (Fig. 40). The central nodule bears a circular, slightly transversely elongated stigma opening and dilated and recurved proximal raphe ends (Fig. 44).

**Type:**—USA. California: San Antonio Creek, Sierra Nevada Mts, 38.2758° N, 120.3361° W, *Nathan Mack, October 9, 2015* (holotype UC2050495 circled specimen on slide; isotype RS! 008, circled specimen on slide and material, CSUSM, USA).

**Etymology:**—The epithet refers to the mountain habitat in the Sierra Nevada, California, where the species was first observed.

**Distribution and ecological notes:**—Found in three sites in the Sierra Nevada Mts, CA (elevation 1226 to 1400 m a.s.l.). Habitats are generally characterized by low nutrients (TN <0.11 mg L<sup>-1</sup>, TP <0.01 mg L<sup>-1</sup>), low conductivity (<53.3 μS cm<sup>-1</sup>), slightly alkaline (15–30 mg L<sup>-1</sup> CaCO<sub>3</sub>), and pH 7.6–8.8 (see Table 1).

**Comments:**—The group of *Gomphonema* species with wide axial areas was reviewed by Reichardt (2005), where he documented 14 taxa with light and scanning electron microscopy. Many of these taxa were from Africa, Southeast Asia and Australasia. Additional taxa in this group were also considered by Metzeltin & Lange-Bertalot (1998, 2007) from South America. While none of the taxa considered in these works resembles *G. sierrianum* in terms of valve shape (most are much more lanceolate-clavate, and lack the distinct protracted head pole), several of them have flap-like occlusions that extend outside the areolar opening (see for example SEM images in Reichardt (2005) for *G. clevei* Fricke, *G. brachyneurum* O. Müller, *G. sundaense* Reichardt, *G. moresbyanum* Reichardt).

Liu *et al.* (2013) described *Gomphonema chinense* Liu & Kociolek from mountains in Northeastern China. This diatom resembles *G. sierrianum* most closely in terms of valve outline (Table 2), but differs by less pronounced headpole, longer striae, and c-shaped areolae openings lacking siliceous flaps (see Liu *et al.* 2013, Figs 16–26).

### *Gomphoneis oreophila* Stancheva & Kociolek, *sp. nov.* (Figs 46–71)

#### LM observations

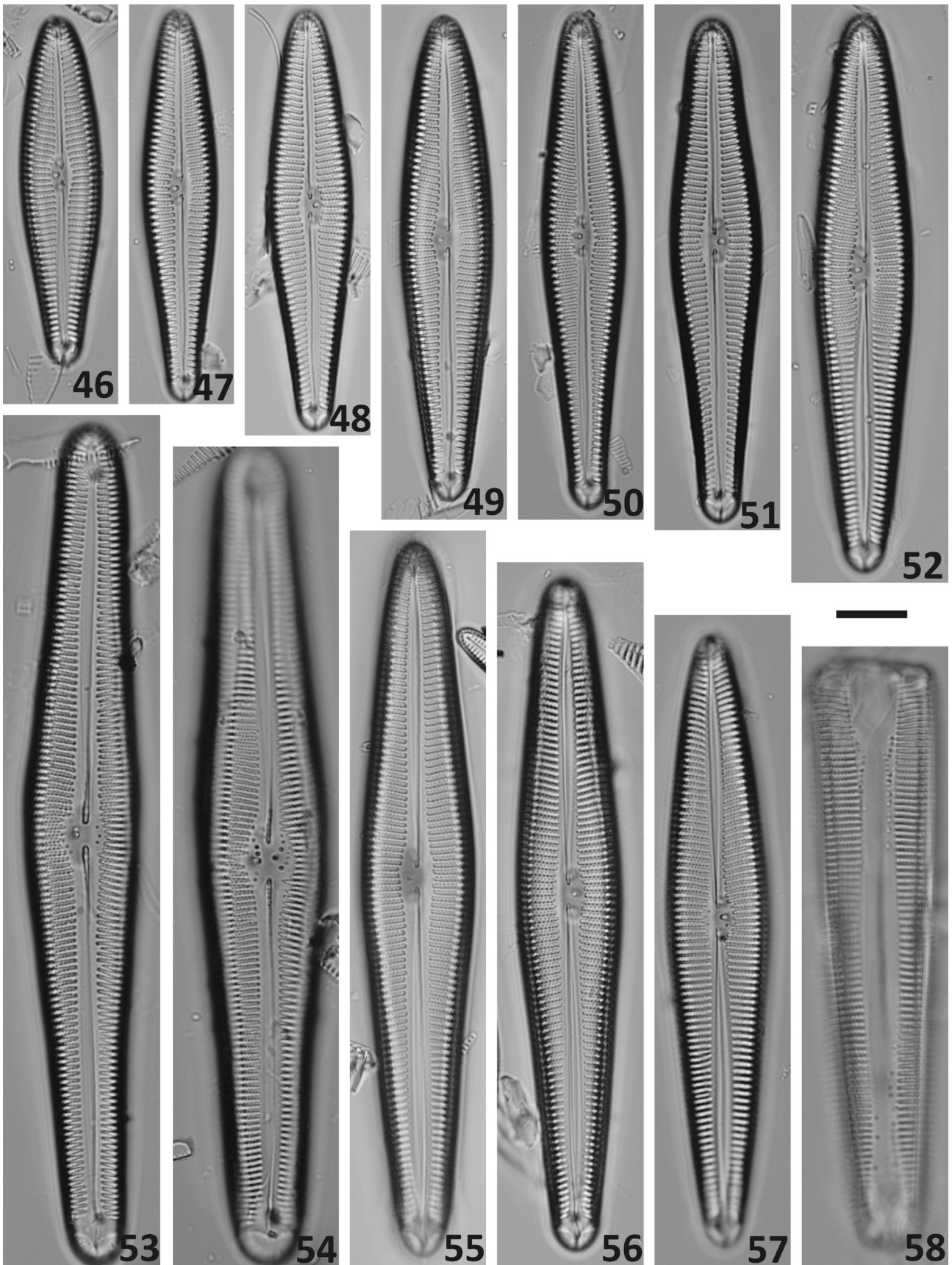
Valves are nearly lanceolate, slightly clavate with rounded head pole and narrower foot pole (Figs 46–57). Margins are straight to convex and tumid at the center. Cells are 10.3–16 μm wide, 51–105 μm long; initial valves 15–17 μm wide, 120–125 μm long (Figs 53, 54). Axial area is relatively narrow, straight and slightly expanded only at the center to form a small linear-elliptical central area bearing one stigma (Figs 46–52). Raphe is slightly arched, distinctly lateral. Striae are nearly parallel to slightly radiate in the center, more strongly radiate toward the poles, 10–12 in 10 μm. Striae distinctly biseriate. Longitudinal lines located close to the valve margin (submarginal, Figs 49, 52, 54, 56) better visible when the valve is tilted. Initial valves bent, tumid in the center, bearing in the central area one stigma and 2 to many areolae (Figs 53, 54). Frustules are cuneate in girdle view with single irregular row of puncta missing in the central part (Fig. 58).

#### SEM observations

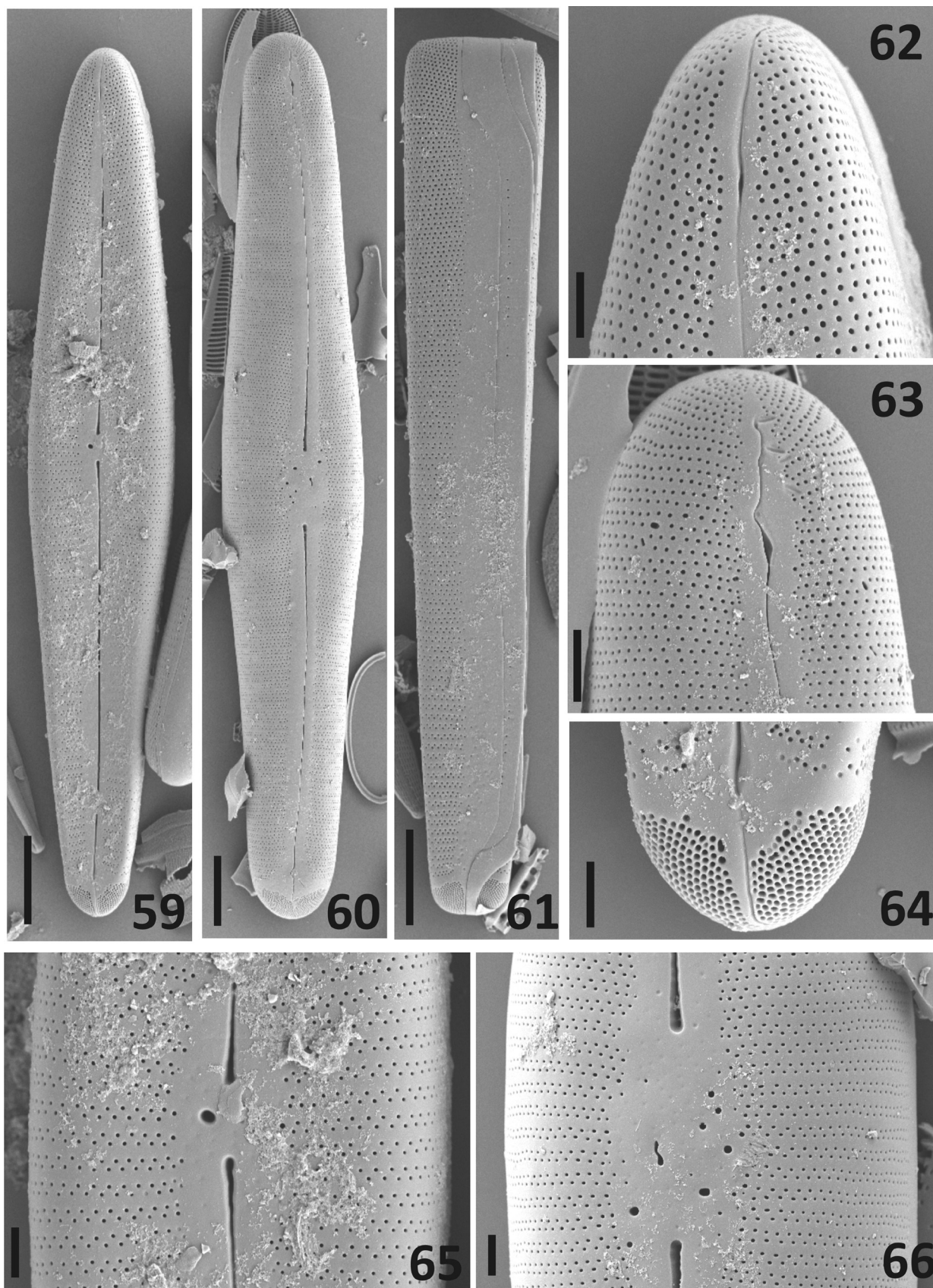
Externally, the striae are composed of double rows of circular puncta which extend onto the mantle (Figs 59, 60, 62). The raphe is more or less straight, or slightly arched, but not undulate (Figs 59, 60). Distal raphe ends are slightly bent before the apices and extend into the mantle (Figs 62, 63). Spines are not present at the head pole (Figs 61–63). At the foot pole the apical pore field is separated from the striae by unornamented silica, and the porelli are distinct and larger than the puncta (Figs 64). Externally, one round stigma is visible in the central area (Fig. 65), accompanied by several areolae in the initial cells (Fig. 66).

Internally, a large axial plate, narrow marginal lamina, and pseudosepta at both poles can be seen (Fig. 67). The axial plate terminates before reaching the helictoglossa at the foot pole (Figs 67, 70) where radiate striae with circular puncta are visible (Figs 70, 71). The marginal lamina is narrower near the central area (Figs 68, 69). The elongate central nodule is slightly raised and bears a slit-like stigma and recurved proximal raphe ends (Fig. 68).

**Type:**—USA. California: Rice Creek, Sierra Nevada Mts, 40.39999° N, 121.44109° W, *Jennifer York, July 1, 2015* (holotype UC2050496 circled specimen on slide; isotype RS! 009, circled specimen on slide and material, CSUSM, USA).



**FIGURES 46–58.** *Gomphoneis oreophila* Stancheva & Kociolek, *sp. nov.* type material from Rice Creek, California, USA (site 518RCNAPC). LM images. Figs 46–52, 55–57 valve view. Figs 53, 54 valve view of initial valves. Fig. 58 girdle view. Scale bar: 10  $\mu\text{m}$ .

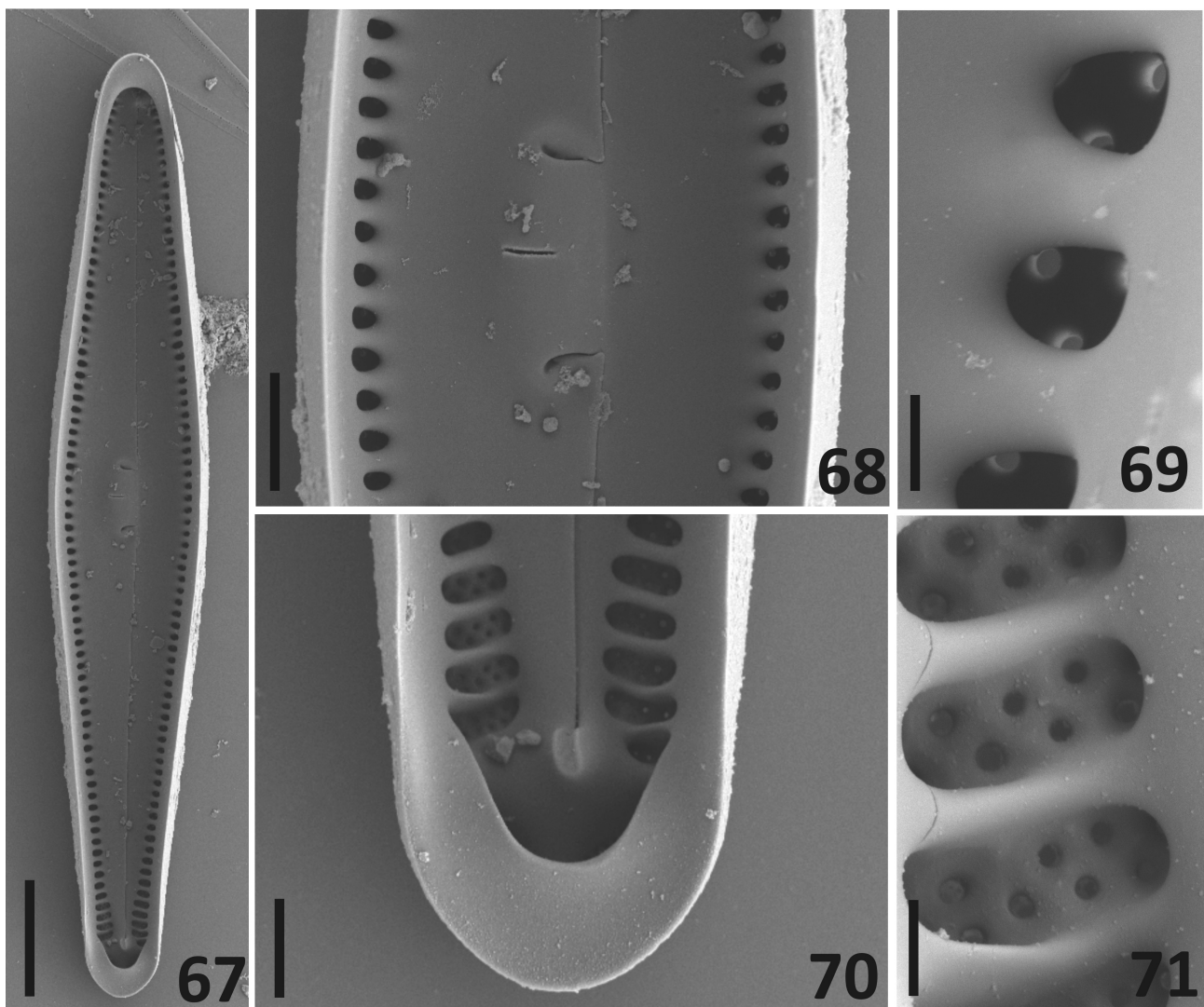


**FIGURES 59–66.** *Gomphoneis oreophila* Stancheva & Kociolek, *sp. nov.* type material from Rice Creek, California, USA (site 518RCNAPC). SEM images. Fig. 59 external valve view. Fig. 60 external valve view of initial cell. Fig. 61 external girdle view. Figs 62 and 63 detail of the head pole. Fig. 64 detail of foot pole with apical pore field. Fig. 65 central area with single circular stigma. Fig. 66 central area of initial cell with single stigma and several sigmoid areolae. Figs showing the same specimen are: 59, 62, 64, and 65; 60, 63, and 66. Scale bars: Figs 59–61 = 10  $\mu$ m; Fig. 63 = 3  $\mu$ m; Figs 62, 64, 65, 66 = 2  $\mu$ m.

**Etymology:**—This species epithet means “mountain-loving” referring to where it has been found.

**Distribution and ecological notes:**—Found only in the type locality which is high elevation site in the Sierra Nevada Mts, CA (1902 m a.s.l.). Habitat is characterized by low nutrients (TN 0.04 mg L<sup>-1</sup>, TP 0.05 mg L<sup>-1</sup>), low conductivity (74.8 μS cm<sup>-1</sup>), slightly alkaline (62 mg L<sup>-1</sup> CaCO<sub>3</sub>), and pH 8.3 (see Table 1).

**Comments:**—The large size and position of the longitudinal lines near the margin suggest a close relationship between this taxon and species such as *G. herculeana* (Ehrenb.) Cleve (1894: 73), *G. mammilla* (Ehrenb.) Cleve (1894: 73) and *G. minuta* (Stone) Kociolek & Stoermer (1988: 56). However, in these species the longitudinal lines are positioned approximately midway between the axial area and margin, or closer to the axial area, and apical spines are common (Kociolek & Stoermer 1988). The lanceolate shape of this species clearly separates it from these large species of the genus, as well as *Gomphoneis linearis* Kociolek & Stoermer (1986: 146–7) in which the longitudinal lines are positioned close to the valve margin. The latter species, known from only a few specimens from a single locality in Oregon (Kociolek & Stoermer 1986), differs in the shape of the valves, being more linear in outline (as opposed to having a distinctly lanceolate outline), has proportionately more broadly rounded headpoles, and is smaller (Table 2). Furthermore, the central striae around the stigma are radial with closely spaced tips in some specimens (see Kociolek & Stoermer 1986, Figs 25 and 26), in contrast to nearly parallel central striae in *G. oreophila*.



**FIGURES 67–71.** *Gomphoneis oreophila* Stancheva & Kociolek, *sp. nov.* type material from Rice Creek, California, USA (site 518RCNAPC). SEM images. Internal valve view of the same specimen. Fig. 67 shows a large axial plate and narrow marginal lamina. Fig. 68 central nodule bears a slit-like stigma and recurved proximal raphe ends. Fig 69 detail of the marginal lamina near the central area. Figs 70 and 71 detail of the marginal lamina at the foot pole. Scale bars: Fig. 67 = 10 μm; Fig. 68 = 3 μm; Fig. 70 = 2 μm; Figs 69, 71 = 0.5 μm.

## Discussion

Ultrastructure of the three new freshwater gomphonemoid diatoms allows comment on morphological variability within both *Gomphonema* and *Gomphoneis*. For *Gomphonema*, the areolar structure of *G. sierrianum* is very similar to other species with wide axial area. Reichardt (2005) has showed species with wide axial areas that also possess not only external volae (flaps) (similar to many other *Gomphonema* species; e.g. Levkov *et al.* 2016), but the volae cover most of the opening of the areolae (leaving a very narrow, c-shaped slit). In *G. sierrianum* the volae extend over the opening and off the surface of the valve (e.g. Figs 43, 45 this study), a feature observed by Reichardt 2005 in *G. moresbyanum* Reichardt (2005), but missing in other species with wide axial areas (Metzeltin & Lange-Bertalot 1998; Kociolek *et al.* 2016). Any phylogenetic (versus ecological) significance of this feature awaits future research.

In *G. californicum*, there are several morphological features that appear beyond the “typical” species of the genus. The first, and perhaps most obvious, is the structure of the areolae. In this species, striae are composed of slit-like openings from the mantle to near the margin of the valve; these appear unoccluded or with slight occlusions. The areolae are round and unoccluded from near the mantle to the axial area. While there are some species where the striae may have single rows of areolae at one point and double rows within the same stria (e.g., *G. pseudoparvulum* Kociolek *et al.* 2016: figs 98, 99), it is very rare to see different areolar morphologies within a single stria. Reichardt (1999) did illustrate both *G. drutelingense* Reichardt (1999: 38) and *G. longilineare* Reichardt (1999:39) with round, unoccluded areolae, but their structure was the same throughout the length of the striae. Also, in *G. californicum* the porelli in the apical pore fields appear to have a narrow, unornamented area present. This, to our knowledge, has not been recorded previously for a freshwater gomphonemoid diatom. Finally, we illustrated the presence of a small, blunt spine at the head pole of this species. Spines are not typical in the genus *Gomphonema*, except in the cases of rather large taxa such as *G. grande* Karthick *et al.* (2016: 188) from Africa, two endemic species from Lake Baikal (Kulikovskiy & Kociolek 2014) and *G. semiapertum* Grunow from fossil deposits in Shasta, California (Schmidt 1899, Tafel 216, Figs 7 and 8).

In *Gomphoneis oreophila*, many of its morphological structures are similar to species in the *Herculeana* group of the genus, including the differentiated apical pore fields, presence of a wide axial plate, presence of marginal laminae, and a slit-like internal stigma opening on the central nodule (Kociolek & Stoermer 1988, 1989). In most members of this group, the external areolae openings are occluded, with volae that are variously-shaped. Unique to this species in the *Herculeana* subgroup, areolar openings are circular without apparent occlusions.

In regards to the distribution of the three new gomphonemoid species described herein, our data show that they are rare and restricted to streams in remote areas of the Sierra Nevada Mountains. Although with limited distributions, all three of the new species were locally abundant. The SWAMP diatom data set, built in the last eight years includes species from over 1600 stream localities in California, but the new taxa have not been previously recorded. It seems that the diatom flora from streams in the Sierra Nevada Mountains is characterized by high endemism among gomphonemoid diatoms. This study, showed that more than one rare gomphonemoid species coexisted in a single stream locality. For instance, *Gomphonema californicum* was recorded in four streams (Table 1 this study), in two of which were together with *Gomphoneis mammilla*, an endemic species for California and Oregon (Kociolek & Rosen 1984), and with the newly described *Gomphoneis oreophila*. Similarly, *Gomphonema sierrianum* so far known from three streams (Table 1 this study), was recorded with another undescribed *Gomphonema* taxon, a publication that is in progress (Marina Potapova, personal communication). Further information about the distribution of the newly described *Gomphonema* and *Gomphoneis* species could be gained by reviewing other existing diatom collections from streams in the west coast and the Sierra Nevada, such as from the large US federal bioassessment programs. Continuous taxonomic studies are needed for more complete knowledge of the diatom flora from relatively unexplored regions of the state of California, which will increase the power of the diatom based stream bioassessment

## Acknowledgements

The California State Water Resources Control Board Surface Water Ambient Monitoring Program provided funding and data. Dr. Lisa Underwood (EcoAnalysts) provided diatom slides from sites 521BTC303 and 518RCNAPC. The authors acknowledge the assistance of Drs Ryan Anderson at the Nano3 Facility at the University of California, San Diego, and Steve Barlow at the San Diego State University Electron Microscopy Facility (NSF instrumentation grant DBI-0959908). We thank the editor Dr. Christopher Lobban, and Loren Bahls for their comments and suggestions which improved the presentation of this paper.

## References

- Agardh, C.A. (1817) *Synopsis Algarum Scandinaviae*. Lund, 135 pp.
- Bahls, L.L. (2011a) *Nupela potapovae* sp. nov. (Bacillariophyta), a lentic alpine species from North America. *Diatom Research* 26 (2): 167–174.  
<https://doi.org/10.1080/0269249X.2011.597586>
- Bahls, L.L. (2011b) Three new species of *Navicula* (Bacillariophyta) from Oregon and Montana and a review of diatom endemism in the Northwest. *Northwest Science* 85 (4): 517–526.  
<https://doi.org/10.3955/046.085.0402>
- Bahls, L.L. (2012a) Five new species of *Stauroneis* (Bacillariophyta, Stauroneidaceae) from the northern Rocky Mountains, USA. *Phytotaxa* 67 (1): 1–8.  
<https://doi.org/10.11646/phytotaxa.67.1.1>
- Bahls, L.L. (2012b) *Navicula whitefishensis*, a new diatom (Bacillariophyta) from the Northern Rockies. *Intermountain Journal of Sciences* 18 (1–4): 1–5.
- Bahls, L.L. (2013) New diatoms (Bacillariophyta) from western North America. *Phytotaxa* 82 (1): 7–28.  
<https://doi.org/10.11646/phytotaxa.82.1.2>
- Bahls, L.L. (2014a) New diatoms from the American West—A tribute to citizen science. *Proceedings of the Academy of Natural Sciences of Philadelphia* 163: 61–84.  
<https://doi.org/10.1635/053.163.0109>
- Bahls, L.L. (2014b) *Neidiopsis hamiltonii* sp. nov., *N. weilandii* sp. nov., *N. levanderi* and *N. wulffii* from western North America. *Diatom Research* 29: 371–386.  
<https://doi.org/10.1080/0269249X.2014.889606>
- Bahls, L.L., Pierce, J., Apfelbeck, R. & Olsen, L. (2013) *Encyonema droseraphilum* sp. nov. (Bacillariophyta) and other rare diatoms from undisturbed floating-mat fens in the northern Rocky Mountains, USA. *Phytotaxa* 127 (1): 32–48.  
<https://doi.org/10.11646/phytotaxa.127.1.7>
- Bahls, L.L. & Potapova, M. (2015) Two new species of *Navicula* (Bacillariophyta, Naviculales) from the Cascade Mountains of the American Northwest. *Phytotaxa* 218 (3): 253–267.  
<https://doi.org/10.11646/phytotaxa.218.3.4>
- Burge, D.O., Thorne, J.H., Harrison, S.P., O'Brien, B.C., Rebman, J.P., Shevock, J.R., Alverson, E.R., Hardison, L.K., Rodrigues, J.D., Junak, S.A., Oberbauer, T.A., Riemann, H., VanderPlank, S.E. & Barry, T. (2016) Plant diversity and endemism in the California Floristic Province. *Madroño* 63: 3–206.  
<https://doi.org/10.3120/madr-63-02-3-206.1>
- Calsbeek, R., ThOMPSON, J.N. & Richardson, J.E. (2003) Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. *Molecular Ecology* 12: 1021–1029.  
<https://doi.org/10.1046/j.1365-294X.2003.01794.x>
- Cleve, P.T. (1894) *Synopsis of the naviculoid diatoms*. Part 1. *Kongliga Svenska-Vetenskaps Akademiens Handlingar* 26: 1–194.
- Ehrenberg, C.G. (1832) Über die Entwicklung und Lebensdauer der Infusionsthier; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der Königlich-Akademie der Wissenschaften zu Berlin*, 1831: 1–154.
- Ehrenberg, C.G. (1849) *Über das mächtigste bis jetzt bekannt gewordene (angeblich 500 Fufs mächtige) Lager von mikroskopischen reinen kieselschaligen Süßwasser-Formen am Wasserfall-Flusse im Oregon*. Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich-Preussischen Akademie der Wissenschaften zu Berlin, Vol: 1849, pp. 76–87.
- Fetscher, A.E., Busse, L. & Ode, P.R. (2009) *Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California*. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP 002. (updated May 2010). Available from: [http://www.waterboards.ca.gov/water\\_issues/programs/swamp/docs/sop\\_algae.pdf](http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/sop_algae.pdf) (accessed 9 December 2016)
- Fricke, F. (1902) *Plate 234*. In: Schmidt, A. (Ed.) *Atlas der Diatomaceen-kunde*. O.R. Reisland, Leipzig.
- Grunow, A. (1860) Ueber neue oder ungenügend gekannte Algen. Erste Folge, Diatomeen, Familie Naviculaceen. *Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien* 10: 503–582
- Karthick, B., Kociolek, J.P., Taylor, J.C. & Cocquyt, C. (2016) *Gomphonema grande* sp. nov., a new diatom (Bacillariophyta) from the Democratic Republic of the Congo, Tropical Africa. *Phytotaxa* 245 (3): 187–196.  
<https://doi.org/10.11646/phytotaxa.245.3.1>
- Kociolek, J.P. (2012a) *Diatoms of the Southern California Bight*. Available from: [http://dbmuseblade.colorado.edu/DiatomTwo/dscb\\_site/index.php](http://dbmuseblade.colorado.edu/DiatomTwo/dscb_site/index.php) (accessed 9 December 2016)

- Kociolek, J.P. (2012b) *Master List of Names for California Freshwater Diatoms*. Available from: [http://dbmuseblade.colorado.edu/DiatomTwo/dscb\\_site/index.php](http://dbmuseblade.colorado.edu/DiatomTwo/dscb_site/index.php) (accessed 9 December 2016)
- Kociolek, J.P. & Kingston, J.C. (1999) Taxonomy, ultrastructure and distribution of some gomphonemoid diatoms (Bacillariophyceae: Gomphonemataceae) from rivers in the United States. *Canadian Journal of Botany* 77 (5): 686–705.  
<https://doi.org/10.1139/b99-007>
- Kociolek, J.P. & Rosen, B.H. (1984) Observations on North American *Gomphoneis* (Bacillariophyceae). I. Valve ultrastructure of *G. mammilla* with comments on the taxonomic status of the genus. *Journal of Phycology* 20: 361–368.  
<https://doi.org/10.1111/j.0022-3646.1984.00361.x>
- Kociolek, J.P. & Stoermer, E.F. (1986) Observations on North American *Gomphoneis* (Bacillariophyceae). II. Descriptions and ultrastructure of two new species. *Transactions of the American Microscopical Society* 105: 141–151.  
<https://doi.org/10.2307/3226386>
- Kociolek, J.P. & Stoermer, E.F. (1988) Taxonomy, ultrastructure and distribution of *Gomphoneis herculeana*, *G. erienne* and closely related species (Naviculales: Gomphonemataceae). *Proceedings of the Academy of Natural Sciences of Philadelphia* 140: 24–97.
- Kociolek, J.P. & Stoermer, E.F. (1989) Phylogenetic relationships and evolutionary history of the diatom genus *Gomphoneis*. *Phycologia* 28: 438–454.  
<https://doi.org/10.2216/i0031-8884-28-4-438.1>
- Kociolek, J.P., Laslandes, B., Bennet, D., Thomas, E., Brady, M. & Graeff, C. (2014) Diatoms of the United States. I. 2014. Taxonomy, ultrastructure and descriptions of fifty new species and other rarely reported taxa from lake sediments in the western U.S.A. *Bibliotheca Diatomologica* 61: 1–188.
- Kociolek, J.P., You, Q., Wang, X. & Liu, Q. (2015) Consideration of some interesting freshwater gomphonemoid diatoms from North America and China, and the description of *Gomphosinica*, gen. nov. *Nova Hedwigia, Beihefte* 144: 175–198.
- Kociolek, J.P., Woodward, J.C. & Graeff, C. (2016) New and endemic *Gomphonema* C.G. Ehrenberg (Bacillariophyceae) species from Hawaii. *Nova Hedwigia* 102: 141–171.  
[https://doi.org/10.1127/nova\\_hedwigia/2015/0296](https://doi.org/10.1127/nova_hedwigia/2015/0296)
- Kulikovskiy, M. & Kociolek, J.P. (2015) The diatom genus *Gomphonema* Ehrenberg in Lake Baikal. I. Morphology and taxonomic history of two endemic species. *Nova Hedwigia, Beihefte* 144: 507–518.
- Kützing, F.T. (1833) Synopsis Diatomacearum oder Versuch einer systematischen Zusammenstellung der Diatomeen. *Linnaea* 8: 529–620.
- Kützing, F.T. (1844) Die Kieselschaligen. Bacillarien oder Diatomeen. Nordhausen, 152 pp.
- Lange-Bertalot, H. (1993) 85 neue taxa und über 100 weitere neu definierte Taxa ergänzend zur Süßwasserflora von Mitteleuropa, Vol. 2/1–4. *Bibliotheca Diatomologica* 27: 164 pp., 134 pls.
- Levkov, Z. (2009) *Amphora sensu lato*. In: Lange-Bertalot, H. (Ed.) *Diatoms of Europe*, Volume 5. A.R.G. Gantner Verlag K.G., Ruggell, 916 pp.
- Levkov, Z., Mitic-Kopanja, D. & Reichardt, E. (2016) The diatom genus *Gomphonema* from the Republic of Macedonia. In: Lange-Bertalot, H. (Ed.) *Diatoms of Europe*, Volume 8. A.R.G. Gantner Verlag K.G., Ruggell, 552 pp.
- Liu, Y., Kociolek, J.P. & Wang, Q. (2013) Six new species of *Gomphonema* Ehrenberg (Bacillariophyceae) species from the Great Xing'an Mountains, Northeastern China. *Cryptogamie, Algologie* 34: 301–324.  
<https://doi.org/10.7872/crya.v34.iss4.2013.301>
- Metzeltin, D. & Lange-Bertalot, H. (1998) Tropical diatoms in South America I: About 700 predominately rarely known or new taxa representative of the neotropical flora. *Iconographia Diatomologica* 5: 1–695.
- Metzeltin, D. & Lange-Bertalot, H. (2007) Tropical diatoms in South America II. Special remarks on biogeography disjunction. *Iconographia Diatomologica* 18: 1–877.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (1999) Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.  
<https://doi.org/10.1038/35002501>
- Nees ad Esenbeck. C.G. (1820) *Horae Physicae Berolinensis*. Bonn, 129 pp.
- Reichardt, E. (1999) Zur Revision de Gattung *Gomphonema*. Die Arten um *G. affine/insigne*, *G. angustatum/micropus*, *G. acuminatum* sowie gomphonemoide Diatomeen aus dem OberligozUan in Böhmen. *Bibliotheca Diatomologica* 8: 1–203.
- Reichardt, E. (2005) Die Identität von *Gomphonema entolejum* Østrup (Bacillariophyceae) sowie Revision ähnlicher Arten mit weiter Axialarea. *Nova Hedwigia* 81: 115–144.  
<https://doi.org/10.1127/0029-5035/2005/0081-0115>
- Reichardt, E. & Lange-Bertalot, H. (1991) Taxonomische Revision des Artenkomplexes um *Gomphonema angustum*-*G. dichotomum*-*G. intricatum*-*G. vibrio* und ähnliche Taxa (Bacillariophyceae). *Nova Hedwigia* 53: 519–544.
- Ress, J., Thomas, E., & Kociolek, J.P. (2016) New and interesting *Gomphonema* and *Gomphosphenia* from two Rocky Mountain Front



- Range streams, Colorado, U.S.A. *Diatom Research* 31: 51–62.  
<https://doi.org/10.1080/0269249X.2016.1141802>
- Schmidt, A. (1899) *Atlas der Diatomaceen-Kunde*. Fues's Verlag (R. Reisland). Series V (Heft 54), pls.213–216.
- Sovereign, H.E. (1963) New and rare diatoms from Oregon and Washington. *Proceedings of the California Academy of Sciences* 4 (31): 349–368.
- Sovereign, H.E. (1958) The diatoms of Crater Lake, Oregon. *Transactions of the American Microscopical Society* 77: 96–134.  
<https://doi.org/10.2307/3224112>
- Spaulding, S.A., Kociolek, J.P. & Davis, D. (2002) A new diatom genus from a playa lake in New Mexico, USA with the description of two new species. *European Journal of Phycology* 37: 135–143.  
<https://doi.org/10.1017/S096702620100350X>
- Stancheva, R., Busse, L., Kociolek, J.P. & Sheath, R.G. (2015) *Standard Operating Procedures for Laboratory Processing and Identification of Stream Algae*. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP 0003. Available from: [http://www.waterboards.ca.gov/water\\_issues/programs/swamp/bioassessment/docs/sop\\_algae\\_lab.pdf](http://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/docs/sop_algae_lab.pdf) (accessed 9 December 2016)
- Stancheva, R., Hall, J.D. & Sheath, R.G. (2012) Systematics of the genus *Zygnema* (Zygnematophyceae, Charophyta) from Californian watersheds. *Journal of Phycology* 48: 409–422.  
<https://doi.org/10.1111/j.1529-8817.2012.01127.x>
- Stancheva, R., Hall, J.D., McCourt, R.M. & Sheath, R.G. (2013) Identity and phylogenetic placement of *Spirogyra* species (Zygnemaophyceae, Charophyta) from California streams and elsewhere. *Journal of Phycology* 49: 588–607.  
<https://doi.org/10.1111/jpy.12070>
- Stepanek, J.G. & Kociolek, J.P. (2013) Several new species of *Amphora* and *Halamphora* from the western USA. *Diatom Research* 28 (1/2): 61–76.  
<https://doi.org/10.1080/0269249X.2012.735205>
- Thomas, E.W. & Kociolek, J.P. (2008) Taxonomy and ultrastructure of two new *Neidium* species (Bacillariophyceae) from lakes in the Sierra Nevada mountains of northern California (U.S.A.). *Diatom Research* 23: 471–482.  
<https://doi.org/10.1080/0269249X.2008.9705770>
- Thomas, E.W. & Kociolek, J.P. (2015) Taxonomy of three new *Rhoicosphenia* (Bacillariophyta) species from California, USA. *Phytotaxa* 204 (1): 1–21.  
<https://doi.org/10.11646/phytotaxa.204.1.1>
- Van Heurck, H. (1880) *Synopsis des Diatomées de Belgique*. Atlas. Ducaju & Cie, Anvers.