

A new freshwater *Gomphonema* Ehrenberg (Bacillariophyta) species from Eastern Himalayas, India

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Abstract: A new freshwater diatom species, *Gomphonema adhikarii* sp. nov., is described from a small road-side pool from the alpine region of Arunachal Pradesh, Eastern Himalayas, India. Detailed valve morphological features, based on both light and scanning electron microscopy, is presented and this taxon is compared with similar species. *Gomphonema adhikarii* has a distinct asymmetry about the apical axis and slightly bent foot pole area. The outline of the valve is slightly undulate, the valve centre is swollen and both apices are broadly rounded. This new species described from the Eastern Himalayas supports the idea of the area being rich in biodiversity, even with respect to the freshwater diatom flora.

Key words: *Gomphonema*, Arunachal Pradesh, Tawang, *Gomphonema adhikarii* sp. nov.

INTRODUCTION

The Eastern Himalayas are rich in biodiversity, positioned within two major biodiversity hotspots, i.e. Indo–Burma and the Himalaya Hotspots (CEPF 2005). This region extends geographically from Nepal to Tibet and Yunnan of China including the north–eastern states of India. A unique geo–climatic complexity in this region supports a wide range of species diversity. This region alone houses more than 1500 endemic plant species (MYERS et al. 2000). Studies on the diatom flora of the Himalayan region have been confined to Nepal (JÜTTNER et al. 2000, 2004, 2010a, 2010b, 2011, 2017, 2018; VAN DE VIJVER et al. 2011; KRSTIĆ et al. 2013), Tibet and China (MERESCHKOWSKY 1906; KOCIOLEK 1992; HUIZHONG & JIAYOU 2000; LI et al. 2010a, 2010b; GONG & LI 2011; HU et al. 2013; GONG et al. 2015; JIANG et al. 2018; LIAO & LI 2018). Studies on the freshwater diatoms from the Indian Territory of the region are limited to a few recent records (DAS et al. 2018; WADMARE et al. 2019). Beyond floristic and biodiversity discovery studies, several efforts have also been made for ecological assessment of streams, rivers and lakes through diatom studies in the adjoining Nepalese Himalayas (ORMEROD et al. 1994; JÜTTNER et

al. 1996, 2003; SIMKHADA & JÜTTNER 2006; SIMKHADA et al. 2006; SHARMA et al. 2012; GURUNG et al. 2018).

Gomphonema Ehrenberg is one of the most taxon–rich diatom genera comprised of more than 940 taxa so far (KOCIOLEK et al. 2020). Members of the genus are identified by linear–lanceolate heteropolar valves with (usually) a single stigma present in the central area (ROUND et al. 1990). More than 130 *Gomphonema* taxa have been documented so far from various freshwater and terrestrial habitats of India, since the first record by EHRENBURG (1845). About 30% of these taxa are endemic (KARTHICK et al. 2011). Some *Gomphonema* species from high elevations of the Eastern Himalayas showed higher endemism (JÜTTNER et al. 2004). The higher endemism in the Himalayas can be inferred from the oligotrophic conditions of the freshwaters there (MOSER et al. 1998; JÜTTNER et al. 2004). We have begun an initial survey of biodiversity discovery and floristics in Northeastern India. DAS et al. (2018) described three new *Gomphonema* species from a river in Western Arunachal Pradesh including *Gomphonema mayamae* Sudipta K. Das, C. Radhakrishnan, Kociolek et B. Karthick (DAS et al. 2018), which had a fimbriate structure in the girdle band near the head pole. This morphological feature is

unique among the freshwater *Gomphonema* species. In the present study, we further our work by describing one new *Gomphonema* species from a small road-side pool in the Tawang region of Arunachal Pradesh state of India.

MATERIALS AND METHODS

Study area. Material was collected from epilithic habitats on submerged stones in a small road-side pool near Nagula Lake, on the way to Bumla (Indo–China border) in the Tawang district of Arunachal Pradesh ($27^{\circ}39'49.7''\text{N}$, $91^{\circ}51'32.6''\text{E}$; elevation 4173 m a.s.l.) (Fig. 1). The sampling site and the adjacent area are part of the Nagula High Altitude Wetland (HAW) Complex, one of the two ecologically crucial HAW complexes located in the Tawang district at an elevation of more than 3700 m (PANIGRAHY et al. 2012). Both Nagula and Bhagajang HAW complexes have alpine to tundra climatic conditions, encompassing more than 360 lakes, along with their

feeding and outflow streams. Apart from the lakes, there are also small pools, which generally turn to small swamps during the summer months. All of the lentic freshwater bodies in this locality are treated as sacred by local religious communities, thus they are mostly pristine.

Diatom collection and treatment. Brown coloured slimy films from small stones were scraped for diatoms using a toothbrush along with the pool water. The resulting brown suspension was transferred to the laboratory in a plastic container. The suspension was cleaned by boiling in concentrated Nitric acid (HNO_3) to oxidize organic matter. Subsequently, the resulting material was alternately centrifuged and rinsed with distilled water several times until the sample attained a neutral pH. The cleaned suspension was air-dried onto coverslips and mounted with Naphrax® mounting medium. Light microscopy (LM) observations were made with an Olympus BX 53 (Tokyo, Japan), equipped with Differential Interference Contrast optics and images were captured with an Olympus DP 73 digital

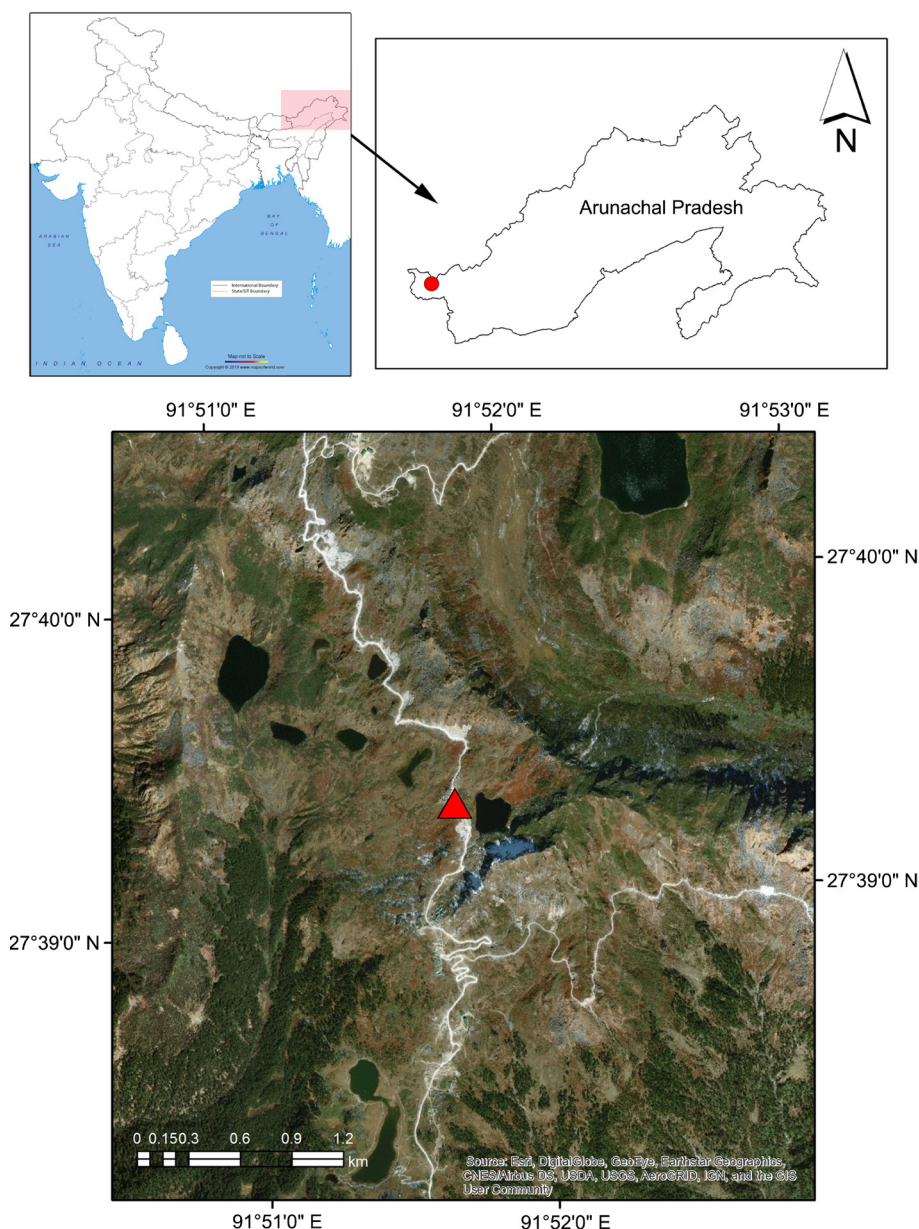


Fig. 1. Location map of the study site in Tawang, Arunachal Pradesh.

camera. The clean material was used for SEM observations. The diatom materials were dried onto glass coverslips and affixed to aluminum stubs with double-sided carbon tape. Stubs with the cleaned material were sputter-coated with gold-palladium with an Emitech K575X sputter coater. SEM observations were performed with an EVO MA15, Carl Zeiss microscope with LaB6 filament. LM and SEM images were processed in GIMP (version 2.8.14) and plates were compiled with Inkscape (version 0.91). Cleaned material and slides are archived at the Diatom Collection at Agharkar Research Institute Herbarium (AHMA).

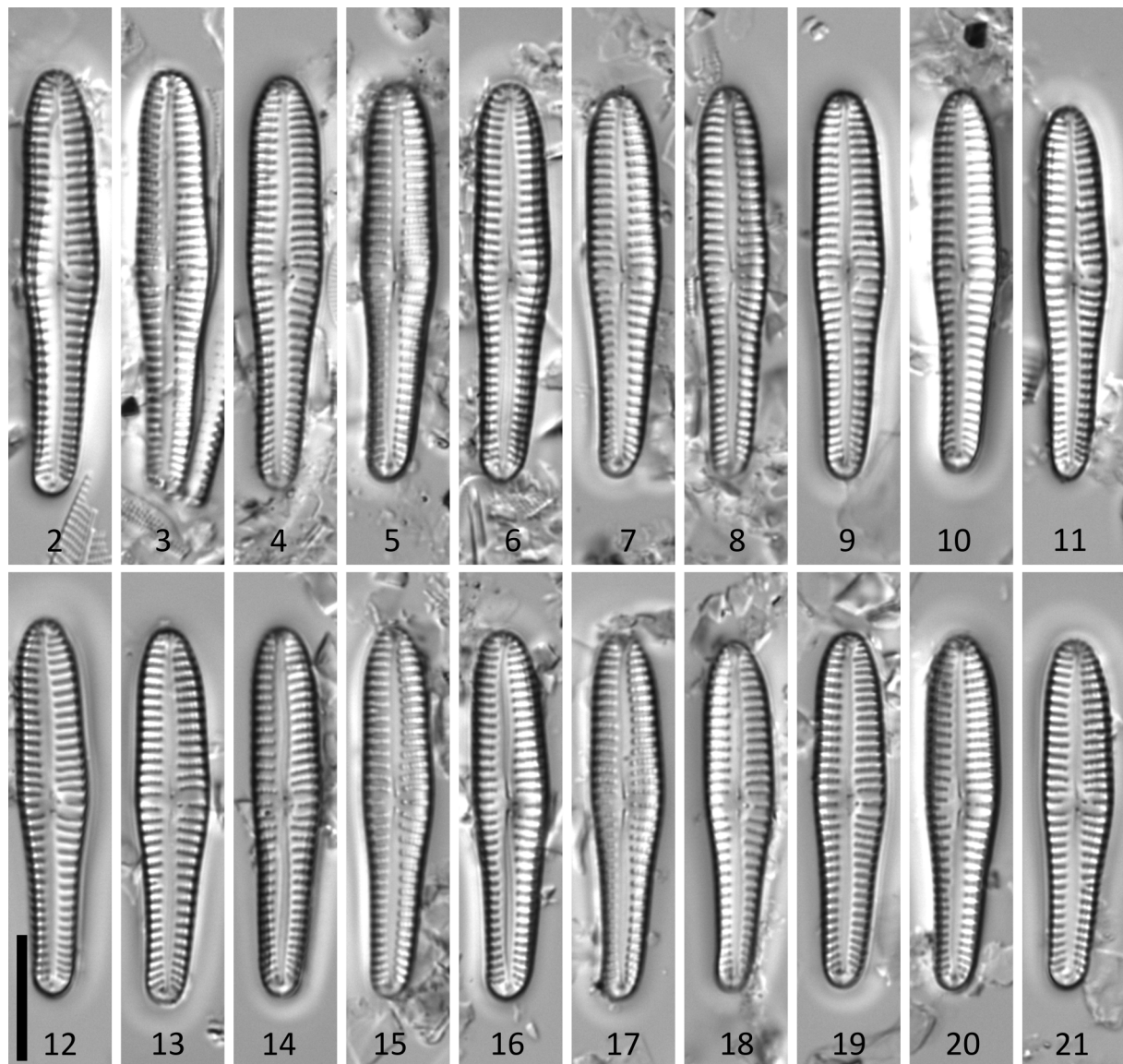
RESULTS

***Gomphonema adiharii* C.Radhakrishnan, Sudipta K.Das, Kocielek et B.Karthick sp. nov. (Figs 2–35)**

LM Description (Figs 2–21): Valves distinctly clavate, clearly asymmetrical about the apical axis, apices broadly

rounded at the head pole, narrowly rounded foot pole. Length (28–34 μm) and breadth (5–6 μm) ($n=72$). Axial area narrow, linear-lanceolate in shape. Raphe lateral, proximal raphe endings slightly undulate and curved distal raphe fissures visible. Isolated round stigma present in the central area. Striae uniseriate, slightly radiate at centre, becoming parallel to radiate near the foot pole (12–14 in 10 μm). The broad central area has one shortened stria on each side. Apical pore field (APF) present at the foot pole.

SEM Description (Figs 22–35): In the SEM, externally, striae are uniseriate, mostly occluded with volae to give “reverse C- or 3-” shaped areolae, which extend to the mantle (Figs 24–26). Raphe undulate, proximal raphe endings deflected to the stigma side (Fig. 26), distal raphe ending curved onto the mantle, in the direction opposite the stigma (Fig. 25). Central area small, an isolated round stigma opening is present (arrow in Fig. 26). The apical pore field is composed of round porelli. Porelli



Figs 2–21. Light microscope images of *Gomphonema adiharii* sp. nov. Valve view showing size diminution series. Scale bar 10 μm .

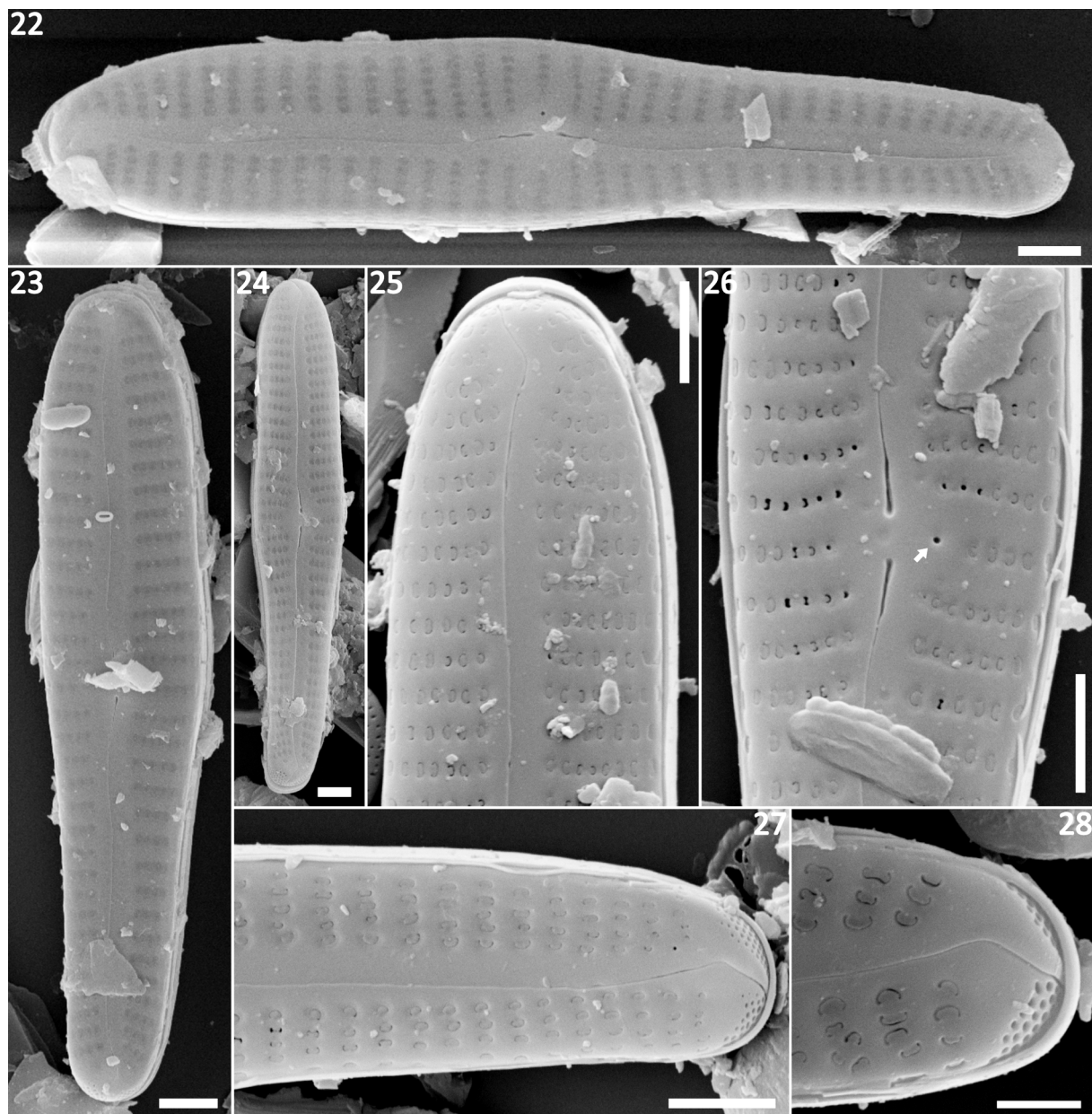
number 56–58/10 μm (measured perpendicular to raphe) (Fig. 28). The pore field is distinctly separate from the areolae, that is it is both morphologically different and physically separated from the areolae (Figs 24, 27–28). Internally, striae are parallel, slightly radiate at the foot pole (Figs 29–31). Raphe almost straight and a prominent helictoglossa is present at the raphe ends (marked as ‘H’ in Figs 32, 34–35). There is a small-sized pseudoseptum present at the headpole and footpole (Fig 32, 34). Internal areolae openings are C- or 3-shaped and present in deep grooves (Figs 32–35). The central nodule is slightly large and elliptical (Fig. 33). Proximal raphe ends are curved. A small elliptical stigma opening is present at one side of the central nodule (Figs 29, 33) (arrow in

Fig. 33). Apical pore fields are covered internally by the pseudoseptum (Figs 34–35).

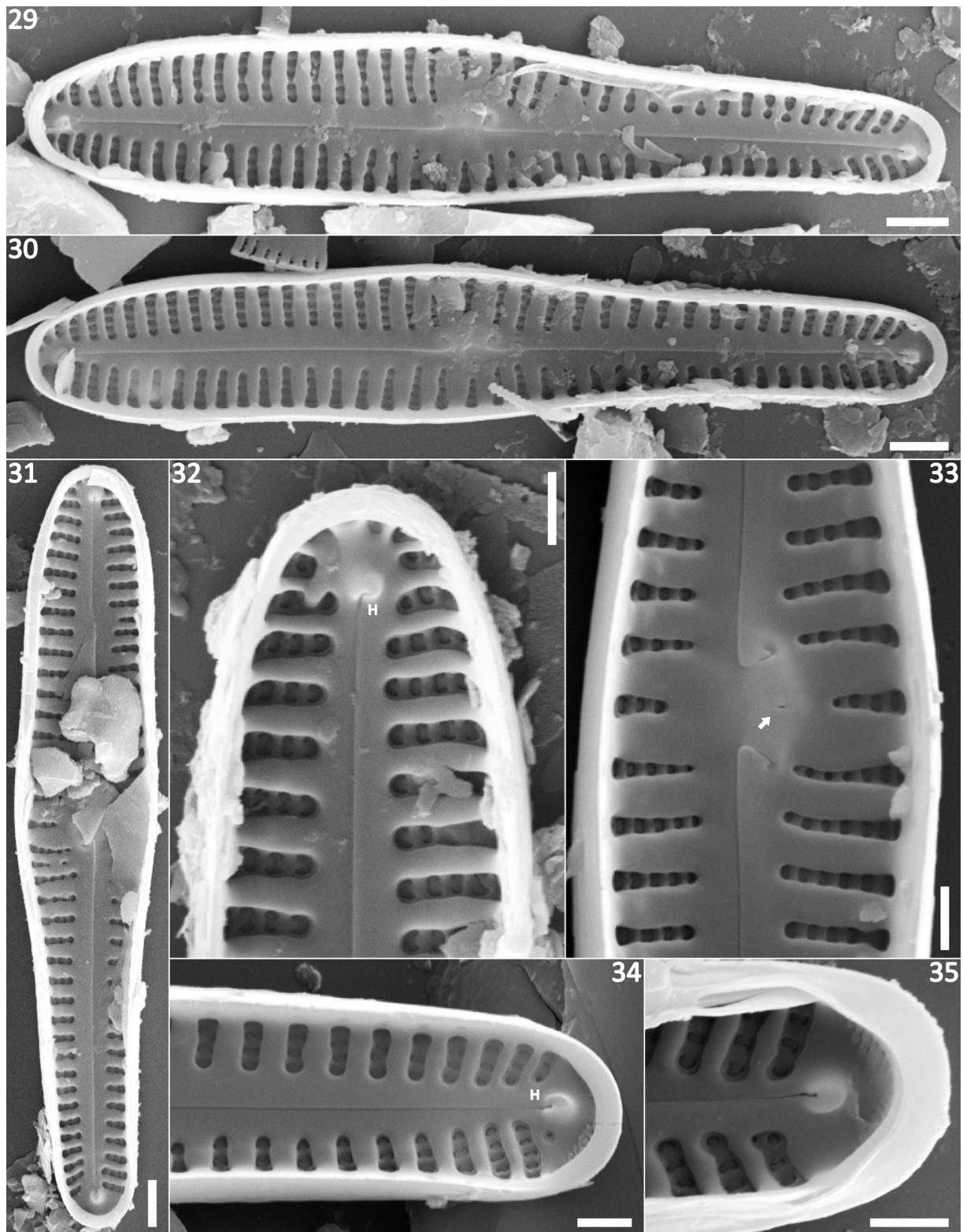
Holotype: Slide #49–065; Sample Accession #2433; deposited at the Diatom Collection, Agharkar Research Institute Herbarium (AHMA), Pune, India.

Type locality: INDIA, Arunachal Pradesh, A pool located at the roadside, On the way to Bumla, Tawang district (27°39'49.7"N, 91°51'32.6"E; elevation 4173 m a.s.l.) (holotype: Agharkar Research Institute Herbarium (AHMA). Collected from an epilithic habitat.

Etymology: Named in the honour of the Indian algologist Prof. Siba Prasad Adhikary, Visva-Bharati, Siksha Bhavana Santiniketan for his phycological contributions in north-eastern India.



Figs 22–28. External view of the scanning electron micrographs of *Gomphonema adhikarii* sp. nov.: (22–24) external view of whole valve showing slightly cymbelloid valves; (25) rounded head pole showing distal raphe end; (26) central area of the valve showing stigma (pointed by an arrow) and proximal raphe ending; (27–28) foot pole showing ‘c’ or rarely ‘3’ shaped areolae and apical pore field. Scale bars 2 μm (22–24), 1 μm (25–28).



Figs 29–35. Internal view of the scanning electron micrographs of *Gomphonema adhikarii* sp. nov.: (29–31) internal view of the whole valve showing helictoglossae and parallel to radiate striae; (32) rounded head pole showing helictoglossae (pointed by 'H') and pseudoseptum; (33) valve centre showing proximal raphe ends and stigma opening (pointed by an arrow); (34–35) foot pole showing helictoglossae (pointed by 'H') and pseudoseptum. Scale bars 2 μm (29–31), 1 μm (32–35).

Table.1 Comparison of morphologically similar species of *Gomphonema adhikarii* sp. nov.

Characters	<i>Gomphonema adhikarii</i> sp. nov	<i>G. mayanae</i>	<i>G. scardicum</i>	<i>G. angusticephalum</i>	<i>G. heilongtunensis</i>	<i>G. jerguckium</i>
Length (µm)	28-34	22.5-36.7	36-63	19.5-49	21-37	20-37
Width at centre (µm)	5-6	5.6-6.9	7.5-9.5	4-6.4	5.1-6.5	5-5.7
Striae (in 10 µm)	12-14	11-13	9-12	10-14	11-14	12
Valve shape	Linear, clavate, undulated margin, slightly cymbelloid due to convex margin on one side near foot pole	Clavate, slightly asymmetrical to apical axis	Linear-lanceolate, largest valve width near mid-valve	Clavate, margin rarely undulate	Clavate, linear	Clavate, lanceolate, slightly cymbelloid
Constriction between head pole and mid-valve	No constriction	No constriction	Weak in larger valves and indistinct in smaller valves	Indistinct to weak	No constriction	No constriction
Head pole	Rounded	Broadly rounded, girdle band with a fimbriate margin	Narrowly rounded to cuncate	Acutely rounded, wider than central area	Wedge shaped acutely rounded	Obtuse, more broadly rounded
Foot pole	Narrowly rounded	Narrowly rounded	Acutely rounded	Acutely round	Narrowly rounded	Narrowly rounded
Raphe	Lateral, proximal endings slightly undulated	Lateral, undulated near proximal end	Lateral, weakly undulated	Lateral, undulated	Lateral, external raphe fissure slightly to moderately undulated	Lateral, slightly undulate
Striae	Uniseriate, parallel to radiate towards foot pole	Uniseriate, slightly radiate at the centre	Uniseriate	Uniseriate, mostly parallel, radiate towards the poles	Uniseriate, parallel in center, with slightly radiate towards apices	Uniseriate, moderately radiate, sometimes almost parallel at the head pole
Areolae	c- or rarely 3- shaped	c- or 3- shaped	c-shaped	c-shaped	c-shaped	c- or weakly 3-shaped
Central area	Broad but small, had one shortened striae on each side	Small with one shortened striae on each side	Small, formed by shortening of single central stria on both valve sides	Small, with single central stria on both valve sides	Small, rounded, with two slightly shortened median striae	Widely roundish, occupying half the width of the valve
Stigmata	Stigma opening round, situated at the end of central striae	Stigma opening round, situated at end of central striae	Stigma opening round, situated at end of central striae	Stigma opening round, in the central area	Stigma clearly separated from striae, close to the central node, stigma opening round	Stigma opening round in external valve. Small and slit-like in internal valve
Axial area	Narrow, linear-lanceolate	Narrow the entire length of the valve	Narrow, linear-lanceolate	Narrowly linear	Narrow at apices, slightly expanded towards the valve centre	Lanceolate, widening towards central area
Reference	Present study	Das et al. 2018	Mitić-Korovina et al. 2014	Reichardt 1999 Kurovskiy et al. 2015	Li et al. 2010b	Reichardt 2009

DISCUSSION

The newly-described *Gomphonema* species has several morphological features such as asymmetrical to apical axis, swollen valve centre, and rounded apices which differentiate it from all other known species, in particular, the species described from the Himalayan region (JÜTTNER et al. 2004, 2018; KARTHICK et al. 2015; DAS et al. 2018). A comparative account of the morphology of *Gomphonema adhikarii* and taxa that appear morphologically similar are presented in the Table. 1.

Our new species is most similar under the LM to *G. mayamae* (fig 1–21 in DAS et al. 2018), a species that is also asymmetric to both the apical and transapical axes and described from the Eastern Himalayan region of India (DAS et al. 2018). The two species differ in valve shape (*G. adhikarii* sp. nov. has a more undulate valve outline, the headpole is less quadrate and the footpole is more broadly rounded than in *G. mayamae*). The two species also differ in that under the electron microscope *G. mayamae* was described to have a fimbriate girdle band structure not described elsewhere amongst the freshwater gomphonemoid diatoms (DAS et al. 2018). This type of girdle band structure is apparently missing in *G. adhikarii* sp. nov.

The newly described species bears some resemblance with *G. heilongtanensis* Li, Kociolek et Metzeltin (fig 35–70 in LI et al. 2010b), described from Tibet. Though the valve shape and dimensions match one another, the distinctly cuneate headpole in *G. adhikarii* differentiates it from the species described from China.

It is common to separate the Cymbellales into those taxa that possess asymmetry about the apical axis ('cymbelloid' diatoms) from those with asymmetry about the transapical axis ('gomphonemoid' diatoms). In fact, in both groups, there are species that possess both symmetry types. In the cymbelloids, both *Gomphocymbellopsis* Krammer and *Didymosphenia* M. Schmidt (e.g. *D. curvirostrum* (Tempère et Brun in Brun et Tempère) M. Schmidt; see KOCIOLEK & STOERMER 1988) have species with asymmetry about the transapical axis. And in the gomphonemoid lineage, *Afrocybella* Krammer, a genus endemic to East African Rift Valley lakes that is more closely related to gomphonemoid diatoms than its name suggests (KOCIOLEK & STOERMER 1988) is diagnosed by having species with both cymbelloid and gomphonemoid symmetry. Within *Gomphonema*, species with asymmetry about the apical axis have been named but considered as possible teratologies by MAYER (1917, 1919) and named by FRICKE (1902). These taxa are known from Europe, Asia and North America (MAYER 1919; FRICKE 1902; DAS et al. 2018). It seems likely that this feature of asymmetry about both the apical and transapical axes in members of the Cymbellales has been achieved independently many times, rather than a feature that is diagnostic for a natural group of taxa.

Recently many novel taxa representing multiple lineages of diatoms were described from Asia, in

particular, tropical Asia (GLUSHCHENKO et al. 2019a, b; THACKER et al. 2019; LIU et al. 2019; ROY et al. 2019; KULIKOVSKIY et al. 2020). These discoveries confirm that Asia is the hotspot of diatom diversification as proposed by KOCIOLEK (2019) and the same finding need to be strengthened using molecular phylogenetic tools.

ACKNOWLEDGEMENTS

Authors thank the Directors of Agharkar Research Institute and Botanical Survey of India for facilities and encouragement. This work was supported by the Department of Biotechnology, India (BT/17/NE/TAX). We are also thankful to the Dept. of Environment & Forests, Govt. of Arunachal Pradesh for providing necessary permission (CWL/Gen/173//2018–19/Pt.VII/4097–109) for sampling. We are also thankful to the reviewer and editor for the constructive comments.

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