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Encyonema keshrii, sp. nov.: a new diatom species (Cymbellales, Bacillariophyceae) from the Indian subcontinent

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Encyonema keshrii, sp. nov.: a new diatom species (Cymbellales, Bacillariophyceae) from the Indian subcontinent

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ABSTRACT
Despite more than 150 years of diatom research in India, cymbelloid taxa have rarely been investigated when compared to the other parts of the world. A new freshwater species of *Encyonema* Kützing was discovered from the mountain ranges of Peninsular India and is described based on light and scanning electron microscopy observations. This paper provides a comparison of the new taxa with morphologically similar taxa of the genus *Encyonema*. *Encyonema keshrii*, sp. nov., is characterized on the basis of distinctive morphological characters including valve and apex shape as well as striae density. *Enccyonema keshrii*, sp. nov., is found to be a wide spread taxon with distribution across the Eastern Ghats, Western Ghats and the Eastern Himalayas of Indian subcontinent.

KEY WORDS
Bacillariophyta,
Encyonema,
India,
ultrastructure,
Himalayas,
new species.

RÉSUMÉ
Encyonema keshrii*, sp. nov. : une nouvelle espèce de diatomées (Cymbellales, Bacillariophyceae) du sous-continent indien.*
Malgré plus de 150 ans de recherche sur les diatomées en Inde, les taxons cymbelloïdes ont rarement été étudiés par rapport aux autres régions du monde. Une nouvelle espèce d'eau douce d'*Encyonema* Kützing a été découverte dans les chaînes de montagnes de l'Inde péninsulaire et décrite sur la base d'observations au microscope optique et électronique à balayage. Cet article fournit une comparaison des nouveaux taxons avec des taxons morphologiquement similaires du genre *Encyonema*. *Encyonema keshrii*, sp. nov., est caractérisé sur la base de caractères morphologiques distinctifs, notamment la forme de la valve et de l'apex ainsi que la densité des striae. *Encyonema keshrii*, sp. nov., est un taxon très répandu, réparti dans les Ghâts orientaux, les Ghâts occidentaux et l'Himalaya oriental du sous-continent indien.

MOTS CLÉS
Bacillariophyta, *Encyonema*, Inde, ultrastructure, Himalaya, espèce nouvelle.

INTRODUCTION

The cymbelloid diatoms are mostly freshwater taxa and are distributed worldwide. Agardh (1830: 1) erected the genus *Cymbella* C.Agardh to incorporate both free-living and stalked cymbelloid diatoms. Shortly after this, Kützing (1833: 61) separated the cymbelloid taxon which live in mucilaginous tubes or in mucilagenous colonies into the monospecific genus *Encyonema* based on the typus generis *E. paradoxum* Kützing (1833: 589). Later, Silva *et al.* (2013) observed the original material and lectotypified *E. paradoxum*. For the first time, Silva & Nogueira (2015) observed and documented the ultrastructural details of *E. leibleinii* (C.Agardh) W.J.Silva, R.Jahn, T.A.V.Ludwig & M.Menezes and *E. lacustre* (C.Agardh) Pantocsek from the type material. Most of the 19th century authors classified *Cymbella* sensu lato based on the life-form concept (i.e. stalked, found in mucilaginous tubes, etc.). Krammer (1982) for the first time established three subgenera *Encyonema*, *Cymbella* and *Cymbopleura* Krammer (1982: 20) within *Cymbella* on the basis of morphological characters like deflection of external distal raphe fissures, the presence of colony formation and presence and position of the stigmata, apical pore fields. Afterwards, Krammer (1997a, b) revised the cymbelloid taxa and (re)established eight cymbelloid genera including *Cymbella* C.Agardh emend. Krammer (1997a: 11), *Encyonema* Kützing (1833: 589), *Cymbopleura* (Krammer) Krammer (1999: 284), *Cymbellopsis* Krammer (1997a: 157), *Encyonopsis* Krammer (1997a: 156), *Navicella* Krammer (1997a: 12), *Gomphocymbella* O.F.Müller (1905: 12) and *Pseudencyonema* Krammer (1997a: 157). Later, Krammer (2003) proposed another four new genera including *Delicata* Krammer (2003: 110), *Gomphocymbellopsis* Krammer (2003: 127), *Afrocymbella* Krammer (2003: 129) and *Navicymbula* Krammer (2003: 123) to replace *Navicella.* Very recently, further two cymbelloid genera were erected: *Oricymba* Jüttner, Krammer, E.J.Cox, Van de Vijver & Tuji (Jüttner *et al.* 2010a: 408) and *Kurtkrammeria* L.Bahls (2015: 170). Among these two, *Oricymba* is well represented across the various biogeographic zones of the Indian subcontinent (Radhakrishnan *et al.* 2018) and descriptions of many new species are awaited from our own work from the Western Ghats and Eastern Himalayas.

Encyonema has more than 400 described taxa (Kociolek *et al.* 2018). This dorsiventral biraphid genus is mainly characterized by a strongly curved dorsal margin, terminal raphe endings bent towards the ventral side, and usually without stigmata or apical pore fields (Round *et al.* 1990, Taylor & Cocquyt 2016). *Encyonema* species are found in freshwater benthic habitats with low electrolyte content (Krammer 1997b). This genus is very distinct from the two other closely related genera i.e. *Encyonopsis* and *Cymbellopsis* (Krammer 2002). *Encyonema* has a high degree of dorsiventrality and can thus be easily distinguishable from *Encyonopsis* and on the other hand *Encyonema* is distinguished from *Cymbellopsis* by the presence of quite irregular areolae and some of them often form clusters and foramina are consist of either delicate apically elongated slots or irregularly x-shaped openings (Krammer 1997b: fig. 198). As cymbelloid diatoms are a highly diverse group, new taxa are regularly described across the world (Rumrich *et al.* 2000; Kellogg & Kellogg 2002; Metzeltin *et al.* 2005; Metzeltin & Lange-Bertalot 2007; Le Cohu & Azémar 2011; Bahls 2013; Bahls 2015; Marquardt *et al.* 2016, 2017).

In spite of over 150 years of diatom study on the Indian subcontinent, most of the cymbelloid taxa remain undocumented. H. P. Gandhi, a pioneer of diatom studies in India, discovered eight new *Cymbella* taxa (Karthick 2009) and Radhakrishnan *et al.* (2018) transferred one of these *Cymbella* taxa into *Oricymba*. Sarode & Kamat (1984) reported another ten new *Cymbella* taxa and recently, Vigneshwaran *et al*. (2019) discovered a new species of *Cymbella* from the Western Ghats. Four new *Oricymba* taxa (Jüttner *et al.* 2010a), four new *Cymbella* taxa (Jüttner *et al.* 2010b) and two new *Cymbopleura* taxa (Van de Vijver *et al.* 2011) were reported from Nepalese Himalayas. However, there has till now not been a single new *Encyonema* reported from India.

In the present paper, we describe and illustrate a new *Encyonema* taxon (*E. keshrii,* sp. nov.) from the Eastern Ghats, India, based on light and scanning electron microscopy. Further, this taxon is also found from the Western Ghats and Eastern Himalayas. Despite considerable literature searches, only few somewhat similar taxa were found with which it is compared.

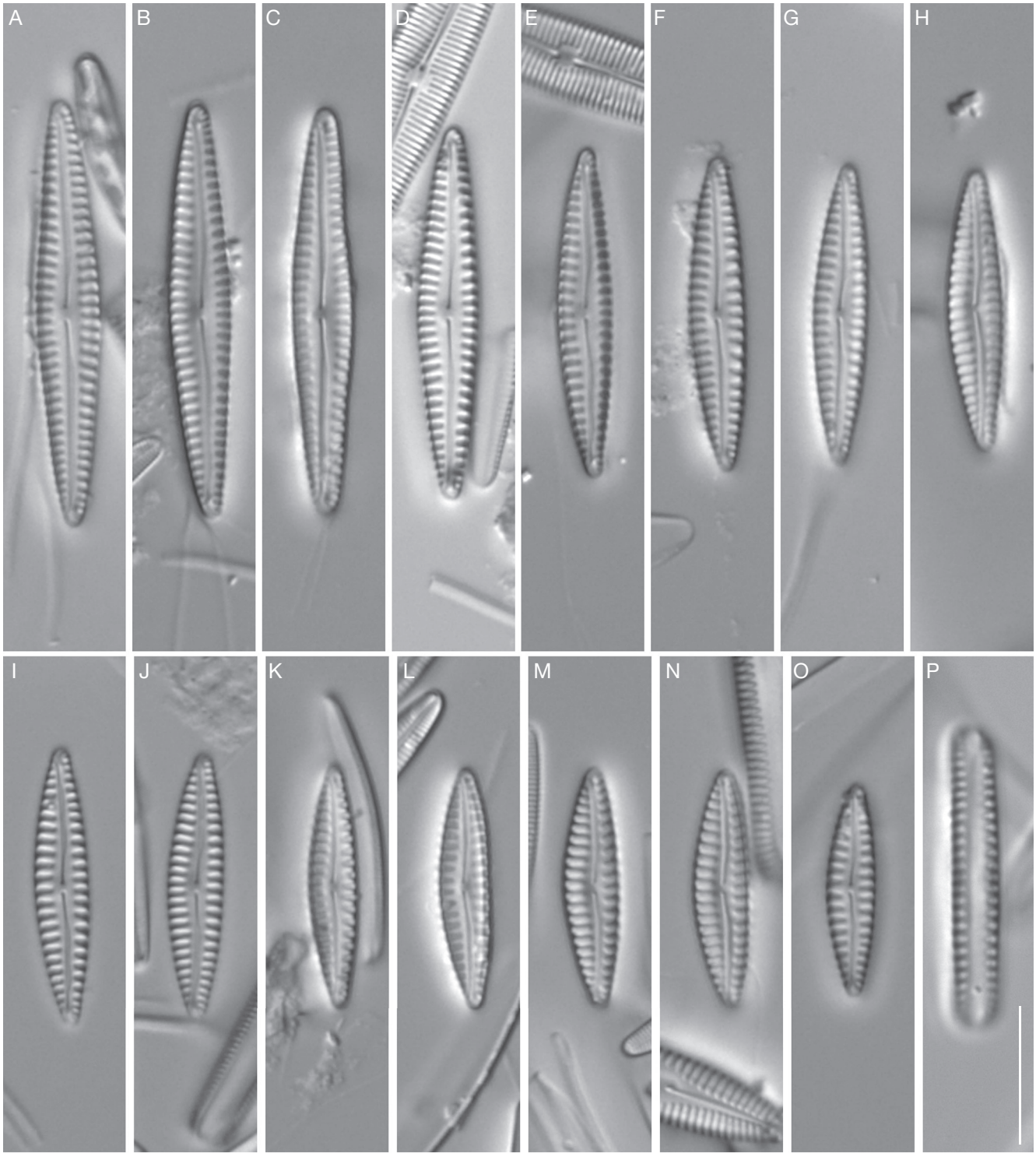

Fig. 1. — Light microscopy of *Encyonema keshrii*, sp. nov., from the type population: **A**-**O**, valve views showing the size diminution series; **P**, girdle view; **F**, holotype. Scale bar: 10 µm.

MATERIAL AND METHODS

The diatom sample used for the description of the new species was collected from the Kolli Hills (locally known as Kolli Malai) of Tamil Nadu State in India. This is a small mountain range which rises to 1300 m a.s.l. in height and covers an area of approximately 280 km² and is the part of Eastern Ghats mountain ranges. Karthick *et al.* (2017) described two freshwater species of *Achnanthidium* Kützing from this site and discussed in detail the geology and geography of the Kolli Hills. The sample examined in the present study was collected from Masilla Waterfalls (11.3034°N, 78.3939°E, altitude 1121 m a.s.l.) of Kolli Hills on 2 December 2017.

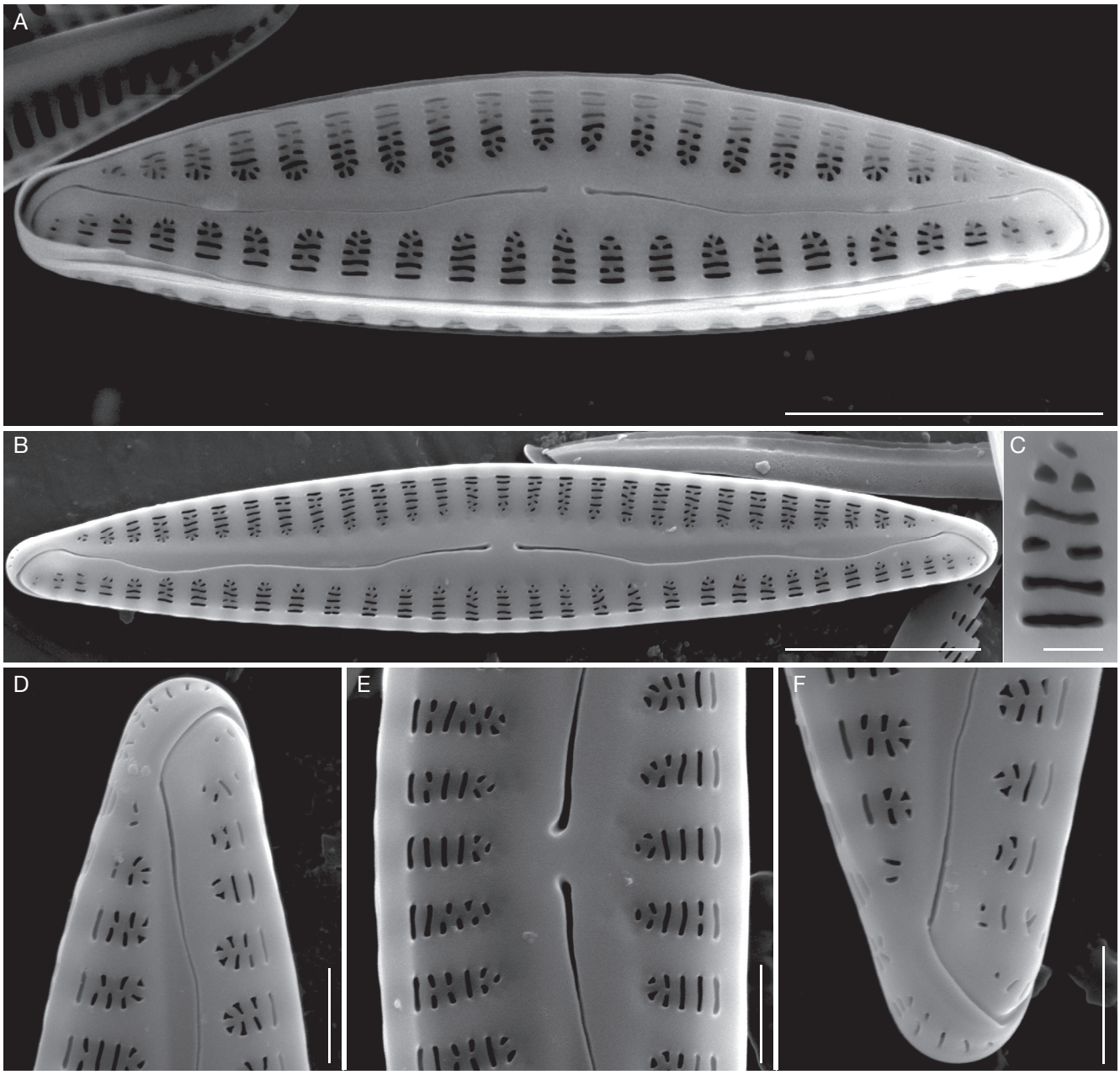

Fig. 2. — Scanning electron micrographs of valve exterior of *Encyonema keshrii*, sp. nov.: **A**, **B**, whole valve showing slightly dorsiventral, lanceolate to linear-lanceolate valves, raphe and striation pattern; **C**, showing unique lineolate areolus; **D**, **F**, details of marginal and apical pole showing distal raphe ends, mantle poroids; **E**, middle portion of the valve showing proximal raphe ends. Scale bars: A, B, 5 µm; C, 0.25 µm; D-F, 1 µm.

Benthic diatom samples were collected by scraping rocks from the Masilla Waterfalls and associated spray zone which were then transferred into Whirl-Pak® storage bags. *E. keshrii*, sp. nov., was also found at Sharavathi River at Padambile village near Jog Falls in Karnataka in the Western Ghats (14.22231°N, 74.82821°E, altitude 479 m. a.s.l.; sample no. AHMA #1557) from an epilithic habitat. The sample from the Western Ghats is discussed in order to give information on the distribution of this taxon; however the description is based solely on the sample from the Eastern Ghats sample. Water quality variables such as pH, water temperature, dissolved oxygen of the Western Ghats site (JogSharavathi River, Karnataka) were measured using HACH (United States; Loveland, Colorado) HQ40D portable multiparameter. Nitrate and phosphate were measured using a portable colorimeter HACH 1900 using HACH standard reagents (NitraVer® 3 Nitrate and NitraVer® 6; PhosVer® 3 Phosphate).

A sub-sample of the collected material was cleaned, or acid digested, by using HNO_3 to remove organic matter. The sample was rinsed 5-6 times with distilled water through repeated centrifugation by REMI R-8C BL (Mumbai, India) at 3000-3500 rpm until the pH was neutral. An aliquot of the cleaned sample was then air dried onto glass coverslips and the coverslips subsequently mounted onto microscope slides using Naphrax® as the mounting medium (Karthick *et al.* 2010).

The mounted slides were observed using a Olympus BX53 microscope (Tokyo, Japan) equipped with an Olympus DP74

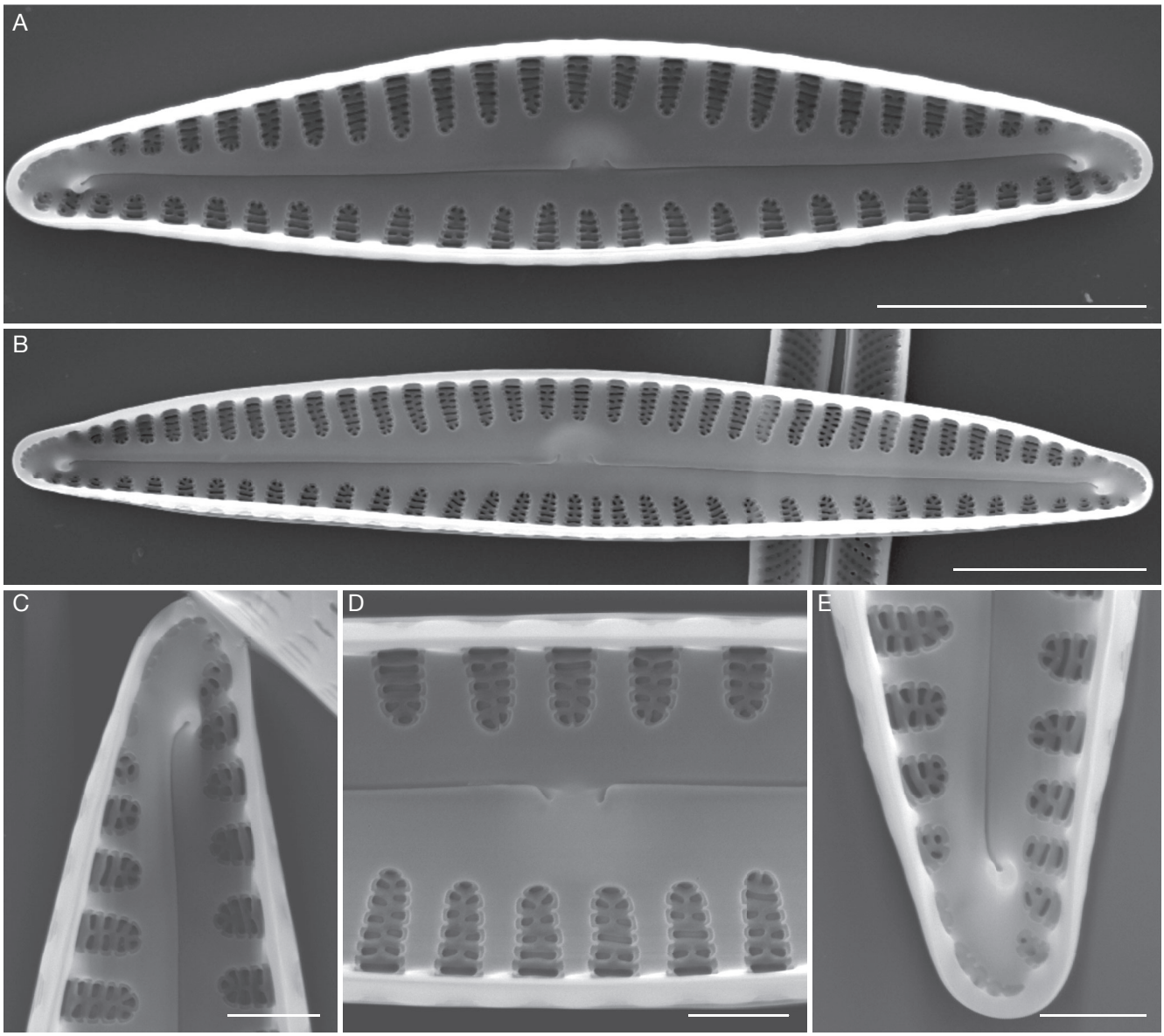

FIG. 3. — Scanning electron micrographs of valve interior of *Encyonema keshrii*, sp. nov.: **A**, **B**, whole valve view, with raphe and striation pattern; **C**, **E** valve apices, with small helictoglossae; **D**, central area, showing proximal raphe ends. Scale bars: A, B, 5 µm; C-E, 1 µm.

digital camera and observed with 100x objective [numerical aperture (n.a.) 1.42] using differential interference contrast (DIC) microscope optics and images were taken with Olympus cellSens standard 1.16 imaging software. For scanning electron microscope (SEM) studies, aliquots of the cleaned material were air dried on a fragment (5 × 5 mm) of vinyl phonograph record (MacGillivary & Ehrman 2011). The vinyl fragment was fixed to aluminium stubs using double sided carbon tape, sputter-coated with gold palladium and specimens observed using a FEI Quanta 2000 F-SEM (10 kV, WD 10 mm). For girdle views (Fig. 4) the vinyl fragment was placed on aluminium stubs with carbon tape and coated with gold using Emitech K575X (Quorum Technologies; Lewes, United Kingdom) sputter coater and observed on a EVO® MA 15 Zeiss (Oberkochen, Germany) scanning electron microscope. Both cleaned material along with original samples, permanent slides are stored in the herbarium (AHMA) at the Agharkar Research Institute, Pune, India.

Morphological terminology used follows Barber & Haworth (1981) and Round *et al.* (1990).

RESULTS

Encyonema keshrii S.Roy, C.Radhakrishnan, J.C.Taylor, Kulikovskiy & B.Karthick, sp. nov. (Figs 1-6)

HOLOTYPE SPECIMEN. — Slide no. #24-73 from sample no. #1187 (Holotype here designated, illustrated in Fig. 1F), Collected on 2 December 2017 and deposited in at the Agharkar Research Institute Herbarium (AHMA), Diatom Section, India.

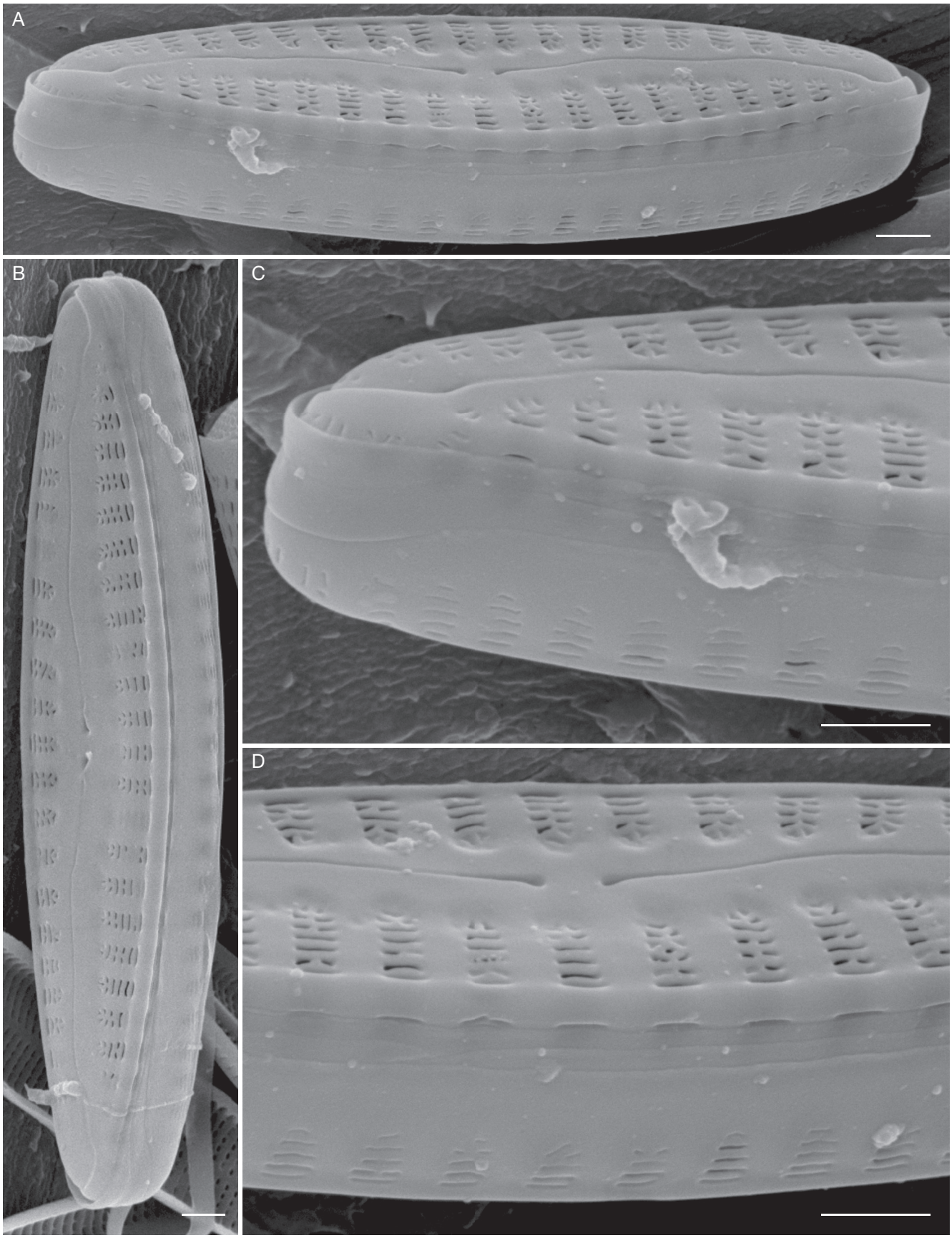

Fig. 4. — Scanning electron micrographs of girdle view of *Encyonema keshrii*, sp. nov.: **A**, dorsal side; **B**, ventral side of whole valve, showing mantle areolae and girdle bands; **C**, valve apex covered with girdle bands and transapical areolae present on the valve mantle; **D**, central area showing the mantle areolae, ribbed valve margin and layers of girdle bands. Scale bars: A-D, 1 µm.

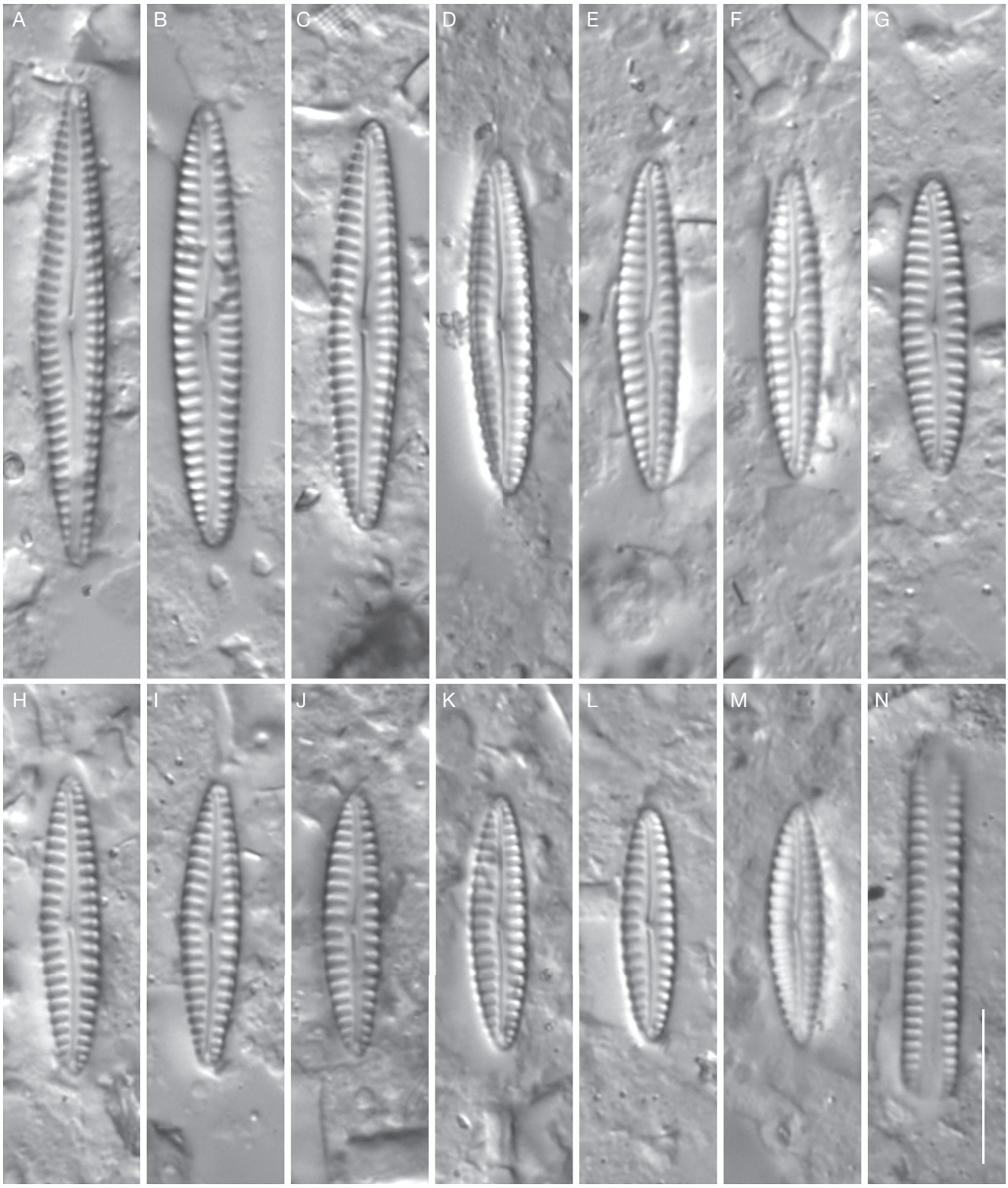

Fig. 5. — Light microscopy of *Encyonema keshrii*, sp. nov., represented from the Sharavathi River sample: **A**-**M**, valve views showing the size diminution series; **N**, girdle view. Scale bar: A-N, 10 µm.

Type Locality. — Masilla Waterfalls (locally known as Masilla Aruvi), Aariyur Panchayat, Kolli Hills (11.3034°N, 78.3939°E, altitude 1121 m above sea level), Namakkal district, Tamil Nadu, India.

Habitat. — Epiphytic in lotic freshwater.

Etymology. — This species is dedicated to the Indian Phycologist Prof. Jai Prakash Keshri (The University of Burdwan, West Bengal, India) whose contribution to Indian phycological research is hereby acknowledged and who was the Ph.D. supervisor of SR.

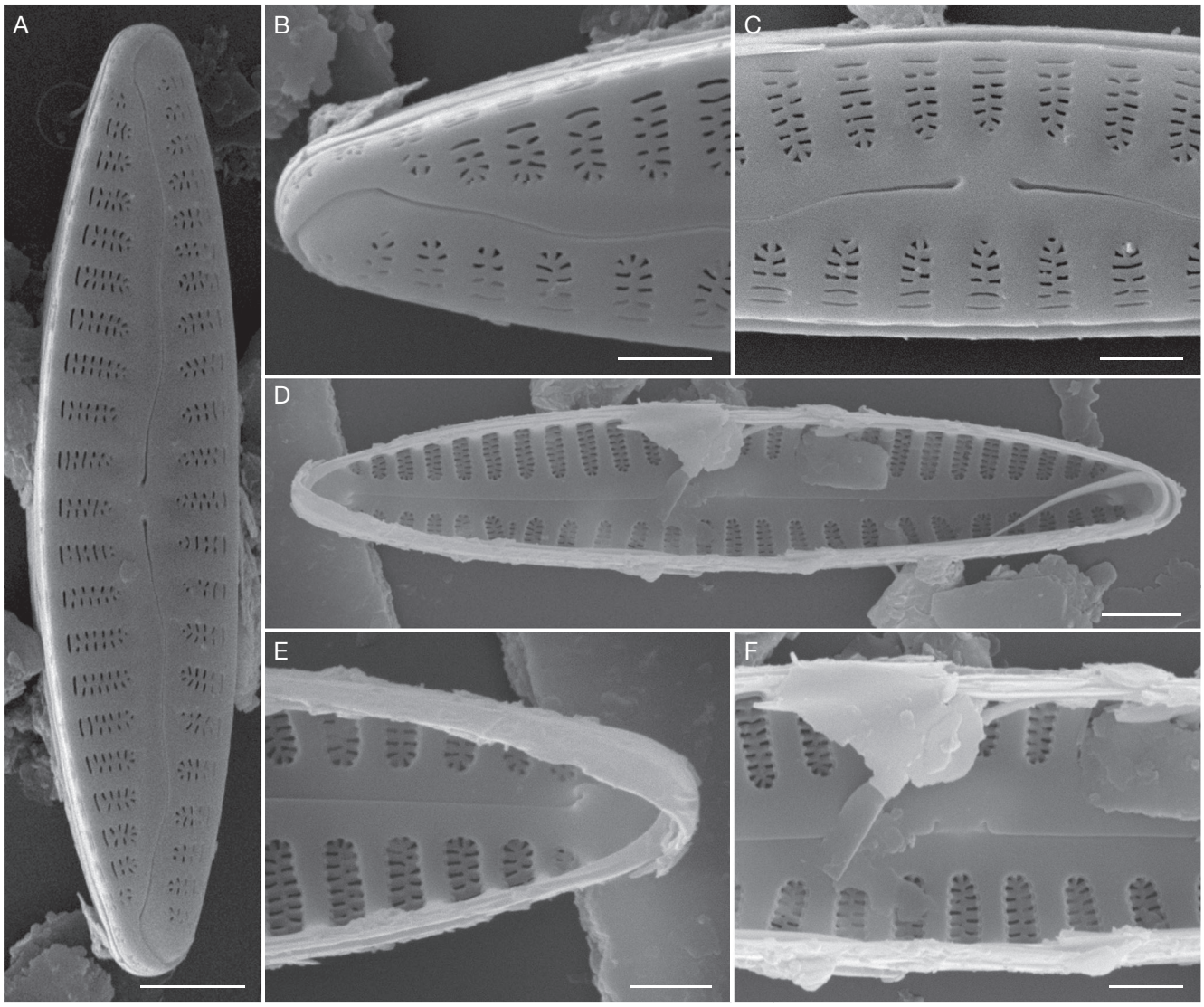

Fig. 6. — Scanning electron micrographs of *Encyonema keshrii*, sp. nov., represented from the Sharavathi River sample: **A**-**C**, valve exterior ultrastructures; **D**-**F**, valve interior ultrastructures. Scale bars: A-F, 1 µm.

Ecology and distribution. — This species is known from the Eastern Ghats (Masilla Waterfalls, Kolli Hills Tamil Nadu), the Western Ghats (Sharavathi River at Padambile village near Jog Falls, Karnataka) and the Eastern Himalayas (stream near Nongstoin Town, Meghalaya). Across all the sites, it occurs in low conductivity and oligotrophic conditions.

Additional material. — Figs 5; 6 show LM and SEM views of the new taxon from Sharavathi River in Karnataka of the Western Ghats. All morphometric data (n = 55) (L = 15.30-31.34 µm, W = 3.56-4.55 µm with 12-14 striae in 10 µm, n = 55), valve shape and characteristic observed under SEM matched with *E. keshrii*, sp. nov. The water quality recorded for the Western Ghats sampling site was pH = 7.72, dissolved oxygen (DO) = 7.66 mg/L, water temperature = 28.6 °C, nitrate = 0.3 mg/L, phosphate = 2.26 mg/L.

Description

LM (Fig. 1)

Valves lanceolate to linear-lanceolate, slightly dorsiventral, weakly heteropolar with rounded to acute apices, never protracted. Valves 15.33-30.82 µm long and 3.50-4.67 µm broad with 13-14 striae in 10 µm (n = 51). Areolae are not discernible in LM. Raphe weakly lateral with proximal endings slightly curved towards the dorsal margin, endings rounded and pore-like. Distal raphe endings sickle-shaped and curved towards the ventral margin. Stigma absent. Axial area narrow, lanceolate widening slightly in the centre but no distinct central area is present.

SEM (Figs 2-4)

In external valve view (Fig. 2) striae composed of one to four linear areolae (Fig. 2A, B) (compare to internal view of valve: Fig. 3C-E). Striae interrupted at the valve margin and then continue on the valve mantle (Figs 2A; 4A, D). Raphe lateral and curving towards the dorsal margin in the central region (Fig. 2B). A higher number of areolae in each striae occur adjacent the axial area with the final areolae near the margin being a single large lineolate areolus (Fig. 2C). Areolae do not appear to be occluded by cribra or hymenes and are 33-41 in 10 µm (Fig. 2A, B). Mantle perforated

TABLE 1. — Comparison of morphometric data of *Encyonema keshrii*, sp. nov. and closely related *Encyonema* taxa from different sources (– denotes "data not available").

	E. africanum	***E. neocaledonicum***	***E. oestrupii***	***E. sparsipunctatum***	***E. subhinzae***	***E. thioense***	***E. yucatanense***	***E. directiforme***	***E. keshrii*, sp. nov.**
References	Krammer (1997b)	Krammer (1997b)	Krammer (1997b)	Krammer (1997a)	Krammer (1997b)	Moser et al. (1998)	Metzeltin & Lange-Bertalot (1998)	Krammer (1997a)	Present study
Length (µm)	18.9-40	22-43	21-31	19-42	24-34	26-42	30-42	23-41	15.33-30.82
Width (µm)	4.8-6.2	5-6.8	4.3-5.2	5-7	4.7-5.7	5.6-6	6.4-6.7	4.7-5.9	3.50-4.67
Striae in 10 µm	12-14	14-16	11-13	11-12	12-13	11-13	9-11	13-17	13-14
Areolae in 10 µm	38-42	28-32	–	16-19	24-26	–	–	32-36	33-41
Valve shape	barely dorsiventral, lanceolate	slightly dorsiventral, linear-lanceolate	slightly dorsiventral, lanceolate	slightly dorsiventral, lanceolate	moderatly to slightly dorsiventral, lanceolate	slightly dorsiventral, lanceolate	slightly dorsiventral, lanceolate	slightly dorsiventral, lanceolate	slightly dorsiventral, lanceolate to linear-lanceolate, weakly heteropolar
Apices	protracted, rounded and slightly curved towards ventral side	not protracted, rounded	protracted and rounded, sometimes slightly curved towards ventral side	not protracted, acuminately rounded or beaked	not protracted, acuminately rounded	protracted, semicapitate and narrowly rounded	not protracted, rounded or subrostrate, slightly bent towards ventral side	protracted, acuminately rounded, slightly curved towards ventral side	never protracted, rounded to acute apices

by small linear poroids at the apices (Fig. 2D, F). Proximal raphe endings curved towards the dorsal margins, pore like and slightly expanded (Fig. 2E). Distal endings curving over the junction of the valve face and mantle (Fig. 2D, F). Internal valve view (Fig. 3) shows striae separated by distinct virgae (Fig. 3A, B). Distal raphe endings terminate in small helictoglossae which are slightly angled towards the ventral margin (Fig. 3C, E), proximal raphe endings simple and curved abruptly towards the dorsal margin (Fig. 3D). In external girdle view (Fig. 4), the cingulum is composed by 2-3 open bands or copulae. Mantle of each valve contains single row of areolae on both dorsal (Fig. 4A, C, D) and ventral side (Fig. 4B).

DISCUSSION

The new species of *Encyonema* described here shares certain morphological characters with eight similar looking species in this genus. Based on the available literature, a summary is presented in Table 1. From Table 1 it is clear that though *Encyonema africanum* Krammer (1997b: 67) does not differ from *E. keshrii*, sp. nov., in respect to length, striae density and areolae density, but it does differ from *E. keshrii*, sp. nov., by higher valve width (4.8-6.2 µm). *E. keshrii*, sp. nov., has non-protracted apices and has a different valve outline compared to *E. africanum*. *Encyonema neocaledonicum* (Manguin) Krammer (1997b: 75) has non-protracted apices and a similar valve shape to *E. keshrii*, sp. nov., but its striae density (14-16 in 10 µm), width (5-6.8 µm) and length (22-43 µm) are higher. Although *Encyonema oestrupii* Krammer & Lange-Bertalot (Krammer 1997b: 68, 191) has a very similar valve shape and length, it can nevertheless be easily differentiated because of protracted and slightly curved apices, higher width (4.3-5.2 µm) and slightly lower striae density (11-13 in 10 µm) than that of *E. keshrii*, sp. nov. *Encyonema sparsipunctatum* Krammer (1997a: 179) can be easily distinguished from *E. keshrii*, sp. nov., by very low areolae (16-19 in 10 µm) and striae (11-12 in 10 µm) density, higher width (5-7 µm) and a lanceolate valve shape. *Encyonema subhinzae* Krammer (1997b: 6) and *Encyonema yucatanense* Metzeltin & Krammer (Metzeltin & Lange-Bertalot 1998: 38) both have non-protracted apices, very high striae density (12-13 in 10 µm) and a somewhat similar valve shape to *E. keshrii*, sp. nov., but *E. subhinzae* has broader valves (4.7-5.7 µm) and low areolae density (24-26 in 10 µm). On the other hand, *E. yucatanense* has longer (30-42 µm) and broader (6.4-6.7 µm) valves with less striae density (9-11 in 10 µm). *Encyonema thioense* Lange-Bertalot & Krammer (Moser *et al.* 1998: 129-130) and *Encyonema directiforme* Krammer & Lange-Bertalot (Krammer 1997a: 179) both have to some extent a similar valve shape as that of *E. keshrii*, sp. nov., but cannot be confused as both *E. thioense* and *E. directiforme* have protracted valve apices. In addition, *E. thioense* has larger (26-42 µm) and broader (5.6-6 µm) valves and slightly low striae density (11-13 in 10 µm) compared to *E. keshrii*, sp. nov., by *E. directiforme* also has a larger (23-41 µm) and broader (4.7-5.9 µm) valves, very high striae density (13-17 in 10 µm) and acuminately rounded apices.

The Eastern Ghats sample (sample no. #1187) i.e. the type sample was collected from the spray zone of a waterfall from epilithic habitats and can be considered tolerant to aerophilic conditions. The Western Ghats sample (sample no. #1557) was collected from the epilithon and the water quality parameters show the stream to be oligotrophic.

This new taxon exhibits rounded to acute apices. The type population has more than 90% of the examined cells having acute valve apices, whereas the Western Ghats population showed majority of cell with rounded apices. Phenotypic plasticity could be the reason behind this type of morphological variation present among different populations spread across the Ghats of the Peninsular India. Additionally, the same taxon was also observed from epilithic habitats in a stream in Meghalaya state of India (sample no. #2078), which rises in the Eastern Himalayas. In type locality the new species co-occurred with *Achnanthidium initium* B.Karthick, J.C.Taylor & P.B.Hamilton, *Achnanthidium linannulum* B.Karthick, J.C.Taylor & P.B.Hamilton, *Navicula obtecta* I.Jüttner & E.J.Cox and two unidentified gomphonemoid diatoms. The Western Ghats sample contains *Achnanthidium catenatum* (Bily & Marvan) Lange-Bertalot, *Achnanthidium linannulum* B.Karthick, J.C.Taylor & P.B.Hamilton, *Gomphonema gandhii* B.Karthick & Kociolek, *Staurosirella pinnata* (Ehrenberg) D.M.Williams & Round and *Humidophila contenta* (Grunow) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová. The Eastern Himalayas sample shows *Navicula obtecta* I.Jüttner & E.J.Cox, *Achnanthidium* cf. *gracillimum*, one another unnamed *Achnanthidium* and *Encyonema* species. These observations confirms that this species is widespread in distribution spanning three biogeographic zones (Eastern Ghats, Western Ghats, and Eastern Himalayas) of the Indian subcontinent and prefers oligotrophic waters from the mountainous regions. The molecular characterization at the population level from various biogeographic zones may demonstrate the real diversity of this species.

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