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Article

Two new species of *Gomphonema* (Bacillariophyceae) from Doon Valley, Uttarakhand, India

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With 31 figures and 1 table

Abstract: This paper describes two new *Gomphonema* species from the lesser Himalayas, India, with their valve morphology using light and scanning electron microscopy. The two species, *Gomphonema juettnerii* sp. nov. and *G. doonensis* sp. nov., were found in Nalota stream in Doon Valley of Uttarakhand State. Main features of *G. juettnerii* are valve outline, shape of the areolae and striae pattern and presence of stigma. Main features of *G. doonensis* are rounded headpole, striae made by doubly punctate striae and features in central area. A detailed discussion on doubly punctate striae in members of gomphonemoid diatoms is presented.

Key words: diatoms, Gomphonemoid, Himalayas, doubly punctate, *Gomphoneis*

Introduction

Pioneering investigations on the diatom flora of the Himalayas date back to the 19th century (Ehrenberg 1854, Dickie 1882). After a long hiatus when few diatom taxa were described from this region, there has been renewed attention to this topic. Recent efforts over the last 20 years include studies on taxonomy and ecology of the Himalayan diatom flora (e.g., Ormerod et al. 1994, Nautiyal et al. 1995, 1996, Jüttner et al. 1996, Rothfritz et al. 1997, Nautiyal & Nautiyal 1999, Wojtal et al. 2010, Jüttner et al. 2010). The focus of the present communication is to further the knowledge of *Gomphonema* flora of the Northern part of Indian subcontinent and describe two new species of this genus from a stream descending from lesser Himalaya into the Doon Valley.

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Materials and methods

Study area

The study area falls in the Outer Himalaya Zone. Geologically, rocks belonging to Siwaliks Group are present in and around the study area. The Siwaliks are overlain by Doon Gravels. Towards the north of the study area, a major tectonics feature, known as Main Boundary Thrust (MBT), is present. The MBT separates the rocks of Siwaliks Group from the rocks of Krol Group (Lesser Himalayan Zone) (Valdiya 1980). Sampling location is at Nalota stream (lat. 30° 22′ 18″ N, long. 78° 04′ 51″ E; altitude 483 m above mean sea level (msl), located in the middle of Doon Valley, along its northern fringe. This stream is located close to Dehradun-Mussorie highway (State Highway-1) near Malsi, Deer Park. Locally this stream is called *Nalota Nala*. The stream channel is narrow, shallow and transparent, with streambed consisting of hard substrate with prismatic edges, including rocks, large boulders, cobbles and pebbles. Overhanging vegetation forms partial canopy over the stream.

Collection and Cleaning

Diatom samples were collected in December 2007 from 20 to 30 cm depth by a scraping $3 \times 3\,cm^2$ area from the cobble surface with the help of razor and brush. Samples were preserved in 4 % formaldehyde, digested using concentrated nitric acid, centrifuged, and rinsed several times with deionized water in order to remove the acid. Cleaned material was mounted onto glass slides with Naphrax® mounting medium and observed with Olympus BX-51 light microscopes (LM) equipped with Differential Interference Contrast (DIC) and 1.4 NA (Numerical Aperture) objectives. Digital images were taken with an Olympus DP-71 digital camera. Scanning electron microscope (SEM) examination was performed using a high vacuum Jeol JSM-7401 F Field Emission SEM at the University of Colorado Nanomaterials Characterization Facility. For SEM examination, cleaned specimens were air dried onto cover glasses, attached to aluminum stubs and sputter-coated with 10 nm of Au-Pd. Specimens were examined at 3 kV accelerating voltage, with a working distance of 8 mm. The terminology and character descriptions of Kociolek & Stoermer (1993a, 1993b) were followed for features found in gomphonemoid diatoms. Physical and chemical parameters of water were assessed as per standard protocol for water and wastewater analysis of the American Public Health Association (APHA, 2005). Water quality data of the type locality is presented in Table 1.

Observations

***Gomphonema juettnerii* B. Karthick, R. Nautiyal & J.P. Kociolek (Figs 1–16)**

Description: Valves clavate to narrowly lanceolate, headpole round and footpole narrowly rounded. Length 13.6–25.0 µm, breadth 3.6–4.8 µm. Axial area broad, lanceolate. Striae taper in

Table 1. Water quality variables of the type locality for the two new *Gomphonema* species, Nalota stream, collected December 2007.

Water quality variables	Nalota Stream, Dehradun
Water Temperature (°C)	20.44 ± 3.57
Velocity ($m \cdot s^{-1}$)	0.30 ± 0.09
pH	7.8 ± 0.13
Dissolved Oxygen (mgL^{-1})	10.78 ± 0.96
Total Hardness (mgL^{-1})	74.56 ± 31.88
Alkalinity (mgL^{-1})	60.11 ± 43.57
Phosphates (mgL^{-1})	0.003 ± 0.001
Silica (mgL^{-1})	0.03 ± 0.03

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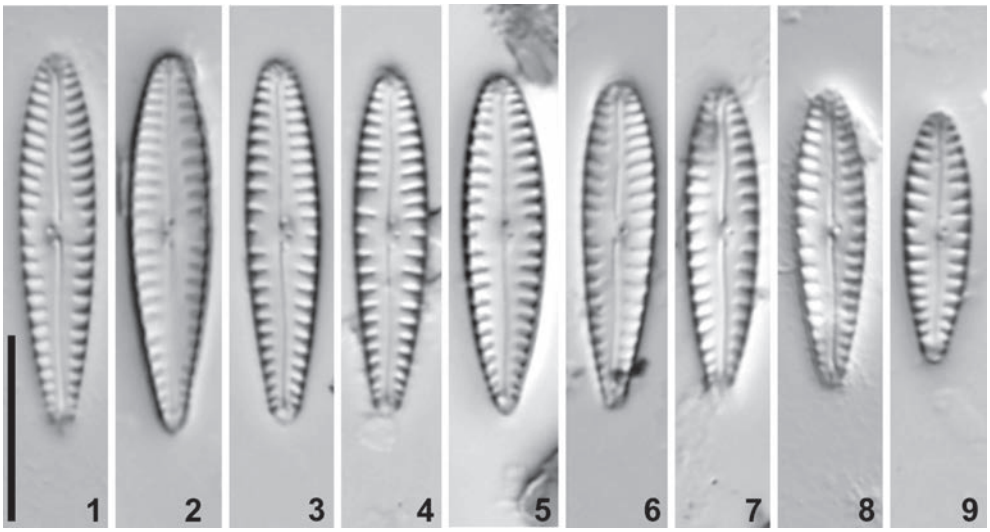


Figs 1–9. Light micrographs of *Gomphonema juettnerii*, from the type population; valve views showing the size diminution series. Scale bar represents 10 µm.

width from the margins to the axial area. Striae are radiate-parallel, strongly radiate at the foot pole, 11–14 in 10 µm. Raphe lateral and undulate. External proximal raphe ends dilated. Stigmal opening is round. Septa and pseudosepta are present at the poles.

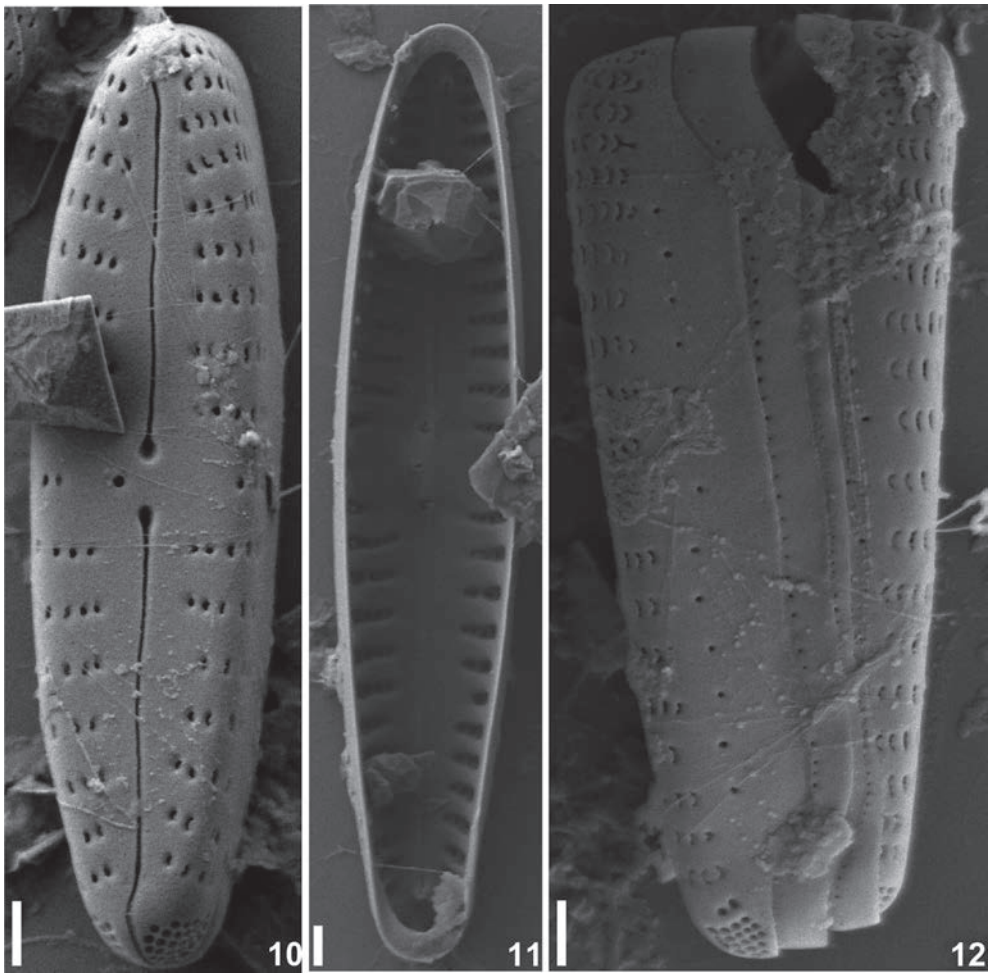
SEM Morphology: In the SEM, the exterior of the valve is dominated by areolae with flaps that form c- or reverse c-shaped openings (Figs 10, 12). The undulate raphe has dilated proximal ends, while the external distal ends are deflected onto the mantle in the same direction (opposite the side bearing the stigma) (Fig. 10). The external stigmal opening is small and round (Fig. 10). The bilobed apical pore field is physically separate, and morphologically distinct, from the striae and composed of rounded porelli that number ca. 60 in 10 µm (Figs 10, 12). Internally, the valve has weakly radiate striae found in deep troughs, separated by thick interstriae (Figs 11–16). A small, slightly raised central nodule, helictoglossae, pseudosepta and the raphe slit are evident (Figs 13–16). The central nodule has recurved proximal raphe ends and a rounded stigmal opening (Figs 13, 15). Helictoglossae are prominent at the head pole (Fig. 14) and foot pole (Fig. 16). Smaller struts help define the apical pore fields, which are covered internally by the pseudoseptum (Fig. 16).

Holotype: Slide No. 552049, Diatom Collection, University of Colorado, Boulder, USA (COLO; Figure 3 = holotype). The valve representing the type is here illustrated as Fig. 3. Cleaned material accession number 9307.

Isotype: Slide No. DS-05, Diatom Collection, Agharkar Research Institute Herbarium (AHMA), Pune, INDIA

Type locality: Nalota stream near deer park, Rajpur Road, Dehradun; Uttarakhand, India (leg. Rachna Nautiyal, December 2007).

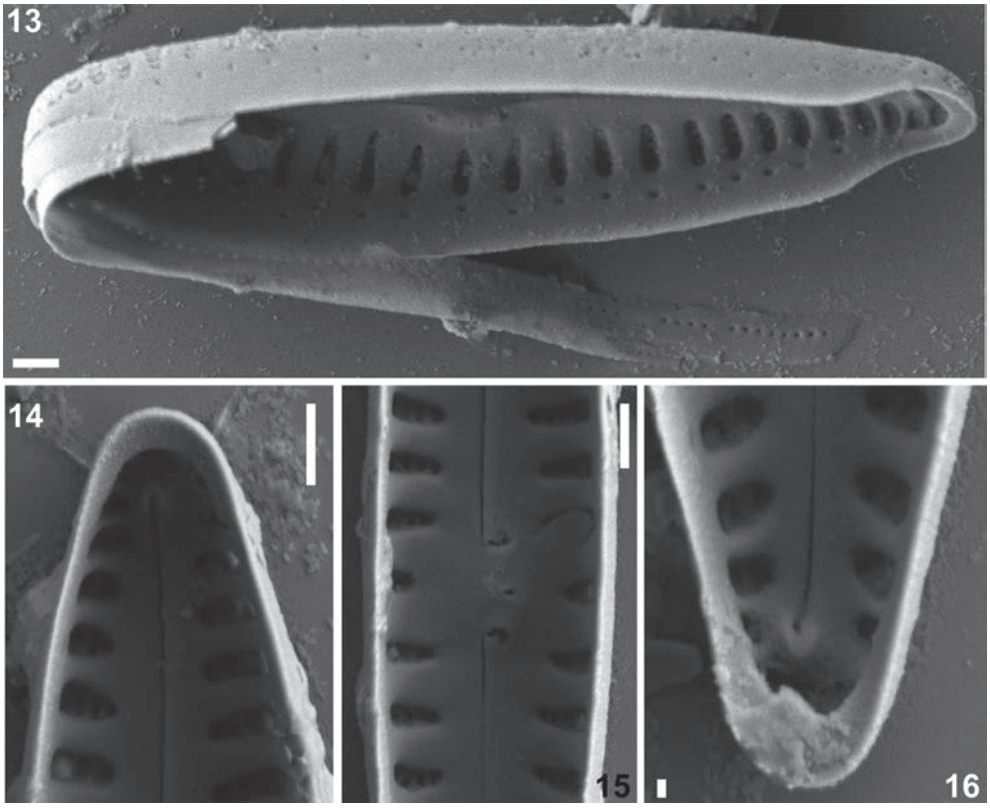
Etymology: This species in named after Dr. Ingrid Jüttner, National Museum of Wales, Cardiff, United Kingdom in recognition of her contribution to the knowledge of Himalayan diatoms.



Figs 10–12. Scanning electron micrographs of *Gomphonema juettnerii*. **10**. External view of whole valve showing "c" or "reverse c" shaped openings. **11**. Internal view of the whole valve showing striae in deep troughs. **12**. External girdle view showing the areolae opening, apical pore fields and girdle bands. Scale bar represents 1 µm.

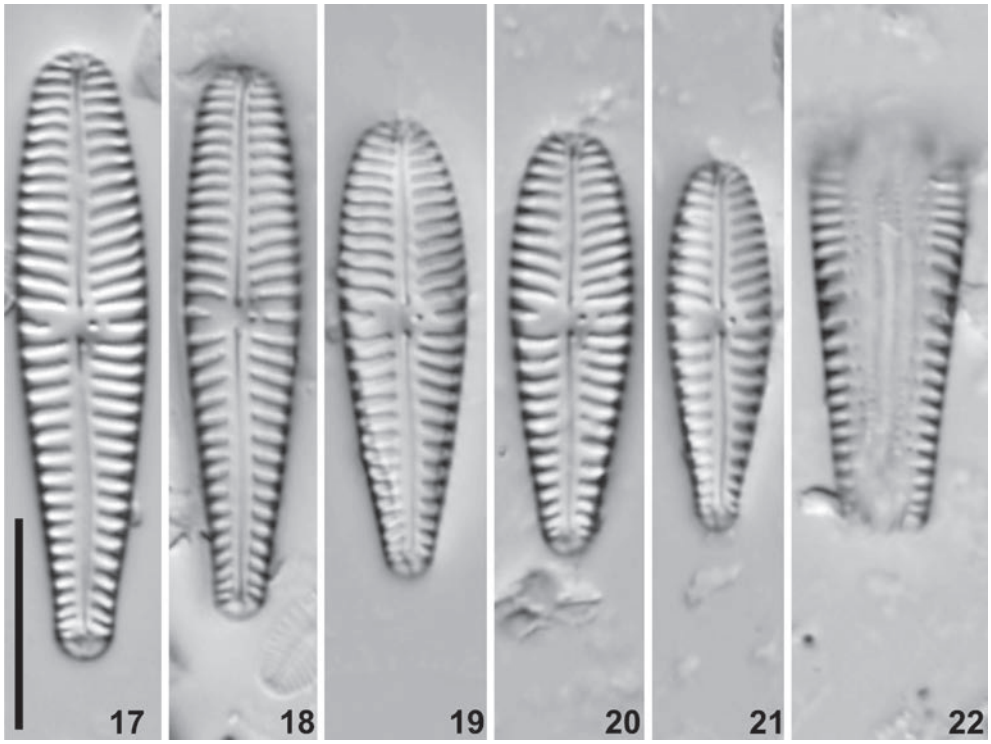
***Gomphonema doonensis* B. Karthick, R. Nautiyal & J.P. Kociolek (Figs 17–31)**

Description: Valves are clavate with a broadly rounded headpole and a narrow footpole. The valve tapers more strongly towards the footpole than to the headpole. Length 14.0–29.2 µm, breadth 4.5–5.5 µm. The axial area is narrow, straight and narrowly lanceolate in shape. The raphe is filiform and lateral. The rectangular-shaped central area is created by shortened striae on both sides of margin. Striae are costate and individual areolae are not visible in the LM. Striae are radiate, strongly radiate at the foot pole, 10–14 in 10 µm. External proximal raphe ends dilated. Stigmal opening is round. Septa and pseudosepta are present at the poles.



Figs 13–16. Scanning electron micrographs of *Gomphonema juettnerii* **13**. Internal view of whole valve showing striae inside deep troughs and central area. **14**. Internal view of the headpole showing prominent helictoglossae. **15**. Internal view of the central area with recurved proximal raphe ends and a rounded stigmal opening. **16**. Internal view of the footpole showing helictoglossae and pseudoseptum. Scale bar represents 1 µm.

SEM Morphology: In the SEM, the exterior of the valve shows striae composed of double rows of areolae across the valve (Figs 23, 25). Areolae are variously shaped, and may be round, tear drop-shaped or ellipsoidal (Figs 23, 25, 26). The undulate raphe has dilated proximal ends, while the external distal ends are deflected onto the mantle in the same direction (Fig. 23). The round external stigmal opening is present in the central area (Figs 23, 27). At the foot pole the distal raphe end bisects the apical pore field. In some specimens short striae composed of a single row of areolae are positioned near the apical pore field (Fig. 28), but mostly striae are biseriate across the valve. The bilobed apical pore field is physically separate, and morphological distinct, from the striae and composed of rounded porelli, 68–70 in 10 µm (Figs 25, 28). Internally, the valve has radiate striae found in deep troughs, separated by thick interstriae (Figs 29–31). A small, slightly raised central nodule has recurved proximal raphe ends and a slit-like internal stigmal opening (Figs 24, 30). Each distal raphe end terminates in a distinct helictoglossa at head pole (Fig. 29) and foot pole (Figs. 31). At the poles pseudosepta are visible (Figs 24, 29, 31).



Figs 17–22. Light micrographs of *Gomphonema doonensis*, from the type population. **17–21**. Valve views showing the size diminution series. **22**. Girdle view of the valve. Scale bar represents 10 µm.

Holotype: Slide No. 552-050, Diatom Collection, University of Colorado, Boulder, USA (COLO; Figure 17 = holotype). The valve representing the type is here illustrated as Fig. 17. Cleaned material accession number 9307.

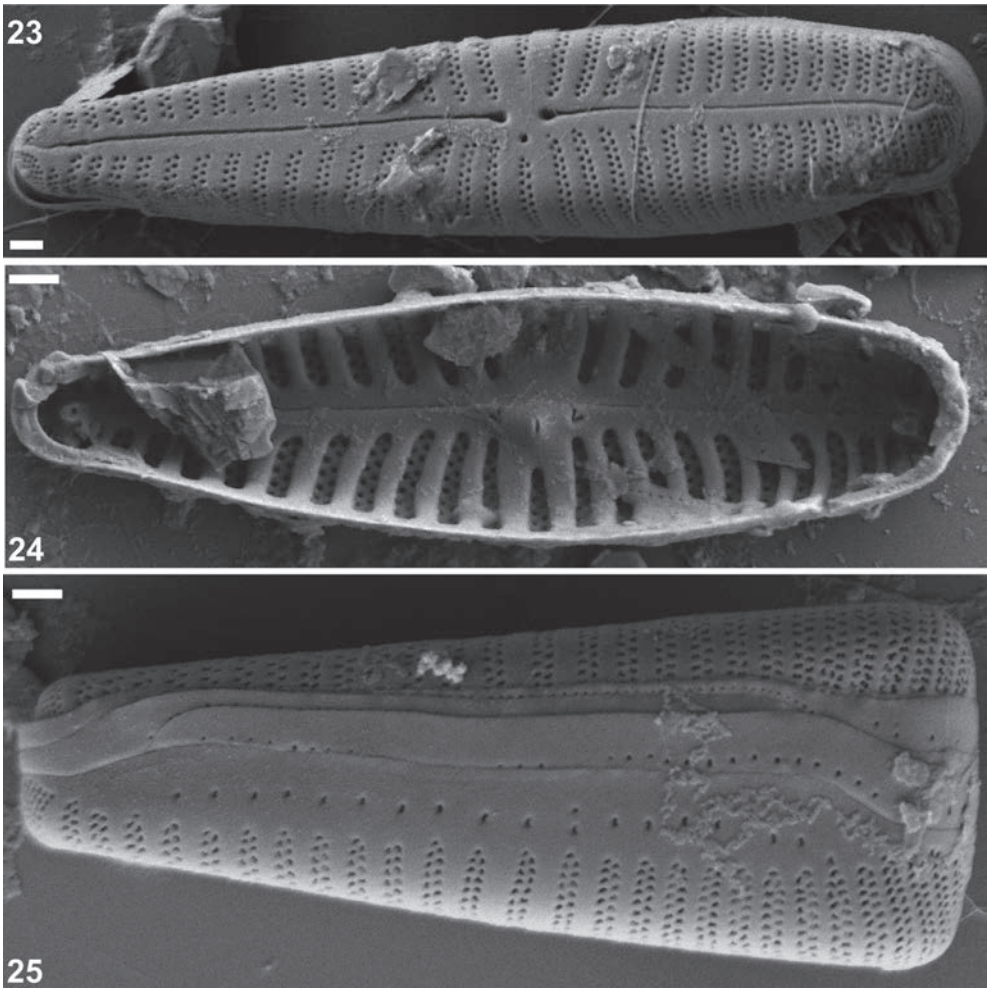
Isotype: Slide No. DS-05, Diatom Collection, Agharkar Research Institute Herbarium (AHMA), Pune, INDIA

Type locality: Nalota stream near deer park, Rajpur Road, Dehradun; Uttarakhand, India (leg. Rachna Nautiyal, December 2007).

Etymology: This species was found abundantly in Doon Valley and is named for its distribution.

Discussion

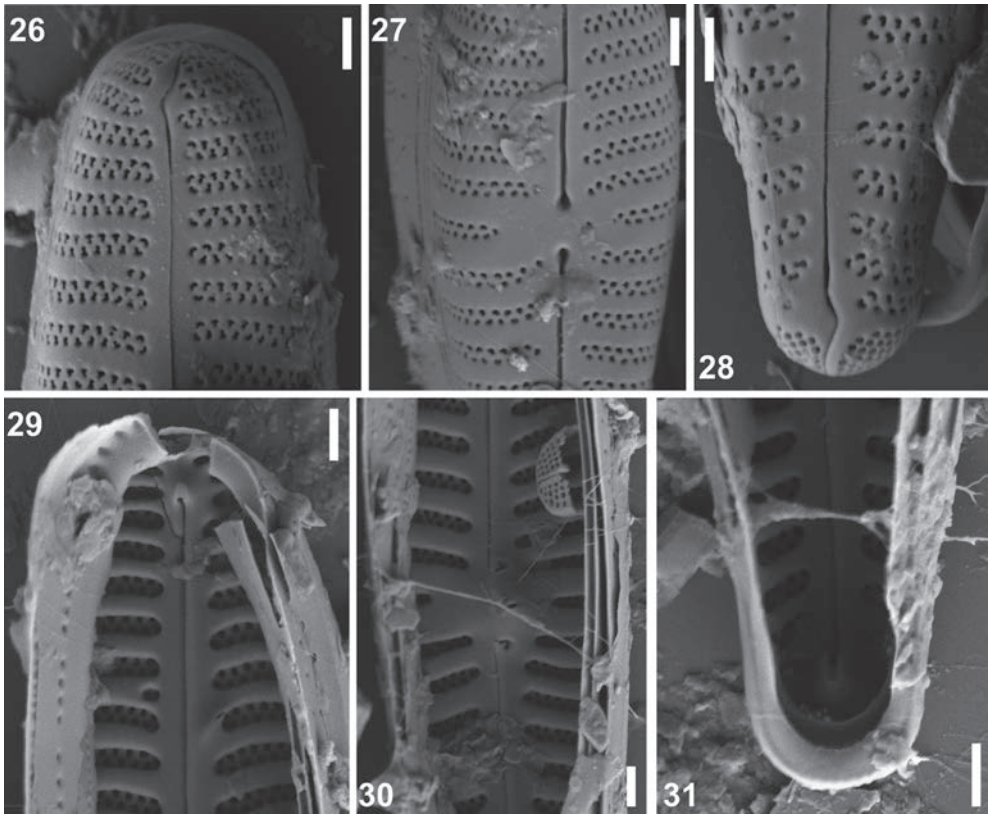
Gomphonema juettnerii resembles *Gomphonema pumilum* var. *elegans* Reichardt et Lange-Bertalot in general shape (Reichardt 1997). It differs, however by less radiate striae, ultrastructure of interstriae trench and external areola structure; areolae of *G. pumilum* v. *elegans* show well developed "C" shape across the valve (Figs 2, 3, Tafel I in Reichardt 1997), whereas *G. juettnerii* shows "C"-shaped structure within the central area, and areolae become reduced to comma shape towards the poles. The central area of *G. pumilum* v. *elegans* is formed by shortened striae (Figs. 1-11, Tafel II in Reichardt 1997), which are absent in *G. juettnerii*. In LM, *G. juettnerii*



Figs 23–25. Scanning electron micrographs of *Gomphonema doonensis.* **23**. External view of whole valve showing double rows of areolae across the valve and undulate raphe. **24**. Internal view of the whole valve showing striae pattern and central area. **25**. External girdle view showing the areolae opening, apical pore fields and girdle bands. Scale bar represents 1 µm.

resembles *G. minutum* Agardh in general valve outline. However, these species differ from *G. juettnerii* by absence of the central area created by shortened striae. Furthermore, *G. minutum* has striae composed of two rows of areolae (Fig. 91 in Kociolek & Kingston 1999). Striae pattern and absence of stigmata in *Gomphonema juettnerii* differentiate it from *G. sinestigma* Reichardt, Jüttner & Cox (Jüttner et al. 2004).

Gomphonema doonensis resembles some of the tropical species of *Gomphonema*, including *G. eurycephalus* Spaulding & Kociolek (Spaulding & Kociolek 1998) described from Madagascar and *G. latistigmata* Kociolek, Passy & Lowe (Passy et al. 1997) described from South Africa. *Gomphonema doonensis* has narrower valves and the presence of shortened striae in the central area and these features distinguish it from *G. eurycephalus*. *Gomphonema doonensis* dif-



Figs 26–31. Scanning electron micrographs of *Gomphonema doonensis*. **26**. External view of the headpole with the external distal raphe end curving onto the valve mantle. **27**. External view of the central area with undulate raphe has dilated proximal ends and round external stigmal opening. **28**. External view of the footpole showing distal raphe end and bilobed apical pore field. **29**. Internal view of the headpole showing striae found in deep troughs, separated by thick interstriae. **30**. Internal view of the raised central area showing recurved proximal raphe ends and a slit like internal stigmal opening. **31**. Internal view of the footpole showing the radiate striae and helictoglossae and pseudoseptum. Scale bar represents 1 µm.

fers from *G. latistigmata* in valve outline and length: the former is generally shorter and clavate, with broadly rounded headpole.

There is considerable confusion as to whether presence of doubly punctate striae alone is both necessary and sufficient to assign gomphonemoid diatoms to the genus *Gomphoneis* Cleve. Doubly punctate striae were first identified by Hustedt (1942, 1945) in several new species of *Gomphonema*. Hustedt noted (1942, p. 121) that there were two features that diagnose *Gomphoneis*, namely doubly-punctate striae and the presence of longitudinal lines. The latter character was not mentioned by Dawson (1982), who documented doubly-punctate striae in the common diatom *Gomphonema olivaceum* Hornemann using transmission and scanning electron microscopy. For this reason, Dawson (1982) subsequently transferred *G. olivaceum* to *Gomphoneis*. Patrick & Reimer (1975, p. 147) further complicated the situation by stating "This genus [*Gomphoneis*] is distinguished by the double rows of decussating puncta alternating with the costae, and the narrow septum near the margins of the valve." Confusion over the taxonomy of this group was expressed

by Lange-Bertalot (1980). In his identification of a large, doubly-punctate *Gomphonema* species from Israel (as "*Gomphoneis herculeana* (Ehrenberg) Cleve", a species known only from North America!) and his argument was that doubly-punctate striae could not diagnose *Gomphoneis*. Kociolek & Stoermer (1989) showed in the context of a formal phylogenetic analysis that *Gomphoneis* is diagnosed by the presence of doubly-punctate striae and the presence of a marginal lamina that gives the impression of the longitudinal lines in primitive members of the genus. Furthermore, Kociolek & Stoermer (1989) documented that two groups may be recognized: the Elegans group (that is the name bearer for the genus, and whose species also either have no stigmata or 4 stigmoids, as well as apical pore fields that are undifferentiated from the areolae) and the Herculeana subgroup (more commonly referenced when discussions of the genus are made). Krammer & Lange-Bertalot (1991) echoed Lange-Bertalot's (1980) mistake when they suggested that some species included the Elegans group of *Gomphoneis* found in Lake Baikal were *Gomphonema* taxa, and made nomenclatural changes reflecting that opinion. See Kociolek et al. (2014) about the diversity and high levels of endemism of *Gomphoneis* species in Lake Baikal. More recently Reichardt (2007) argued on similar lines, while recognizing some gomphonemoid species with doubly-punctate striae and assigning them all to *Gomphonema*, without considering other features. To be clear, as Kociolek (1998) noted, we should refrain from thinking typologically about how species are included into groups (such as genera), and we have to recognize that there may be the loss or gain of evolutionary novelties. Formal phylogenetic analyses will guide these determinations, not a priori determinations made without such analyses. To date, a morphology-based phylogeny of the freshwater gomphonemoid diatoms (Kociolek & Stoermer 1993a) suggests that a suite of features diagnose Gomphonema. These features include: differentiated apical pore fields, a single, "typical" stigma (or in a few cases no stigma), no marginal laminae and no axial plates, and with doubly-punctate striae with flaps occluding the areolae. Diatoms described here from the Himalayas are placed firmly in the genus *Gomphonema*. We are far past the time to be constrained by typological, non-evolutionary, approaches to taxonomy and systematics.

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