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Humidophila manipurensis sp. nov. and the first record of *Humidophila bigibba (Hustedt)* Lowe, Kociolek, Johansen, Van de Vijver, Lange–Bertalot et Kopalová from Northeast India

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Abstract: A new aerophilic diatom species, *Humidophila manipurensis* sp. nov., is described from the Sadu Chiru waterfalls, Manipur, India, a part of the Indo–Burma hotspot region. This species is characterized by having its valve shape slightly inflated at middle, terminal raphe ends that are slightly deflected, round areolae and a central depression. Further, we also recorded *Humidophila bigibba* from Meghalaya State, Northeast India, and this population is compared with the type population from Indonesia and Chinese populations. Fine structural details of both taxa were examined using light and scanning electron microscopy and compared with morphologically similar taxa in this genus. Addition of new species from this region will further add to the biodiversity richness of the Indo–Burma biodiversity hotspot. Further, this study confirms the presence of *H. bigibba* for the first time in India. This species has a deep constriction in the middle of the valve which is not found in the populations from the Indonesia and China.

Key words: Bacillariophyceae, diatoms, Indo–Burma hotspot, Manipur, new species, typology

Introduction

Northeast India forms a junction between the Eastern Himalayas and Indo–Burma biodiversity hotspots with a mixture of Southeast Asian and Indian biota (Chatterjee et al. 2006). The region is often called a gateway of biotic exchange between the Indian subcontinent and Southeast Asia (Roy et al. 2015). This province supports a large number of endemic organisms from various groups, including mammals, plants, fishes, mollusc, lichens, and microorganisms such as rotifers (Upadhaya et al. 2003; Goswami et al. 2012; Sharma & Sharma 2014; Das & Das 2015; Tripathi et al. 2016). The microbial diversity in these hotspots has yet to be assessed in detailed (Bora et al. 2016). Among the microorganisms, algae of the Northeast Indian region have received considerable attention in terms of new records and discoveries (Biswas 1930, 1934; Bhâradwâja 1963; Das et al. 2019). For example, several new diatom species have been described from this locality, belonging to the genera *Stauroneis* (Wadmare et al. 2019), *Pinnularia* (Das et al. 2020), *Gomphonema* (Radhakrishnan et al. 2020), and *Diploneis* (Bhatt & Karthick 2020). The present study describes a new species of *Humdiophila, H. manipurensis*, and reports the presence of another taxon *H. bigibba* for the first time in India.

Humidophila is predominantly an aerophilic genus with 65 taxa that were previously included in the genus *Diadesmis* Kützing 1844 (Chattová et al. 2017). Lowe et al. (2014) separated these taxa from *Diadesmis* (in the subgenus *Paradiadesmis* Lange–Bertalot et Le Cohu) and appraised them as a new genus *Humidophila*. *Humidophila* as a genus has been reported from every continent on earth (Lowe et al. 2014, 2017; Chattová et al. 2017; Kochman–Kedziora et al. 2016; Nikolić et al. 2019) and, moreover, *Humidophila* species typically inhabit aerial and sub–aerial habitats. While some species such as *H. contenta* are widely distributed all over the world, other species, such as *H. aerophila* and *H. crozetikerguelensis*, are considered endemic for certain

regions (Vijver et al. 2002; Lowe et al. 2014; Kopalová et al. 2015; Kochman–Kędziora et al. 2016; Lowe et al. 2017; Chattová 2018).

Only a few species of *Humidophila* have been documented from the Northeast Region of India. Moreover, species such as *H. contenta,* were reported from the Western Ghats (Kodaikanal Hills, Tamil Nadu) (Suxena 1984) and Northeastern Region (Meghalaya) (Ghosh & Gaur 1998) as *Navicula contenta* Grunow. Additionally, Foged (1959, 1976) and Gandhi (1966) have reported *H. biceps,* and its various forms (such as *Navicula contenta* f. *biceps* (Grunow in Van Heurck) Hustedt) from Afghanistan, Sri Lanka, and India (Furey et al. 2020; Hamilton et al. 1992).

In this study, a new aerophilic species, *Humidophila manipurensis*, is described from Sada chiru waterfalls in Manipur, Northeast India, and *Humidophila bigibba* is recorded for the first time in India from the Mawsmai cave located in the state of Meghalaya, Northeast India. For the latter taxon, we compare specimens from India with the type population from Indonesia as well as a population from China to document the minor morphological different across these three populations.

Material and methods

The material for this study originated from two states of Northeast India. Study sites are depicted in Fig. 1. The map presented here is prepared using QGIS version 2.18.5 with the open– source layers. Collection information of the epilithic samples on which our observation is detailed in Table 1. Samples were collected and stored in a Whirl–Pak® sampling bag for

Table 1. Site and sample details of the two species.

Taxa	Site details	Geo–coordinates	Sample collection
Humidophila manipurensis	Sadu chiru waterfalls Bishnupur district of Manipur	24.74141° N, 93.74541° E, 1191 m a.s.l.	Epilithic – Brown biofilm scraped using a toothbrush on the rock in a water–spray zone area
Humidophila bigibba	Mawsmai cave, Cherrapunji, East Khasi Hills of Meghalaya	25.244573° N, 91.724051° E, 1203 m a.s.l.	Epilithic – Scraping greenish–brown biofilm using a toothbrush on the rock in a well light area

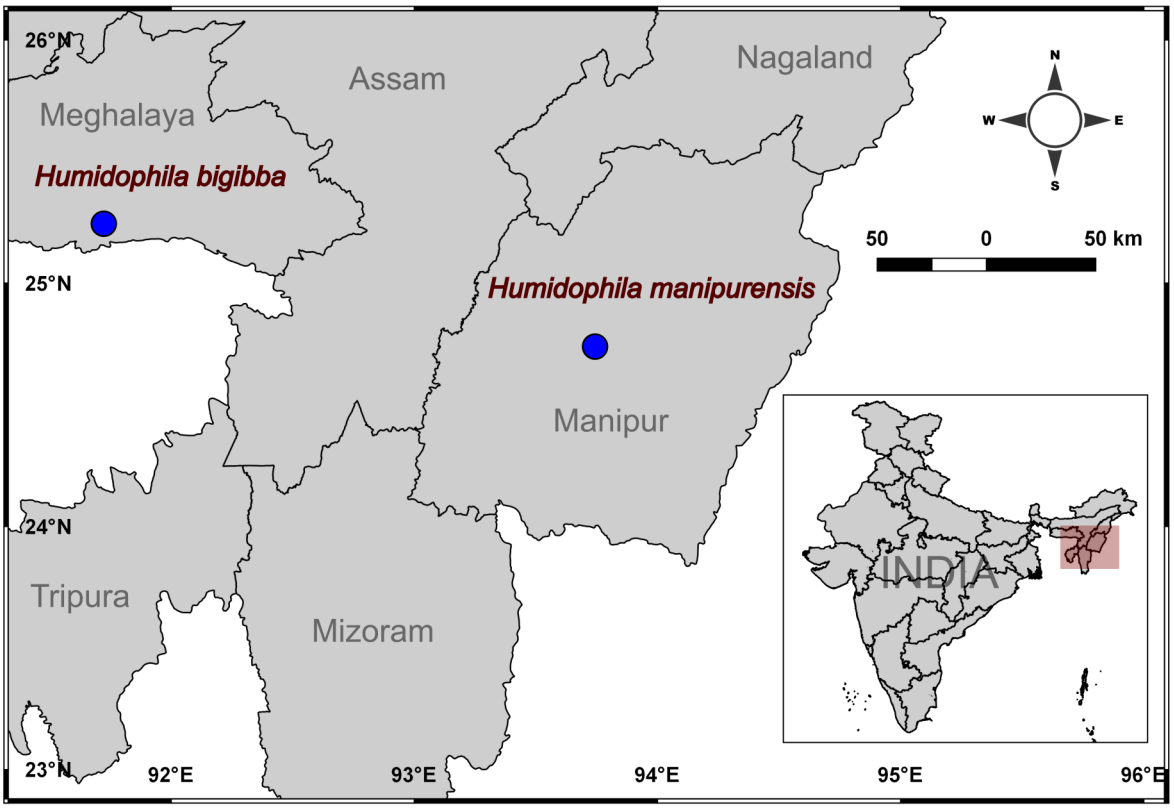

Fig. 1. Map showing the sampling sites of *Humidophila manipurensis* sp. nov. and *Humidophila bigibba* from the Northeast region of India with topography.

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transportation. Onsite measurements were taken for pH, water temperature, electrical conductivity (EC), total dissolved solids (TDS), and dissolved oxygen (DO), using a HACH HQ 40d multimeter (USA). On the same day of collection, Nitrates and Phosphates were measured using a HACH DR1900 Portable Spectrophotometer (Loveland, USA).

Diatom samples were processed by boiling the material with concentrated nitric acid (HNO_3) for 1.0–1.5 hours. The material was then centrifuged at 5000 rpm for 5 mins and rinsed several times with distilled water until it reaches neutral. The cleaned materials were air–dried overnight onto coverslips to prepare permanent slides with Naphrax® for further examination. Light microscopic observations were achieved by an Olympus BX 53 microscope (Tokyo, Japan) equipped with Differential Interference Contrast (DIC, Nomarski) optics with a 100× 1.4 oil immersion objective (1000×). Images were taken using an Olympus DP 74 digital camera with a cellSens standard 1.16 imaging software. Associated taxa were determined by calculating the relative abundance using 600 individual counts. For SEM observation, the cleaned materials were dried onto small glass coverslips and taped to aluminium stubs. Stubs were sputter–coated with gold–palladium using an Emitech K575X sputter coater. SEM observations were carried out using a Zeiss EVO MA15 (Oberkochen, Germany) microscope with LaB6 filament. LM and SEM images were processed in GIMP (version 2.8.14) and plates were compiled with Inkscape (version 0.92). Cleaned materials and slides are archived at the Diatom Collection at Agharkar Research Institute Herbarium (AHMA). The terminology of the valves follows Lowe et al. (2014) and Vouilloud et al. (2022).

Results

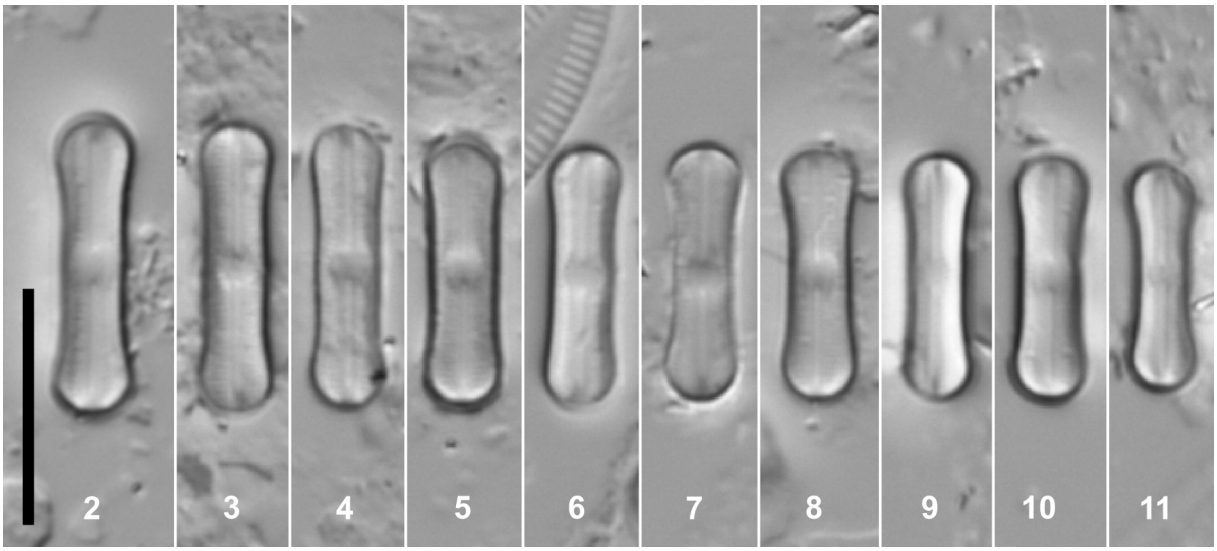
Class **Bacillariophyceae** Haeckel 1878 emend. D.G. Mann in Round et al. 1990
Subclass **Bacillariophycidae** D.G. Mann in Round et al. 1990
Order **Naviculales** Bessey 1907
Family **Diadesmidaceae** D.G. Mann in Round et al. 1990
Genus ***Humidophila*** (Lange–Bertalot et Werum) Lowe, Kociolek, Johansen, Van de Vijver, Lange–Bertalot et Kopalová 2014

Humidophila manipurensis **sp. nov. C.Radhakrishnan, M.Yogehswaran, Kociolek et B.Karthick (Figs 2–18)**
LM Description (Figs 2–11): Valves are linear, with almost parallel margins, in the valve middle slightly inflated in the larger valves, and apices are broadly rounded, wider than the valve centre. Valve dimensions (n=20): length 9.5–13.0 µm, width 2.0–3.0 µm. Axial area narrow and linear, slightly narrower towards to apices. Raphe is straight, with slightly expanded proximal and inconspicuous distal raphe ends. Fascia at centre rectangular in shape. Striae visible, but difficult to resolve with light microscopy.
SEM Description (Figs 12–18): Externally, striae parallel to weakly radiate (Figs 12–14), and composed of round to transapically–elongated areolae (50 in 10 µm). Striae usually terminating near the distal raphe ends (Figs 14–15). Mantle areolae positioned on the shallow depression running across the valve (Fig. 14). Raphe branches straight, with expanded proximal raphe ends (Fig. 16). Shallow grooves may be present on both sides of the proximal and distal raphe ends. Distal raphe ends slightly deflected to same side of the valve (Fig. 15). Proximal raphae ends almost straight (Fig. 16). Internally, a well–developed central nodule present at centre of the valve (Fig. 17). Proximal raphe ends are straight. Distal raphe ends are simple and terminating on weakly developed helictoglossae. Areolae covered by hymenes (Figs 17–18). A vallum is positioned at the margin on both sides of the axial area, with the virgae elevated above the valve face (Fig. 17).

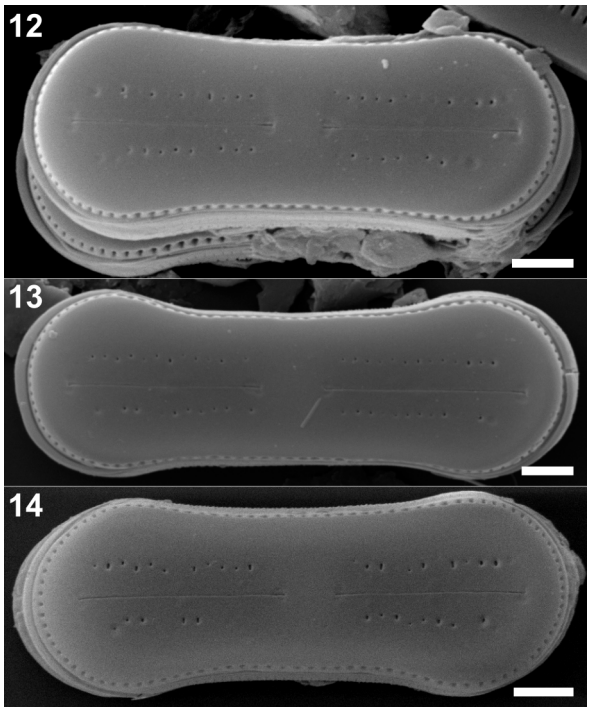
Holotype: Slide #39–091; Sample Accession #1946; deposited at the Diatom Collection, Agharkar Research Institute Herbarium (AHMA), Pune, India.
Etymology: The species name refers to the name of the Indian state where the new species was discovered: Manipur.
Type Locality (designated here): Sadu chiru, Bishnupur District, Manipur, India 24.74141° N, 93.74541° E, elevation 1191 m a.s.l.

Ecology: This species was found in the brown biofilm scraped on a rock, from the spray zone close to a waterfall, with a water temperature of 19.9 °C. The electrical conductivity was 36.20 µs.cm^{-1} with 7.73 ppm of dissolved oxygen; the nitrate and phosphate of the water collected at waterfalls were 0.12 ppm and 0.3 ppm, respectively. *Humidophila manipurensis* sp. nov. occurred with *Humidophila contenta* (Grunow) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange–Bertalot et Kopalová, *Sellaphora saugerresii* (Desmazières) C.E.Wetzel et D.G.Mann, *Sellaphora atomoides* (Grunow) Wetzel et Van de Vijver, and *Platessa hustedtii* (Krasske) Lange–Bertalot in this sample.

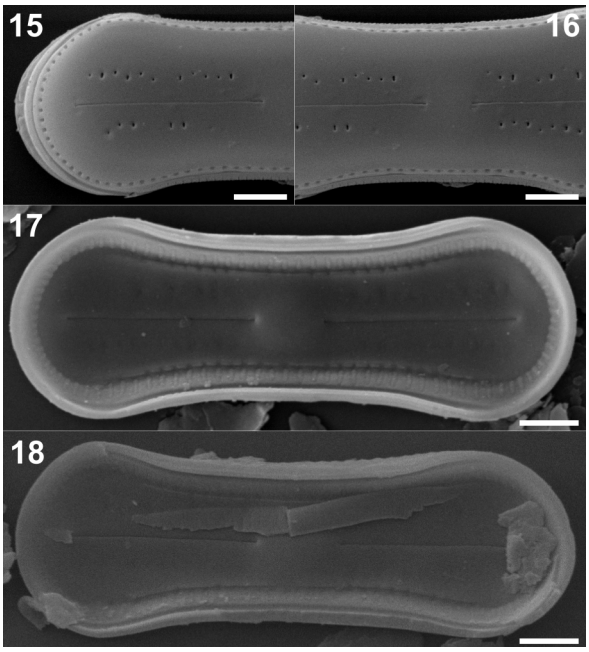
Humidophila bigibba **(Hustedt) Lowe, Kociolek, Johansen, Van de Vijver, Lange–Bertalot et Kopalová (Figs 19–35)**
LM Description (Figs 19–28): Valves are linear, biundulate, inflation in apical axis, margins with a deep constriction in the middle, and termination of broadly rounded valve apex. Valve dimensions (n=30) length 12.0–18.0 µm, width 2.0–3.5 µm at center, and 3.0–4.5 µm at the broadest portion of the valves. Axial area linear and central area rounded. Striae 29–31 in 10 µm, parallel throughout the valve. Both proximal and distal raphe ends straight and not expanded at ends, especially the distal raphe endings terminate before the last stria near the apices.
SEM Description (Figs 29–35): Valves are deeply constricted in the middle and have shallow depressions on the external valve faces (Fig. 29). Areolae near the raphe are narrowly elliptical. At the central area areolae are small and rounded. Areolae near the apices are broadly



Figs 2–11. Light microscope images of *Humidophila manipurensis* sp. nov. Valve view showing size diminution series. Scale bar 10 µm.



Figs 12–14. External view of the scanning electron micrographs of *Humidophila manipurensis* sp. nov.: (12–14) whole showing the external raphe ends and striae patterns. Scale bar 2 µm.



Figs 15–18. External and internal view of scanning electron micrographs of *Humidophila manipurensis* sp. nov.: (15–16) external view of central and apices showing the proximal and distal raphe endings along with mantle areolae patterns; (17–18) internal view of whole valve showing the helictoglossae and areolae structure. Scale bar 2 µm.

elliptical and larger, compared to the rest of the areolae and continued around apices without interruption (Fig. 32). Mantle areolae are narrowly rectangular, running across the apices (Fig. 31). A special appearance of apical openings at periphery of valve face and mantle junction (Figs 29–31). Both externally and internally, the distal raphe ends are straight to slightly tilted to one side (Figs 29, 30, 33, 34). Areolae are covered with hymens, and a vallum is present on either side of the axial area (Figs 33–35).

Ecology: *Humidophila bigibba* was found in the greenish–brown epilithic biofilm growing on rock inside the Mawsmai cave Meghalaya, India. The stone was wetted by alkaline water (pH 7.71) with temperature of 22.3 °C, the electrical conductivity was 232 µs.cm^{-1} with 7.14 ppm of dissolved oxygen; nitrate and phosphate were 0.06 ppm and 0.26 ppm, respectively. The species was accompanied by *Humidophila contenta* (Grunow) R.L.Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange–Bertalot et Kopalová 2014 and *Sellaphora nigri* (De Notaris) C.E.Wetzel et

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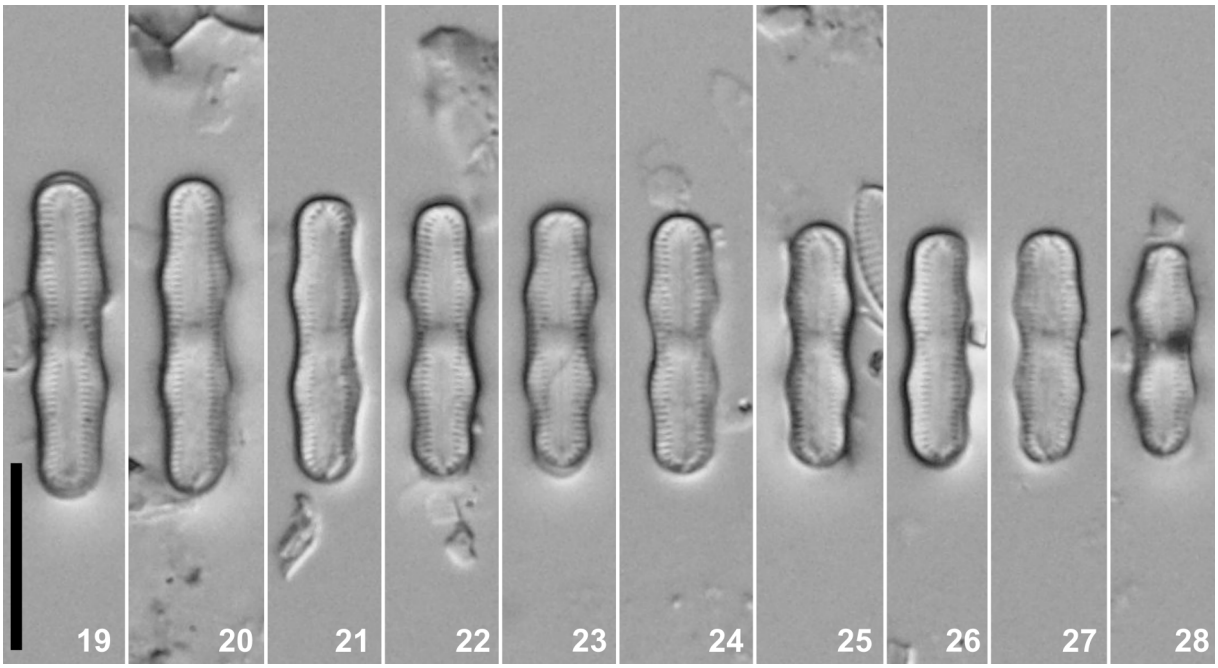


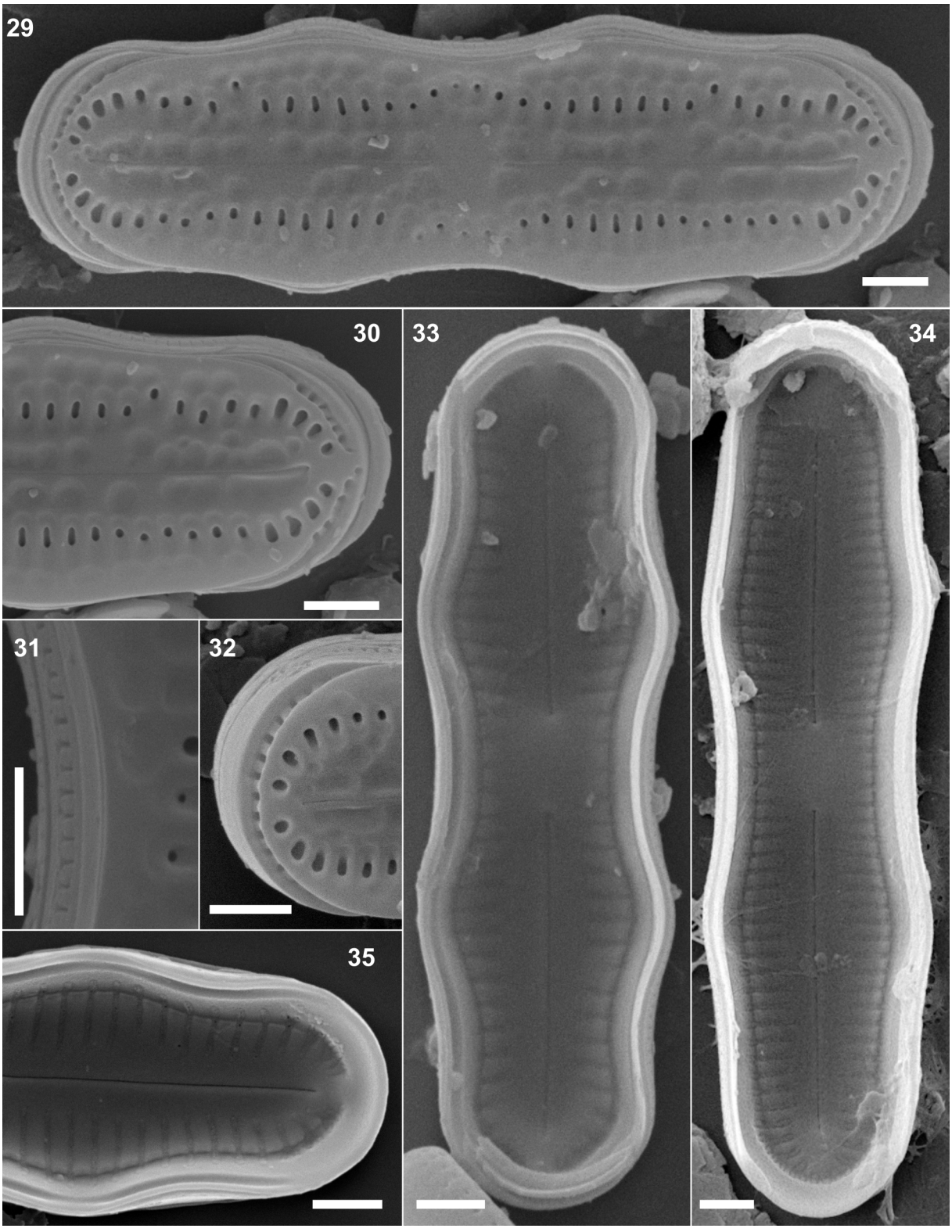
Figs 19–28. Light microscope images of *Humidophila bigibba*. Valve view showing size diminution series. Scale bar 10 µm.

L.Ector 2015. These two other species were abundant with more than 80% in this sample.

Discussion

Humidophila manipurensis sp. nov. is similar to several other *Humidophila* species based on the valve outline, dimensions, striae pattern, and raphe structure. The new species from Northeast India is compared and contrasted with several other species worldwide based on this list of features in Table 2. *Humidophila manipurensis* is more similar to *Humidophila simplex* in terms of overall dimensions. The valve length and width of *Humidophila manipurensis* is 9.8–13.0 µm and 2.1–3.0 µm, similar to *Humidophila simplex* which has a length of 6.0–11.5 µm and width of 2.1–2.9 µm. However, *H. manipurensis* characterized by broadly round apices, whereas *H. simplex* has rounded apices and triundulate margins. In addition, lower striae density (34–38 in 10 µm) and the lack of depressions on both raphe ends also distinguish *H. simplex* from the new species. *Humidophila manipurensis* clearly differs from *Humidophila contenta* (Grunow) R.L.Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange–Bertalot et Kopalová (Lowe et al. 2014) by valve shape (elliptic with parallel margins) and external raphe endings (T–shaped proximal and distal ends) of *H. contenta*. *Humidophila undulocontenta* Lowe, Kociolek et You (Lowe et al. 2017) differs from *Humidophila manipurensis* by the shape of the valve (triundulate margins), striae (interrupted centrally) and central fascia (may or may not be present). Striae–like depressions occurr in centre of the external valve and internally central areolae are absent. *Humidophila manipurensis* clearly differs from *Humidophila paracontenta* (Lange–Bertalot et Werum) Lowe, Kociolek, Johansen, Van de Vijver, Lange–Bertalot et Kopalová (Lowe et al. 2014) based on orientation of the striae (radiate), with lower density (27–30 in 10 µm), both raphe ends clearly marked, internal proximal ends curved, and elongated areolae in *H. paracontenta*. Further, *Humidophila discordabilis* (Gerd Moser, Lange–Bertalot et Metzeltin) Lowe, Kociolek, Johansen, Van de Vijver, Lange–Bertalot et Kopalová (Lowe et al. 2014) has no undulation at the middle of the valve, slightly radiate striae, and areolae clearly elongated; the striae of *H. manipurensis* are mostly rounded and rarely elongated. *Humidophila manipurensis* distinctly differs from *H. deceptionensis* Kopalová, Zidarova et Van de Vijver (Kopalová et al. 2015). *Humidophila deceptionensis* has concave margins, lower striae density (30–32 in 10 µm), slightly expanded proximal ends, transapically elongated areolae and no fascia at the center.

Navicula brekkaensis var. *bigibba* was described by Hustedt from Indonesia (Hustedt 1937) and subsequently transferred to the genus *Humidophila* as *Humidophila bigibba* (Hustedt) Lowe, Kociolek, Johansen, Van de Vijver, Lange–Bertalot et Kopalová (Lowe et al. 2014). Moreover, only limited morphological details were included in the original description of *Navicula brekkaensis* var. *bigibba* (Hustedt 1937), such as valve dimensions and almost no additional details about the type material were offered by Simonsen (1987). Recently, Lowe et al. (2017) reported the same species from Libo, Guizhou Province, China, and provided greater details on the valve ultrastructure. However, certain minor morphological variations exist between the populations from India, Indonesia and China. Comparative morphological details between these three populations are given in the Table 3.



Figs 29–35. External and internal view of scanning electron micrographs of *Humidophila bigibba*: (29–35) external view of apices and central valve showing the proximal and distal raphe endings and areolae structure; (29–32) valve central and apices showing the mantle areolae patterns (31); (33–35) internal view of whole valve and apices showing the helictoglossae and areolae structure. Scale bar 2 µm.

The populations from India and Java (Indonesia), appear to have a more pronounced degree of undulation than the population from China, as illustrated by Lowe et al. (2017). Moreover, the population from India showed a valve width of 2.3 µm at the constricted area to a maximum of 4.5 µm at the broadest part of the valve, but the populations from Indonesia and China were 3.5 and 4 µm broad, respectively (Hustedt 1937; Lowe et

al. 2017). Here, the ratio of width in the constricted and broader areas is twice that of the population from India (Meghalaya) but not the Chinese population. In terms of valve outline and morphometrics, the Indian population more closely resembles type population described by Hustedt from Indonesia than it does with the Chinese population. The difference between these populations may be related to the environment (subtropical/tropical in India and Java versus temperate in Guizhou, China). Further observations on this species from other localities may substantiate or suggest other reasons for the variation expressed among the populations of this distinct species.

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Table 2. Comparison of morphologically similar species of Humidophila manipurensis sp.nov.

Characters	***Humidophila manipurensis* sp.nov.**	***Humidophila simplex***	***Humidophila contenta***	***Humidophila undulocontent*a**	***Humidophila paracontenta***	***Humidophila discordabilis***	***Humidophila deceptionensis***
Length	9.5–13.0 µm	6.0–11.5 µm	6.1–11.6 µm	7.5–10.5 µm	7.0–15.0 µm	16–20 µm	9.0–12.5
Width	2.0–3.0 µm	2.1–2.9 µm	2.1–2.9 µm	2.5–3.5 µm	2.8–3.8 µm	5.5–6.5 µm	2.7–3.1 µm
Striae (in 10 µm)	45–50	34–38	34–49	40–42	27–30	28–32	30–32
Valve outline	Linear with slightly swallow at middle	Linear, round triundulate margin	Elliptical with parallel margins	Triundulate	Linear with slightly swallow at middle	Linear, no undulation at middle	Linear, distinctly concave margin
Raphe	Raphe filiform, straight	Filiform and straight	Raphe filiform, straight	Straight and filiform	Straight and filiform	Straight and filiform	Straight
Raphe ends	Slightly marked terminal raphe ends with deflected and shallow depression	Both ends curved to same side	T–shaped proximal and terminal endings	Slightly marked endings (shallow depressions in the valve)	Clearly marked ends, internally proximal raphe ends are curved	Slightly marked endings	Slightly expanded proximal ends
Striae	Parallel	Parallel	Transapical striae parallel, interrupted centrally	Parallel, interrupted centrally (May or may not be present)	Strongly radiate, more convergent towards to apices	Not Parallel, slightly radiate	Parallel
Areolae	Small round to slightly elongated	Small round to elongated	Rounded to elongated	Rounded to elongated	Elongated	Clearly Elongated	Transapically elongated areola
Fascia	Present	Present	Clearly present	May or may not be present (areolae absent on internal valve)	Clearly present	Absent	Absent
Habitat	Water spray zone, wet wall.	Moss	Moss	Wet wall	Freshwater, and mosses	–	Moss
Reference	Present study	REICHARDT (2018)/ LOWE et al. (2014)	CHATTOVÁ et al. (2018)	LOWE et al. (2017)	LOWE et al. (2014)	LOWE et al. (2014)	KOPALOVÁ et al. (2015)

Table 3. Comparison of morphological characteristics of Humidophila bigibba from three populations across Asia.

Characters	***India*** **(Meghalaya)**	***China*** **(Libo, Guizhou province)**	***Indonesia*** **(Ranau Lake, Java)**
Length	12–18 µm	13–26 µm	16.5 µm
Width	2.0–3.5 µm (Centre) 3–4.5 µm (Broad area)	4.0–5 (Broad area) µm	3.5 µm
Striae (in 10 µm)	29–31	29–30	30
Valve shape	Linear with higher degree of undulation	Linear with slightly undulate margins	Linear with higher degree of undulation
Constriction between head pole and mid–valve	Deep constriction in the middle valve with true undulation	Slightly constricted valve middle, slightly triundulate	Deep constriction in the middle valve with true undulation
Raphe	Straight and linear, Simple straight proximal and distal endings	Straight and linear Not expanded at either the proximal or distal ends	Straight and linear
Striae	Parallel throughout the entire valve but slightly radiate at the apical end	Parallel throughout the entire valve slightly radiate at the apical end	Parallel throughout the entire valve slightly radiate at the apical end
Areolae	Elongated, Continuing around the apices without interruption	Elongated, Continuing around the apices without interruption	—
Central area	Circular	Circular	Circular
Habitat	Wet wall, Mawsami Caves, Meghalaya, India	Wet wall, Maolan Natural Reserve, Libo County, Guizhou province, China	Sumatra, Ranau
Reference	Present study	Lowe et al. (2017)	Hustedt (1937)

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