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Two new aerophilic species of *Stauroneis* Ehrenberg (Bacillariophyta) from the Eastern Himalayas

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ARTICLE

Two new aerophilic species of *Stauroneis* Ehrenberg (Bacillariophyta) from the Eastern Himalayas

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ABSTRACT

Two new diatom taxa belonging to the genus *Stauroneis* Ehrenberg were discovered from an aerophilic habitat in Sikkim, India, a part of the Eastern Himalayas. The morphology of *Stauroneis sikkimensis sp. nov*. and *Stauroneis lepchae sp. nov*. are illustrated with light and scanning electron micrographs and compared with similar species in the genus. *S. sikkimensis* is characterised by lanceolate valves with a median constriction, bluntly rounded apices, radiate striae, and a H-shaped stauros. *S. lepchae* has broadly elliptical to lanceolate valves with obtusely rounded apices, radiate striae, and a characteristic bow-tie shaped stauros. These are the first two diatom species described from the Eastern Himalayas of India.

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Biraphid diatom; bryophyte; Gangtok; Sikkim; *Stauroneis sikkimensis sp. nov*; *Stauroneis lepchae sp. nov*.; ultrastructure

Introduction

The Eastern Himalayan region encompasses the area lying between 82.70°E and 100.31°E longitude and 21.95°N to 29.45°N latitude. The Eastern Himalayas stretch across Nepal, Bhutan, states of East and North-East India, and parts of Yunnan province in South-Western China (Negi 1998). Sikkim, an Indian state that completely lies in the Eastern Himalayas, is among the richest phyto-geographical regions of India and has a diverse flora and fauna. Lower plant groups from the region have also been investigated (Awasthi 1965; Singh, Dey, and Singh 2007; Dey, Singh, and Singh 2008; Mehra and Bir 2008; Das 2010; Das, Van de Putte, and Buyck 2010; Sinha and Ram 2011). But among the photosynthetic organisms, the algal flora (including diatoms) are perhaps the least studied from Sikkim (Prasad and Khanna 1987; Bhakta, Das, and Adhikary 2010; Das and Keshri 2016). Our knowledge of the diatoms from the Himalayas is very limited (Jüttner, Cox, and Ormerod 2000; Jüttner, Reichardt, and Cox 2004; Jüttner, Chimonides, and Cox 2011; Van de Vijver et al. 2011; Krstić et al. 2013; Das et al. 2018).

With over 1200 names listed (Kociolek et al. 2018), *Stauroneis* Ehrenberg (1843, 311) is one of the most species-rich genera. The genus was typified by Boyer (1927) and Round, Crawford, and Mann (1990, 128), placed it into the sub-order Naviculineae, though more recent studies have shown *Stauroneis* and its allies to form a monophyletic group that does not include *Navicula* Bory (Kulikovskiy et al. 2018). Species of the genus are differentiated by cell size and shape, shape of the apices, density and structure of striae, structure of the proximal raphe endings, shape and size of central stauros, and the presence or absence of pseudosepta (Taylor and Cocquyt 2016). This genus is a widespread and morphologically diverse group of raphid diatoms. *Stauroneis* taxa mainly live in freshwater environment, preferring epipelic, mossy or aerophilic habitats (Round, Crawford, and Mann 1990, 592; Bahls 2010), but species have been described from marine and coastal environments as well (Fourtanier and Kociolek 2011). This genus has a very interesting distribution – occurring in polar, temperate and tropical habitats.

In recent years, based on classical taxonomy, many new *Stauroneis* species have been discovered from various parts of globe and improved our knowledge of the diatom tree of life (Moser, Lange-Bertalot, and Metzeltin 1998; Lange-Bertalot et al. 2003; Van de Vijver, Beyens, and Lange-Bertalot 2004; Metzeltin, Lange-Bertalot, and Soninkhishig 2009; Bahls 2010). From Asia, Rioual et al. (2013) described two new species of *Stauroneis* from volcanic lakes in North-Eastern China. All of the above studies highlight local or regional endemism present within the genus. Nearly 120 *Stauroneis* taxa are known from the Indian subcontinent, with 93 being from freshwater habitats and rest from saline associated environments.

During this investigation two new species of *Stauroneis* were discovered from Sikkim, India, and described using light (LM) and scanning electron microscopy (SEM).

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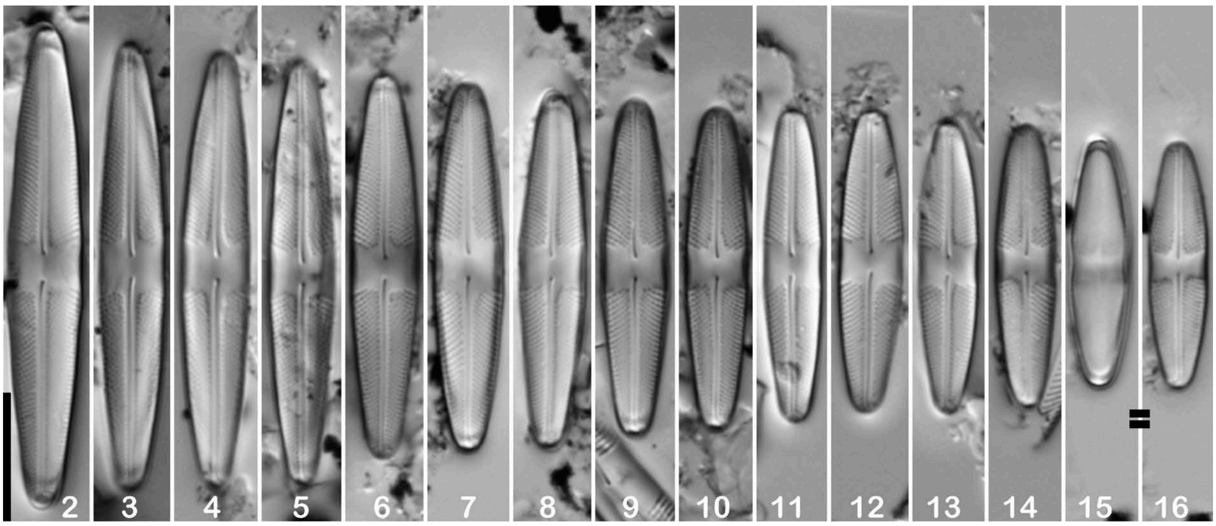
Figure 1. Collection site showing the moss growth on the rock at Kashi Raj Pradhan Road, Vishal Gaon, Gangtok, Sikkim, India.

Material and methods

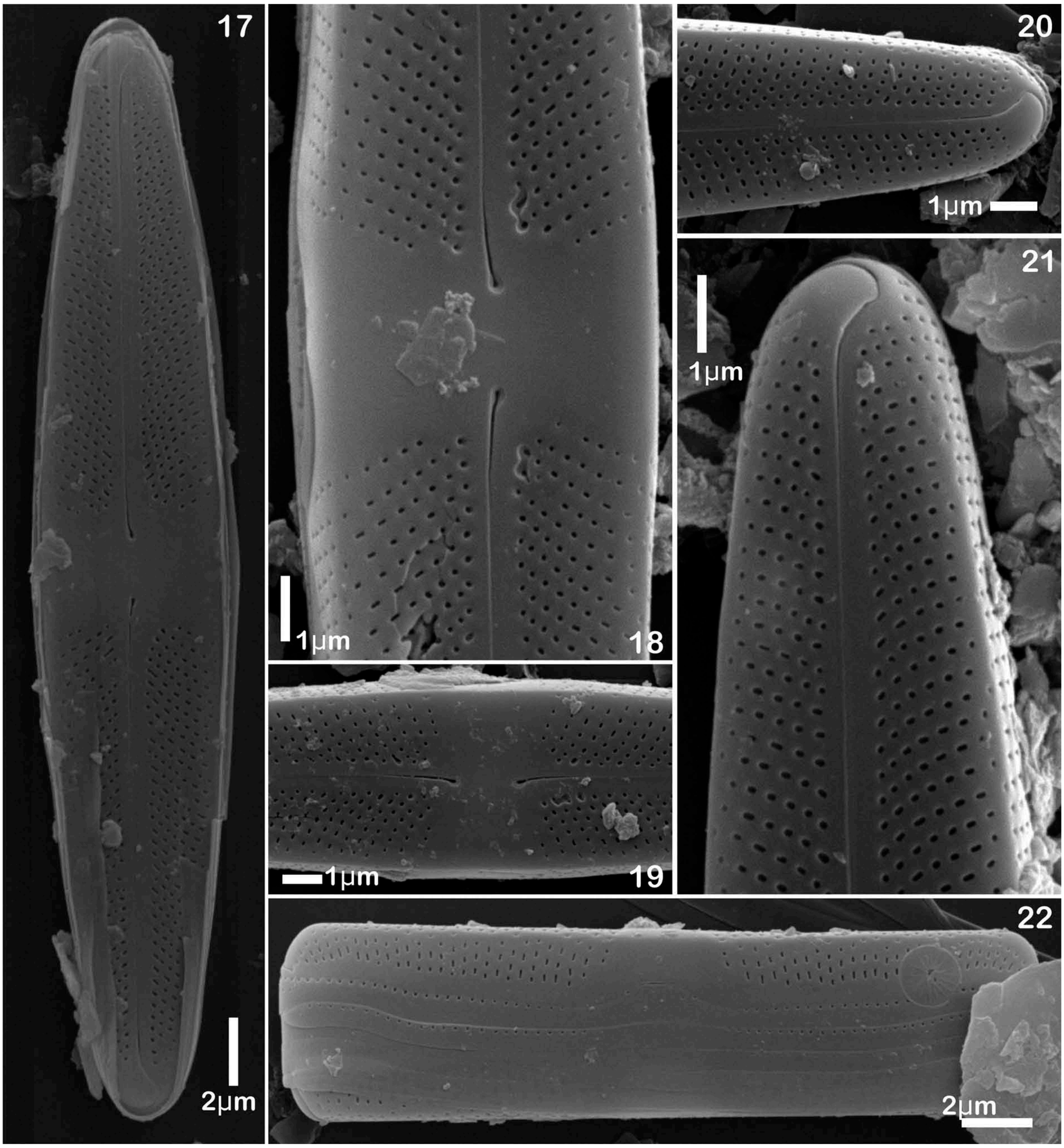
Study area. The holotypic material was collected from a rock located at the roadside in Kashi Raj Pradhan Road, Vishal Gaon, Gangtok, Sikkim (27.321712°N, 88.612653°E) (Figure 1). The big rock is located under the shade of the trees and covered with moss.

Sample collection and treatment

The sample was collected by scraping the moss growing on the rock using a plastic spoon. The moss sample was stored in Whirl-Pak® sampling bag. We were unable to get any physio-chemical parameters as the sample is aerophilic in nature. A portion of sample was cleaned by acid digestion using concentrated HNO_3, alternately centrifuged and rinsed with distilled water 2–3 times until the pH reached 7. The cleaned material was used for light microscopy (LM) and scanning electron microscopy (SEM) analyses. For LM, the cleaned material was mounted onto glass slides using Naphrax®. The slides were studied at X1000 magnification under oil immersion using an Olympus BX53 (Tokyo, Japan) microscope equipped with Differential Interference Contrast (Nomarski) optics and images were taken with an Olympus DP 74 camera. For SEM analysis, due to a large amount of debris in the sample, individual cells were picked under Olympus CKX 53 (Tokyo, Japan) inverted microscope at X400 magnification. The picked cells were dried onto glass cover slips which were affixed to aluminium stubs with double sided carbon tape. Stubs were sputter-coated with gold-palladium with an Emitech K57SX sputter coater (Quorum Technologies, United Kingdom). SEM observations were performed with a Zeiss EVO MA 15 (Oberkochen, Germany), microscope with LaB6 filament. LM and SEM images were processed in GIMP (version 2.8.14) and plates were compiled in Inkscape (version 0.92). Cleaned materials and slides are archived at the Diatom collection at Agharkar Research Institute Herbarium (AHMA). Morphological terminology follows Ross et al. (1979) and Barber and Haworth (1981).



Figures 2–16. *Stauroneis sikkimensis sp. nov*. LM (DIC). Valve views with size diminution series. Holotype- Figure 10. Scale bar- 10 µm.



Figures 17–22. SEM images of External valve of *Stauroneis sikkimensis sp. nov*. Figure 17. External view of an entire valve. Figures 18, 19. Close up of the middle region with teardrop shaped proximal raphe end, highlighting shortened striae next to stauros. Figure 21. Valve apex showing sickle-shaped distal raphe end. Figures 21, 22. Areolae round to transapically elongated continuing onto mantle. Figure 22. Girdle view showing single row of areolae. Scale bar- 2 µm (17, 22), 1 µm (18–21).

Results

Stauroneis sikkimensis N.Wadmare, S.Roy, Kociolek and B.Karthick, *sp. nov.*

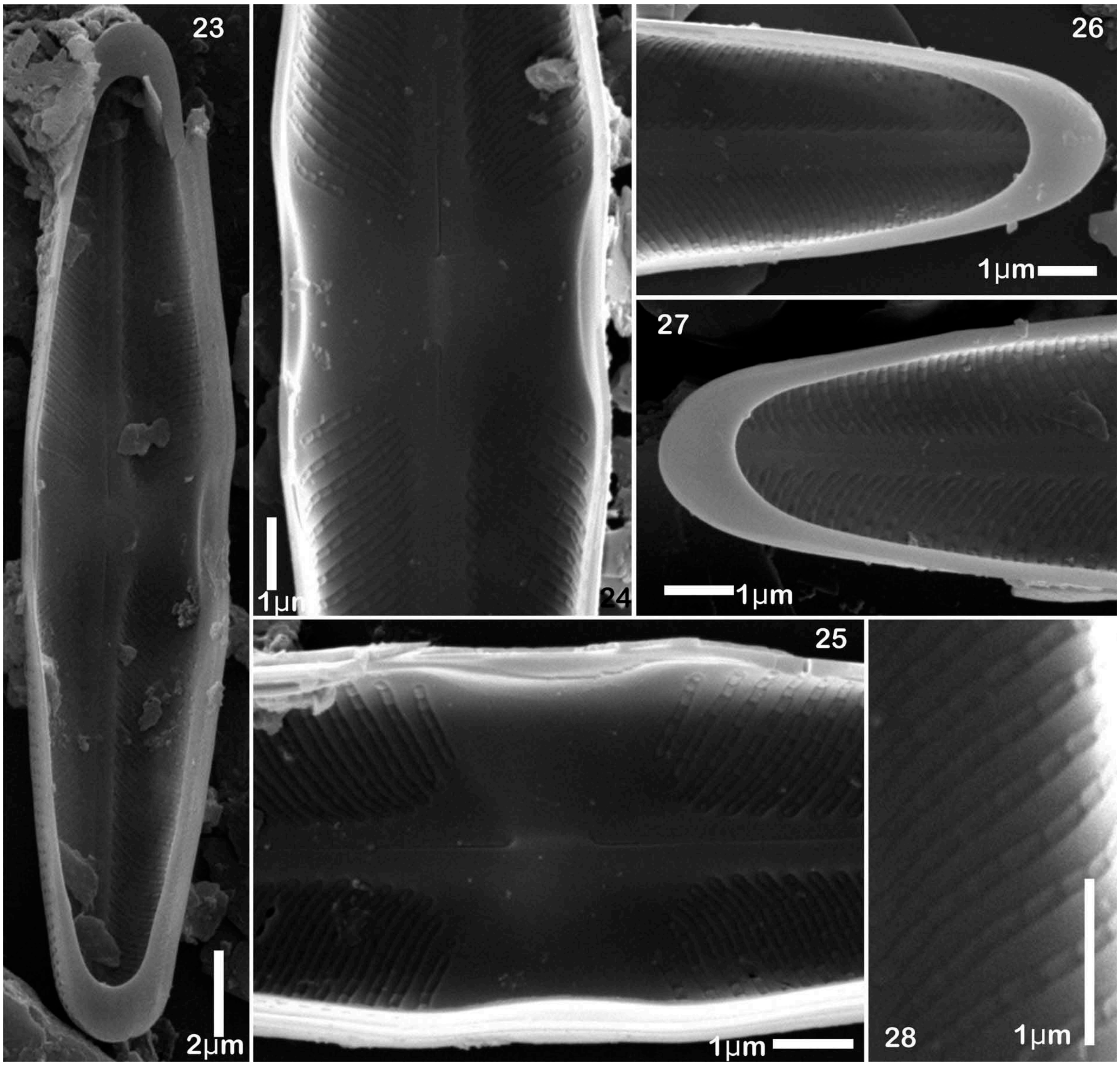
(Figures 2–28)

LM Description (Figures 2–16)

Valves linear-lanceolate with obtusely rounded non-protracted apices. Length 17.9–37.6 µm, breadth 4.1–5.8 µm. Axial area narrow, linear just widening towards the central area. Valves constricted at the central stauros. Central area comprised of a H-shaped stauros. Raphe lateral, external proximal raphe ends tear-drop shaped and slightly deflected in the same direction. Both the external distal raphe ends deflected in the same direction. Striae radiate throughout, 24–28 in 10 µm. Shortened striae present next to the stauros. Pseudosepta distinct. (n = 123).

SEM Description (Figures 17–28)

Externally, valves lanceolate with narrow axial area which is slightly wider at the centre (Figure 17). Central stauros H-shaped without any striation.



Figures 23–28. SEM images of Internal valve of *Stauroneis sikkimensis sp. nov*. Figure 23. Internal view of an entire valve. Figures 24, 25. Valves with depression at the central stauros. Central area with ridge bearing deflected proximal raphe end. Figures 26, 27. Valve apex with distinct pseudosepta hiding the distal raphe ends. Figure 28. Areolae covered with hymen. Scale bar- 2 µm (23), 1 µm (24–28).

Striae next to the stauros are short (Figures 18, 19). Proximal raphe ends tear-drop shaped and bent slightly in the same direction (Figures 18, 19). Distal raphe ends sickle shaped, turned towards the secondary side and continuing onto the mantle (Figures 20, 21). Striae continue onto mantle and composed of round to transapically elongated areolae (Figures 21, 22). Single row of porelli present on the girdle bands (Figure 22).

Internally, valve mantle constricted at the central stauros (Figures 23–25). Proximal raphe ends slightly deflected and terminating onto a well-developed broad stauros (Figures 24, 25). Distinct pseudosepta present at each valve apex obscuring the distal raphe end (Figures 26, 27). Areolae baciliform and covered with hymenate occlusions (Figure 28).

Holotype

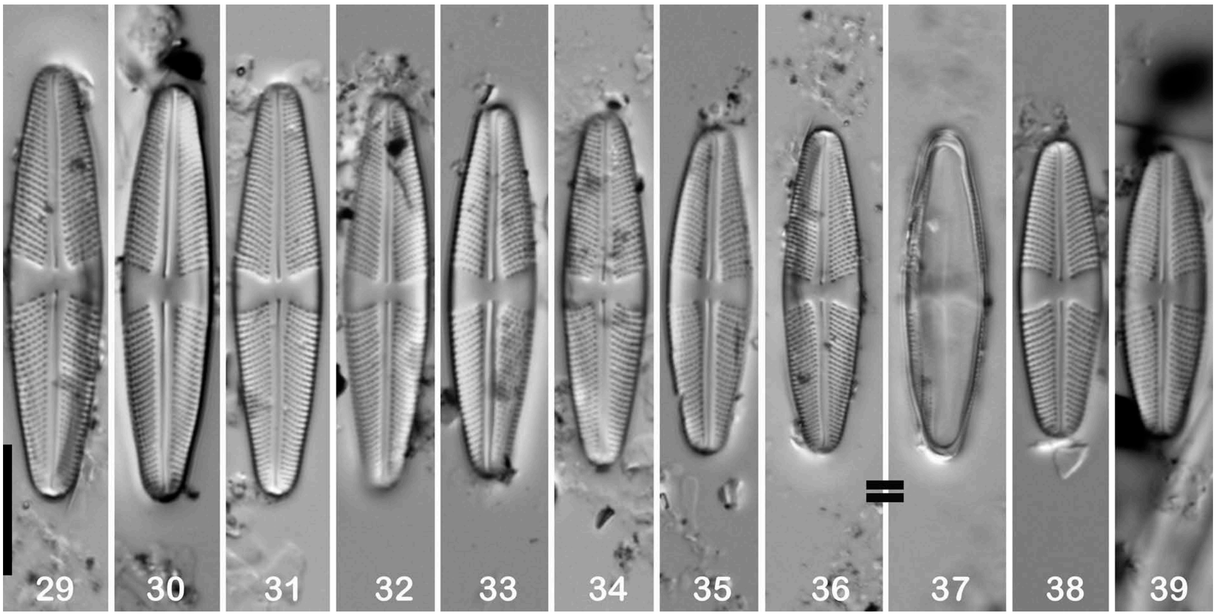
Marked holotype deposited at the Agharkar Herbarium of Maharashtra Association (AHMA) located at Agharkar Research Institute, Pune, India (Illustrated in Figure 10; Slide no. 48–055, Sample # 2378).

Type locality

Kashi Raj Pradhan Road (27.321712°N, 88.612653°E), Vishal Gaon, Gangtok, Sikkim, India.

Etymology

The species name refers to the name of the Indian state from where the new species is discovered: Sikkim.



Figures 29–39. *Stauroneis lepchae sp. nov*. LM (DIC). Valve views with size diminution series. Holotype- Figure 32. Scale bar- 10 µm.

Stauroneis lepchae N.Wadmare, S.Roy, Kociolek and B.Karthick, *sp. nov.*
(Figures 29–48)

LM Description (Figures 29–39)

Valves broadly elliptical to lanceolate with obtusely rounded apices. Length 20.5–33.1 µm, breadth 6.0–7.2 µm. Axial area narrow, linear widening towards the centre. Central area with bow-tie shaped stauros. Raphe filiform, proximal raphe ends straight. Both distal raphe ends deflected in the same direction. Pseudosepta distinct. Striae radiate throughout the valve, 20–22 in 10 µm (n = 48).

SEM Description (Figures 40–48)

Externally, valves lanceolate, raphe filiform mostly, near proximal ends appearing broader (Figure 40). The bow-tie shaped central stauros widens towards the mantle (Figure 41). Proximal raphe ends distinctly curved, terminating in drop-like pores (Figures 40, 41). Distal raphe ends hooked (Figures 42, 43). Areolae transapically elongated, dash-like, continuing onto the mantle (Figure 44).

Internally, valve lanceolate, raphe filiform, proximal raphe ends straight (Figures 45, 46). Striae radiate, composed of dash-like areolae covered by hymenate occlusions (Figure 45). Pseudosepta distinct at each apex covering the distal raphe ends (Figures 47, 48).

Holotype

Marked holotype deposited at the Agharkar Herbarium of Maharashtra Association (AHMA) located at Agharkar Research Institute, Pune, India (Illustrated in Figure 32; Slide no. 48–055, Sample # 2378).

Type locality

Kashi Raj Pradhan Road (27.321712°N, 88.612653°E), Vishal Gaon, Gangtok, Sikkim, India.

Etymology

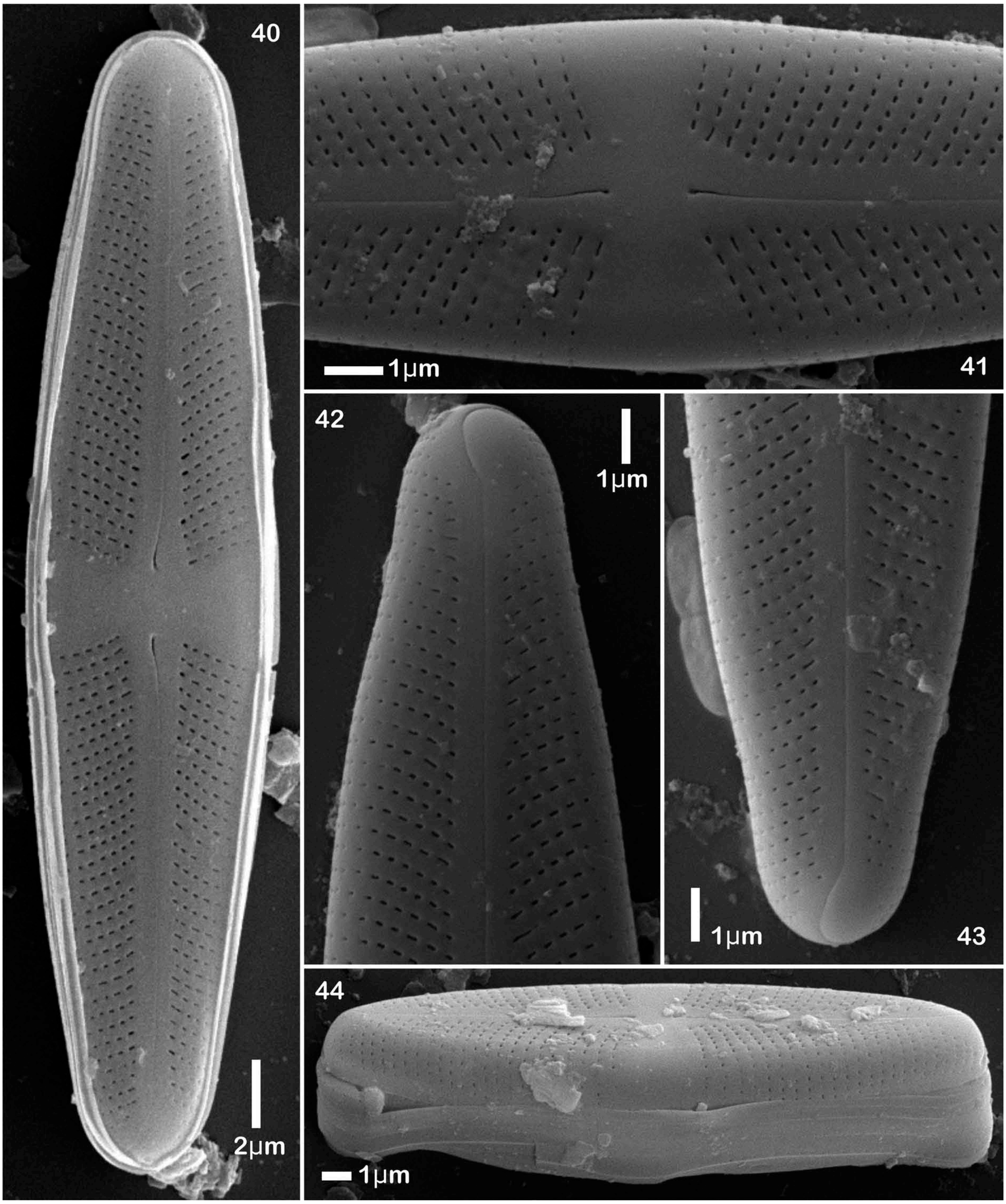
The specific epithet is dedicated to the Lepcha people of Sikkim who are the earliest ethnic group to have settled in Sikkim. They are said to be the original inhabitants of Sikkim.

Remarks

Among these two new species, *S. sikkimensis* is more abundant than *S. lepchae* in the type material. They co-occurred with profuse numbers of *Hantzschia amphioxys* (Ehrenberg) Grunow, *Pinnularia borealis* Ehrenberg, *Caloneis* cf. *bacillum* (Grunow) Cleve, one unidentified species of *Eunotia* Ehrenberg and a rare, unnamed *Stauroneis*.

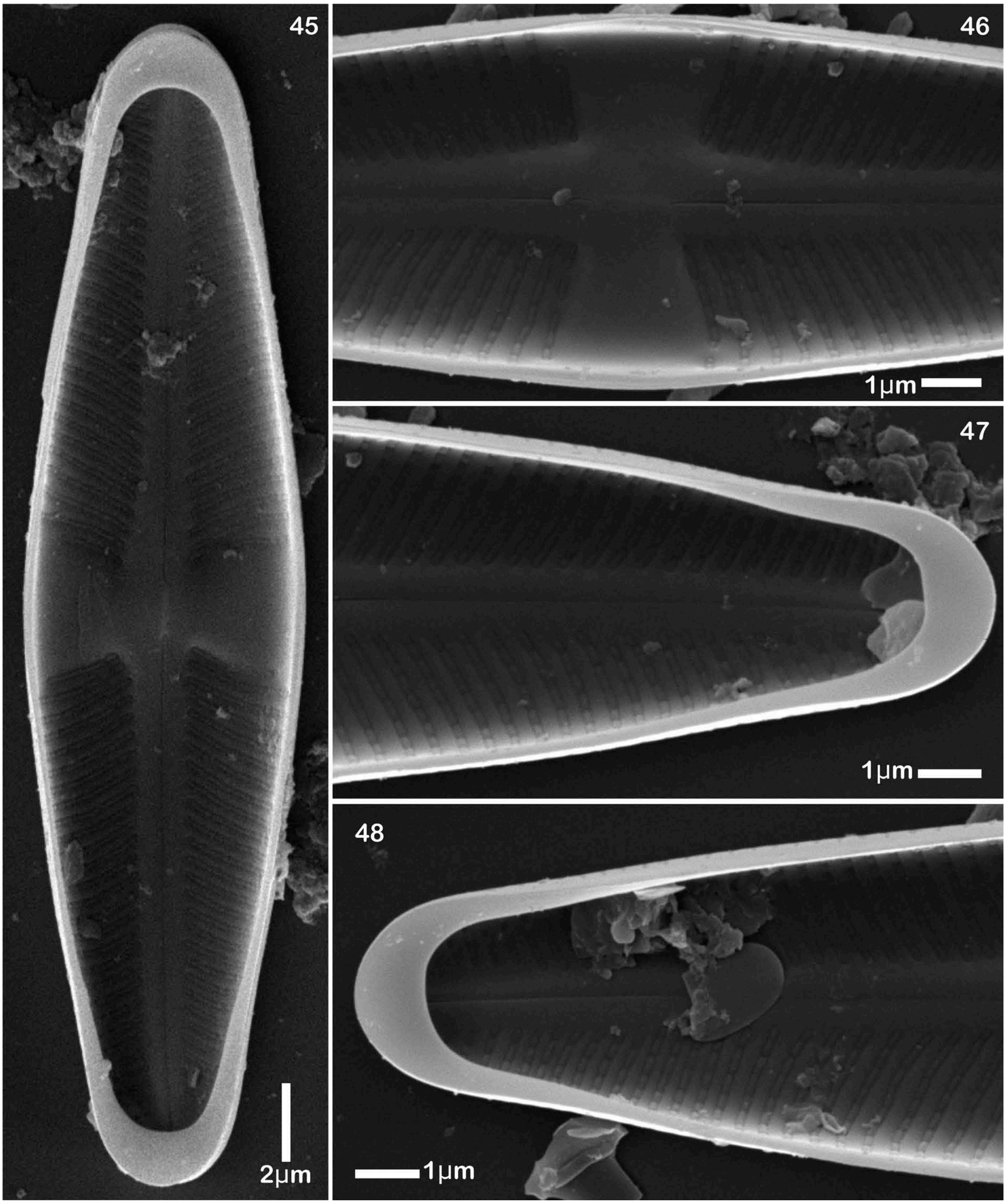
Discussion

The two *Stauroneis* species described here are, compared to many other species of the genus, relatively small but have distinct pseudosepta at the apices. Several decades ago, that would mean comparing them to *S. distinguenda* Hustedt (1937, 226) and its allies, especially as conceived by Hustedt (1937) in his treatment of the genus. *Stauroneis sikkimensis* can be



Figures 40–44. SEM images of External valve of *Stauroneis lepchae sp. nov*. Figure 40. External view of an entire valve. Figure 41. Bow-tie shaped stauros with tear drop proximal raphe ends. Areolae transapically elongated. Figure 42, 43. Valve apex with hooked distal raphe ends. Figure 44. Girdle view with striae continuing onto the mantle. Scale bar- 2 µm (40), 1 µm (41–44).

compared with the group of similar *Stauroneis* species that are relatively smaller and have a median constriction (Table 1). *Stauroneis atacamae* var. *fuegensis* Cleve-Euler (1948, 30) differs from *S. sikkimensis* with respect to valve length (33–40 µm) and shape (linear), as well as areolar density (20 in 10 µm). *S. sikkimensis* resembles *S. distinguenda* Hustedt and *S. cataractae* Moser, Lange-Bertalot, and Metzeltin (1998, 215–216) but *S. cataractae* differs from both of them by striae density (30–36 in 10 µm) and valve shape (linear elliptical), and *S. sikkimensis* differs from *S. distinguenda* in relation to valve shape (linear-lanceolate), valve length (42–-57 µm) and by having curved central raphe ends. We made observations on approximately 123 valves of *S. sikkimensis* and the length of this taxon was always shorter than *S. distinguenda*. Based on general morphological features, *S. sikkimensis* and *S. distinguenda* appear to be closely related, and may represent sister taxa, perhaps the result of geographical isolation. *S. parvula* var. *undulata* Kisselev and Vozzhennikova (1950, 322) and *S. sphagnophila*



Figures 45–48. SEM images of Internal valve of *Stauroneis lepchae sp. nov*. Figure 45. External view of an entire valve. Figure 46. Central bow-tie shaped stauros with ridge having slightly deflected proximal raphe end. Figure 47, 48. Pseudosepta present hiding the distal raphe ends. Scale bar- 2 µm (45), 1 µm (46–48).

Krasske (1948, 428) are similar to *S. sikkimensis* with respect to shape of the valve apex (bluntly or obtusely rounded), striation pattern (radiate throughout) and absence of striae in central area . But *S. parvula* var. *undulata* valves are smaller (15–22 µm), have straight central raphe endings that are small and pin-point like, and a valve shape that is lanceolate having undulate margins as compared to *S. sikkimensis*. *S. sphagnophila* differs from *S. sikkimensis* by possessing a bow-tie shaped stauros, is less broad (4–5 µm) and lacks pseudosepta. *S. borrichii* Lund (1946, 63) differs in respect to valve apex (truncate, weakly rostrate-truncate), striation density (19–25 in 10 µm) and valve length (10–23 in 10 µm). *S. javanica* f. *lapponica* Hustedt (1959, 815) valves are much longer (50–80 µm) and wider (12–18 µm) and the central raphe endings are hooked-shaped as compared to *S. sikkimensis*.

Features used to separate *S. lepchae* from a group of morphologically similar *Stauroneis* species are given in Table 2. *S. lepchae* can be distinguished from *S. australobtusa* Zidarova, Kopalova, and Van de Vijver

Table 1. Comparison of morphometric data of *Stauroneis sikkimensis sp. nov*. and closely related *Stauroneis* taxa from different sources

	(A) *atacamae* var. *fuegensis*	*S. distinguenda*	*S. cataractae*	*S. borrichii*	*S. javanica* f. *lapponica*	*S. parvula* var. *undulata*	*S. sphagnophila*	*S. sikkimensis*
Length (μm)	33–40	42–57	22–28	10–23	50–80	15–22	16–26	17.9–37.6
Breadth (μm)	7–7.5	6	4.8–5.2	3–4	12–18	3.4–4	4–5	4.1–5.8
Valve shape	linear, with median depression	linear lanceolate, with median depression	linear elliptical, with slight median depression	linear- lanceolate, with median depression	linear-lanceolate, with median depression	lanceolate having undulate margins, with median depression	linear lanceolate with median depression	lanceolate with median depression
Apex	bluntly rounded	bluntly rounded	broadly rounded and tapered	truncate, weakly rostrate-truncate	bluntly rounded	obtusely rounded	obtusely rounded	bluntly or obtusely rounded, non-protracted
Striation pattern	radiate throughout	radiate throughout	radiate throughout	radiate throughout	moderately radiate	radiate throughout	radiate throughout	radiate throughout
Striae (in 10 μm)	20	20–28	30–36	19–25	18	25	26	24–28
Striae in the central area	absent	absent	absent	absent	absent	absent	absent	absent
Shape of the areolae	rounded to slightly elongated	slightly elongated	rounded to slightly elongated	rounded	rounded to elongated	slightly elongated	rounded	rounded to elongated
Central raphe endings, central pore	curved, small pin-point-like	curved, weakly expanded	slightly curved, small pin-point-like	straight	hooked shaped, bent central pores	straight, not inflated, small pin-point-like	straight, not inflated small pin-point-like	slightly curved, expanded small pin-point-like
Central area (stauros)	broad, expanded	broad, strongly expanded	broad, strongly expanded	broad, bow-tie shaped	widened	broad, widened and truncate outwards	bow-tie shaped	broad, expanded or H-shaped
Presence/absence of pseudosepta	absent	present	present	present	present	present	absent	present
References	Cleve-Euler 1948	Hustedt 1937, Simonsen 1987	Moser, Lange-Bertalot, and Metzeltin 1998	Lund 1946	Hustedt 1959	Kisselev and Vozzhennikova 1950	Krasske 1948	Present study

Table 2. Comparison of morphometric data of *Stauroneis lepchae sp. nov*. and closely related *Stauroneis* taxa from different sources

	S. australobtusa	*S. pseudoschimanskii*	*S. subhyperborea*	*S. beyensii*	*S. laterostrata*	*S. chasei*	*S. hannae*	*S. lepchae*
Length (µm)	34.1–58.0	24–30	43–70	27–30	25	20–25	15–33	20.5–33.1
Breadth (µm)	8.0–12.0	6–7	11–13.5	6.5–7.5	8	4.5–5	3–7	6–7.2
Valve shape	narrowly lanceolate with slight convex margins	lanceolate	strictly lanceolate	lanceolate	elliptic-lanceolate with undulate margins	linear elliptical	narrow elliptical	broadly elliptical to lanceolate
Apex	weakly protracted, obtusely rounded	shortly or broadly subrostrate	slightly protracted, subrostrate to broadly rounded	shortly protracted, rostrate to subcapitate	protracted, subcapitate	broadly rounded, non-protracted	weakly protracted, obtusely rounded	non-protracted, obtusely to broadly rounded
Striation pattern	weakly to moderately radiate in the valve middle	moderately radiate	radiate throughout	moderately radiate throughout	radiate throughout and slightly curved	radiate throughout	slightly radiate throughout	radiate throughout
Striae (in 10 µm)	18–20	23–24	18–20	28–30	16–24	36–38	20–25	20–22
Striae in the central area	absent	absent	absent	absent	absent	absent	absent	absent
Shape of the areolae	rounded to slightly elongated	rounded to slightly elongated	rounded to slightly elongated	round	rounded	not mentioned	not mentioned	nearly round to slightly elongated transapically
Areolae (in 10 µm)	18–23	20–25	15–18	38–40	not mentioned	not mentioned	not mentioned	27–35
Central raphe endings, central pore	laterally curved, drop-like pores	weakly curved, small but distinctly inflated	curved, Somewhat deflected drop-like pores	straight with slightly expanded, indistinct	somewhat straight, drop-like pores	somewhat straight, indistinct	somewhat straight, indistinct	slightly curved, small pin-point-like
Central area (stauros)	broad, butterfly shaped	distinctly expanded	moderately broad, moderately widened outwards	narrow and rectangular, weakly widened outwards	broad, bow-tie shaped	broad, butterfly shaped	moderately broad, rectangular, weakly widened outwards	broad, bow-tie shaped
Presence/absence of pseudosepta	present	present	present	present	present	present	present	present
References	Zidarova, Kopalová, and Van de Vijver 2014	Van de Vijver, Beyens, and Lange-Bertalot 2004	Van de Vijver, Beyens, and Lange-Bertalot 2004	Van de Vijver, Beyens, and Lange-Bertalot 2004	Hustedt 1943	Cholnoky 1954	Patrick and Freese 1961	Present study

(2014, 193) by its smaller size (length 20.5–33.1 μm, breadth 6.0–7.2 μm), shape of the valve (broadly elliptical to lanceolate) and apex (non-protracted, obtusely to broadly rounded), striation pattern (radiate throughout), and areolae density (27–35 in 10 μm). *S. pseudoschimanskii* Van de Vijver, Beyens, and Lange-Bertalot (2004, 57) differs from *S. lepchae* by the shape of valve (lanceolate) and apex (shortly or broadly subrostrate), striae orientation (moderately radiate), areolar density (23–24 in 10 μm), and shape of the stauros (distinctly expanded). *S. subhyperborea* Van de Vijver, Beyens, and Lange-Bertalot (2004, 72–73) and *S. beyensii* Van de Vijver, Beyens, and Lange-Bertalot (2004, 24–25) both appear somewhat similar to *S. lepchae* in terms of valve shape (lanceolate) and striation pattern (radiate throughout), but *S. subhyperborea* differs in having larger valves (length 43–70 μm, breadth 11.0–13.5 μm), slightly protracted ends and lower areolar density (15–18 in 10 μm). *S. beyensii* has short-protracted apices, higher striae density (28–30 in 10 μm) and areolar density (38–40 in 10 μm), straight central raphe endings and a more narrower, rectangular stauros as compared to *S. lepchae*. *S. laterostrata* Hustedt (1943, 155) is clearly differentiated from *S. lepchae* by having broader valves (8 μm), undulate margins and protracted apices. Though *S. chasei* Cholnoky (1954, 244) has rounded, non-protracted apices and a stauros that has the same shape as *S. lepchae*, this species has linear-elliptical valves that are narrower (4.5–5.0 μm) and have higher stria densities (36–38 in 10 μm). Finally, *S. hannae* Patrick and Freese (1961, 184) differs in the shape of valve (narrow elliptical) and apices (weakly protracted, obtusely rounded), striation pattern (slightly radiate throughout) and density (20–25 in 10 μm) and stauros shape (broad, rectangular).

Past "force-fitting" of taxa from diverse biogeographic regions (Tyler 1996) into European species is well-known, and the idea of many freshwater diatoms having defined patterns of distribution (Kociolek and Spaulding 2000) is more widely accepted. For the genus *Stauroneis*, Van de Vijver, Gremmen, and Beyens (2005) demonstrated that the diatom floras of the Arctic and Antarctic regions are quite different, and they also established a clear biogeographic circumscription of the genus *Stauroneis* from the (Sub-) Antarctic region. Even though a distinct, monophyletic lineage of taxa that includes *Stauroneis* has been identified (Kulikovskiy et al. 2018), relationships within the genus have not been established. Kociolek et al. (Accepted) have discussed whether the presence of a stauros is adaptive for the more than 1,000 taxa assigned to the genus (Kociolek et al. 2018) or if the feature has evolved independently several times.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Surajit Roy, did his Ph.D. at The University of Burdwan, West Bengal. Now he is a SERB National Post-Doctoral Fellow at Agharkar Research Institute, Pune, India. He is now working on taxonomy and ecology of rocky pool diatoms of the Western Ghats, India. Contribution: Wrote introduction, discussion, tables and developed the manuscript.

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