A Taxonomic Investigation on Heterocytous Cyanobacteria of West Bengal, India

Sreemanti Banerjee, Akanksha Singh and Ruma Pal*

Phycology Laboratory, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata–700019, West Bengal, India

ABSTRACT

The current effort was mainly focused on the exploration of heterocytous morphotypes of Cyanobacteria from a wide range of habitats throughout West Bengal. Thorough sampling has been carried out in rice fields as well as terrestrial locales of different areas of the state. A total of 35 heterocytous species from 19 genera were reported which dominantly belong to Nostocaceae followed by Scytonemataceae, Rivulariaceae, Microchaetaceae and Hapalosiphonaceae. Nine species namely *Isocystis infusiona, Tolypothrix willei, Aulosira thermalis, Anabaena turkestanica, Ophiothrix epidendron, Cylindrospermum skuje, Trichormus rotundosporus, Brasilonema octagenarum* and *Hassallia boutellei* have been reported for the first time from India, and one species of *Hapalosiphon* namely *H. pumilus* was recorded for the first time from West Bengal.

Key words: Cyanobacteria, Heterocytous, New reports, West Bengal

*Author for correspondence: Ruma Pal, e-mail: rpalcu@rediffmail.com

Introduction

Cyanobacteria containing specialized nitrogen fixing cells (heterocytes: Komárek & Anagnostidis 2005), akinetes and filaments showing true and false branching, are classified under Nostocales (Komarek 2013). This group mainly favour unpolluted habitats for their growth in comparison to other Cyanobacterial members. Favourable habitat of the group includes rice fields, wet surfaces of soil and tree barks (Tirkey & Adhikary, 2005; Bhakta et al., 2016). The members are ecologically significant for their nitrogen fixing capacity and well known to increase fertility of soil. In modern taxonomy, a polyphasic approach (including molecular, ultrastructural and ecological features) has been considered in cases when morphological identification cannot be conclusive enough for identification of unicellular and oscillatorian morphotypes. However, heterocytous members have very distinct vegetative cell morphology as well as akinetes and heterocytes whose size, shape, position and numbers can serve as highly specific characters to identify up to species level (Komarek, 2013). Observable characteristics of true and false branching patterns are also taxonomically significant.

In India several authors have worked on Cyanobacteria of rice fields which mainly included heterocytous members along with very few unicellular and oscillatorian morphotypes (Tiwari, 1972; Laloraya & Mitra, 1974a, 1974b; Venkataraman, 1975; Sarma & Kanta, 1979, 1980; Kanta & Sarma, 1980; Saha & Mandal, 1980; Sardeshpande & Goyal, 1981; Bongale, 1981; Singh et al., 1986; Anand & Hopper, 1987; Anand, 1989; Singh et al. 1997a, 1997b). Reported dominant taxa included Aulosira sp., Cylindrospermum sp., Nostoc sp., Anabaena sp., Hapalosiphon sp., Tolypothrix sp. etc. Nikam et al. (2010) studied cyanobacterial diversity of Western Ghats of Maharastra. They have investigated 627 soil samples and reported 94 cyanobacterial species including several heterocytous species. Presence of Scytonema, Calothrix, Aulosira and Nostoc on stone monuments and sculptures was explored by Keshri and Adhikary, 2013. Molecular characterization has been implemented to characterize ten Anabaena species (Ezhilarasi & Anand, 2009). New species from Scytonema and Nostoc were reported from Chhattisgarh (Singh et al., 2017) and Jabalpur (Bagchi et al., 2017) respectively. In West Bengal several groups have investigated members of Nostocales earlier (Mukhopadhyay & Chatterjee, 1981; Sinha & Mukherjee, 1975a, 1975b, 1984; Santra, 1993) but very few studies are available regarding the present status of morphotypes and distribution of heterocytous Cyanobacteria from West Bengal.

The present authors have already worked on diverse Cyanobacterial morphotypes from West Bengal (Banerjee & Pal, 2017). The current study was aimed

to investigate heterocytous morphotypes from different habitats throughout West Bengal including scanning electron microscopic (SEM) investigation of some selected forms.

Material and Methods

Samples were collected from 15 districts of West Bengal (Figure 1, Table 1). Sampling was done from rice fields, soil crusts, tree barks, and stagnant waters of grasslands, with the help of scalpel and forceps from March to September 2017-2019. Samples were preserved in 4% formalin; voucher specimens were properly labelled and deposited in Calcutta University Herbarium. Aerial or subaerial samples were recorded under Voucher number (CUH/AL/AE/CYANO) and fresh water samples were recorded under Voucher number (CUH/AL/FW/CYANO). Samples were observed under Carl-Zeiss Axiostar microscope. Photomicrographs were taken using Canon T2-T2 1, 6x SLR 426115. The morphotaxonomic identification of the samples has been done using available literatures viz. Desikachary (1959) and Komarek (2013). The present status of the names was also confirmed from Algaebase, a listing of the world's algae (http://www.algaebase.org) (Guiry & Guiry, 2014).

For SEM study, samples were washed 2-3 times with phosphate buffer and then centrifuged at 10000 rpm for 10 min. A drop of washed material was taken on a glass cover slip (Blue Star) and dried. The samples were coated with gold using a Quorum (Q 150 TES) gold coater. Samples were examined and photographed at different magnifications using Carl Zeiss EVO 18 (EDS 8100) microscope with Zeiss Inca Penta FETX 3 (Oxford instruments) attachment.

Results

In this study, a total of 43 heterocytous forms were collected, and include 35 different species from 19 genera and 5 families. Among the collected samples, the dominant genus was *Anabaena* followed by *Scytonema*, *Cylindrospermum* and *Nostoc*. Among all the collected specimens the highest number was found in *Anabaena*, of which *A. iyengarii* is the most frequent species. Nine species were reported new to India. The collected species along with their collection sites have been enlisted in Table 1.

In Scytonemataceae, Microchaetaceae and Hapalosiphonaceae, heterocytes are not observed in scanning electron microscopy (SEM) as they are enclosed within a thick and continuous sheath, but in Nostocaceae and Rivulariaceae, the heterocytes can be distinguished. In the present study, *Anabaena iyengarii*, *Anabaena turkestanica*, *Nostoc carneum* and *Nostoc linckia* have been represented with their SEM photomicrographs as they have shown diverse morphologies of apical cells and heterocytes (Fig. 8).

The detailed taxonomic descriptions of the collected specimens are given below.

PHYLUM – CYANOBACTERIA CLASS – CYANOPHYCEAE ORDER – NOSTOCALES

FAMILY - NOSTOCACEAE

Anabaena iyengarii Bharadwaja [Fig. 2A-I, N, Fig. 3D-G, Fig. 8A-F, M, S-U]

References – Komarek 2013, p. 826, fig. 1040; Desikachary 1959, p. 406, pl.78, fig. 2

Holotype – *Anabaena oscillarioides* Bory ex Bornet & Flahault

Description – Filaments solitary; trichomes straight or irregularly curved, intensely constricted at cross walls, not attenuated towards ends. Cells barrel shaped, usually shorter than wide up to isodiametric, rarely longer, 3-6.4×3-6.3 μ m; terminal cells conical rounded to elongated. Heterocytes spherical to barrel shaped, \pm isodiametric, 7-9.6×7-7.2 μ m. Akinetes ellipsoid to widely oval, single on both sides of heterocytes, 5.5-21.4×3.4-16.8 μ m with smooth colourless, yellowish exospores.

SEM observations (Fig. 8A-F, M, S-U): Scanning electron microscopy revealed barrel shaped cells with distinct and diverse apical cell types. Sharp conical, obtuse conical as well as elongated apical cells were distinctly observed. Heterocytes show cell wall with reticulate pattern of surface thickening. Akinetes were ellipsoidal as observed in light microscopy.

Place of collection – Amta (Howrah), Baruipur (South 24 pgs), Kandi (Murshidabad), Dankuni (Hoogly), Kharagpur (West Midnapore), Gabgachhi (Malda)

Habitat - Rice fields, stagnant clear water bodies, mats from wet surfaces of soil

Voucher No. CUH/AL/FW/CYANO-31, 37, 83, 84, 170; CUH/AL/AE/CYANO-2, 76

Johanseninema constrictum (Szafer) Hasler, Dvorák & Poulícková [Fig. 3K, M].

Reference - Komarek 2013, p. 835, fig. 1058



Figure 1. Map of West Bengal showing study sites (black dots showing districts from where collections were done).

Holotype – *Johanseninema constrictum* (Szafer) Hasler, Dvorák & Poulícková.

Homotypic synonym – *Anabaena constricta* (Szafer) Geitler

Description – Trichomes cylindrical. Cells short, barrel shaped, $2-3\times3-7$ µm. Heterocytes spherical with diameter 4-5 µm. Absence of akinetes.

Place of collection – Gazole (Malda)

Habitat – Stagnant water body

Voucher No. CUH/AL/FW/CYANO-147

Anabaena torulosa Lagerheim ex Bornet & Flahault [Fig. 2J-M, Fig. 3I, J]

References – Komarek 2013, p.801, fig. 999; Desikachary 1959, p. 415, pl. 71, fig. 6

 $\label{eq:continuous} \mbox{Holotype-$Anabaena oscillarioides} \mbox{ Bory ex Bornet} \\ \& \mbox{ Flahault}$

Description – Trichomes flexuous. Cells barrel shaped, $1.5-2\times2.5-5~\mu m$; terminal cells conical. Heterocytes ellipsoidal, $4.5-10\times4~\mu m$. Akinetes joined to both

sides of heterocytes, solitary or in pairs, $5.2-24\times4.3-12~\mu m$.

Place of collection – Sirkabad (Purulia)

Habitat - Stagnant water in grass lands

Voucher No. CUH/AL/FW/CYANO-114,147

Anabaena turkestanica (Kiselev) Komárek [Fig. 3A-C, Fig. 8G-I]

Reference - Komarek 2013, p.831, fig.1053

Holotype – *Anabaena oscillarioides* Bory ex Bornet & Flahault

Homotypic Synonym – Anabaena oscillarioides var. turkestanica Kiselev

Description – Filaments solitary; trichomes \pm straight, clearly constricted at cross walls, attenuated gradually towards ends. Cells barrel shaped, shorter than wide 1.5-2×3.6-5 μm; terminal cells narrowed and rounded. Heterocytes barrel shaped, 3.2-4.8×4.8-5 μm. Akinetes widely oval 5.3×5 μm; attached from both sides to heterocytes, solitary or in pairs with colourless smooth exospores.

SEM observations (Fig. 8G-I): Barrel shaped cells and heterocyte with flanking akinetes were observed. Apical cell was narrowed and elongated.

Place of collection – Gazole (Malda)

Habitat – Stagnant unpolluted water bodies

Voucher No. CUH/AL/FW/CYANO-150

Nostoc calcicola Brébisson ex Bornet & Flahault [Fig. 4A, B]

Reference - Komarek 2013, p. 984, fig. 1280

Lectotype – *Nostoc commune* Vaucher ex Bornet & Flahault

Description – Colonies irregular, flat, gelatinous. Filaments freely entangled, sheaths usually indistinct, more distinct at colonial margin, colourless. Cells barrel shaped or almost sphaerical, pale blue green, 1.5-2.5×2.5-3 μm. Heterocytes spherical, 4-4.5×3.8-5 μm in diameter. Akinetes almost spherical or slightly elongated, 5-7×3-5μm with smooth, yellow cell wall.

Place of collection - Sumendu Lake (Darjeeling)

Habitat – sub-aerophytic on the surface of wet soil surface

Voucher No. CUH/AL/FW/CYANO-11

Nostoc linckia Bornet ex Bornet & Flahault [Fig. 4 P, Q, T, U, Fig. 8N-R].

Table 1

Voucher No.	Place of collection	Co-ordinates	Species name
CUH/AL/AE/CYANO 2	Amta, Howrah	22.576451 °N, 88.010854 °E	Anabaena iyengarii
CUH/AL/AE/CYANO 11	Sumendu Lake, Darjeeling	26.890014 °N, 88.180904 °E	Nostoc calcicola
CUH/AL/AE/CYANO 12	Sumendu Lake, Darjeeling	26.889140 °N, 88.185307 °E	Scytonema stuposum
CUH/AL/FW/CYANO 30	Serampore, Hoogly	22.756044 °N, 88.210710 °E	Dichothrix baueriana
CUH/AL/FW/CYANO 31	Baruipur, South 24 pgs	22.379151 °N, 88.436015 °E	Anabaena iyengarii
CUH/AL/FW/CYANO 37	Kharagpur, West Midnapore	22.20106 °N, 87.14086 °E	Anabaena iyengarii
CUH/AL/AE/CYANO 38	Baruipur, South 24 pgs	22.373939 °N, 88.435851 °E	Scytonema drilosiphon
CUH/AL/FW/CYANO 40	Telberia, Bankura	22.8334 °N, 86.6724 °E	Iscocystis infusiona
CUH/AL/AE/CYANO 52	Siliguri, Jalpaiguri	26.550596 °N, 88.714170 °E	Scytonema zellerianum
CUH/AL/AE/CYANO 54	Siliguri, Jalpaiguri	26.540371 °N, 88.697088 °E	Scytonema malaviyanense
CUH/AL/AE/CYANO 67	Rasikbill, Koochbihar	26.413994 °N, 89.737037 °E	Calothrix fusca f.durabilis
CUH/AL/AE/CYANO 70	Siliguri, Jalpaiguri	26.732370 °N, 88.407537 °E	Scytonema(Myochrotes)pratii
CUH/AL/AE/CYANO 76	Kandi, Murshidabad	23.951099 °N, 88.041150 °E	Anabaena iyengarii
CUH/AL/AE/CYANO 80	Kandi, Murshidabad	23.954804 °N, 88.042931 °E	Coleodesmium wrangelii
CUH/AL/AE/CYANO 82	Kandi, Murshidabad	23.951099 °N, 88.041150 °E	Scytonema twymanianum
CUH/AL/FW/CYANO 83	Amta, Howrah	22.570507 °N, 88.006133 °E	Anabaena iyengarii
CUH/AL/FW/CYANO 84	Dankuni, Hoogly	22.680119 °N, 88.297635 °E	Anabaena iyengarii
CUH/AL/FW/CYANO 97	Serampore, Hoogly	22.752326 °N, 88.352108 °E	Aulosira thermalis
CUH/AL/FW/CYANO 101	NilNirjon, Birbhum	23.828552 °N, 87.393622 °E	Tolypothrix willei
CUH/AL/FW/CYANO 114	Sirkabad, Purulia	23.272468 °N, 86.196478 °E	Anabaena torulosa
CUH/AL/AE/CYANO 116	Kharagpur, West Midnapore	22.19022 °N, 87.12360 °E	Brasilonema octagenarum
CUH/AL/FW/CYANO 117	Saheb bandh, Purulia	23.338726 °N, 86.358946 °E	Anabaenopsis hispanica
CUH/AL/FW/CYANO 118	Lower dam, Purulia	23.193190 °N, 86.086775 °E	Aulosira doliispora
CUH/AL/FW/CYANO 147	Gazole, Malda	25.213636 °N, 88.193414 °E	Anabaena torulosa,
			Cylindrospermum gregarium,
GT TT / 1 T / 1 T / GT / 1 1 0	a	0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	Johanseninema constrictum
CUH/AL/AE/CYANO 148	Gazole, Malda	25.213636 °N, 88.193414 °E	Cylindrospermum michailovskoense
CUH/AL/FW/CYANO 150	Gazole, Malda	25.214259 °N, 88.189659 °E	Anabaena turkestanica
CUH/AL/FW/CYANO 153	Chakvrigu, Uttar Dinajpur	25.227341 °N, 88.767815 °E	Hapalosiphon pumilus
CUH/AL/AE/CYANO 154	Barasat, North 24pgs	22.718228 °N, 88.470645 °E	Ophiothrix epidendron
CUH/AL/FW/CYANO 163	Adina, Malda	25.137019 °N, 88.153600 °E	Cylindrospermum skuje
CUH/AL/FW/CYANO 165	Gabgachhi, Malda	24.966940 °N, 88.133247 °E	Nostoc linckia
CUH/AL/FW/CYANO 166	Gabgachhi, Malda	24.966512 °N, 88.130629 °E	Trichormus rotundosporus Gloeotrichia raciborskii
CUH/AL/FW/CYANO 168	Gabgachhi, Malda	24.962271 °N, 88.133161 °E	Cylindrospermum stagnale
CUH/AL/FW/CYANO 169	Gabgachhi, Malda	24.962271 °N, 88.133161 °E	Gloeotrichia pisum; Nostoc carneum
CUH/AL/FW/CYANO 170	Gabgachhi, Malda	24.966940 °N, 88.133247 °E	Anabaena iyengarii
CUH/AL/FW/CYANO 238	Bongabari, Purulia	23.375630 °N, 86.404334 °E	Nostoc linckia
CUH/AL/AE/CYANO 243	Raghunathpur, Purulia	23.552625 °N, 86.679207 °E	Scytonema ocellatum
CUH/AL/AE/CYANO 249	Manicktala, Kolkata	22.587996 °N, 88.370794 °E	Scytonema hofmanii
CUH/AL/AE/CYANO 256	Duff Street, Kolkata	22.587944 °N, 88.370993 °E	Scytonema millei
CUH/AL/AE/CYANO 257	Jadavpur, Kolkata	22.497759 °N, 88.379319 °E	Hassallia boutellei

References – Komarek 2013, p. 968, fig. 1260; Desikachary 1959, p. 377, pl. 67, fig. 1

Lectotype – *Nostoc commune* Vaucher ex Bornet & Flahault.

Homotypic Synonym – *Stratonostoc linckia* (Bornet ex Bornet & Flahault) Elenkin

Description – Colonies fine thin mucilaginous, free floating in the form of irregular amorphous, disintegrating dirty olive green gelatinous clusters. Filaments flexuous, densely entangled, especially in young colonies. Sheaths colourless. Cells shortly barrel shaped, blue green, 1.5-2×2.5-4 μm. Heterocytes almost spherical, 2.5-3×2.3-6 μm. Akinetes not observed.

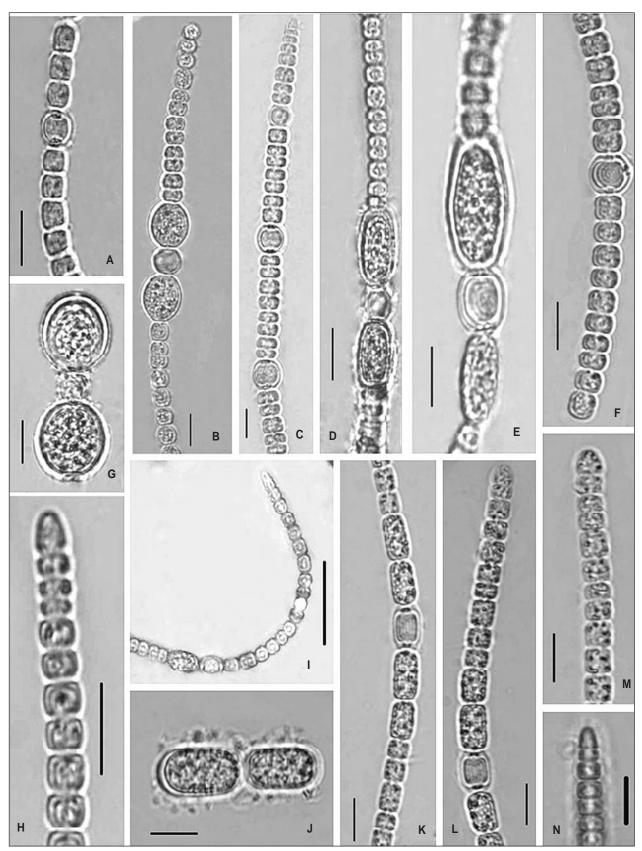


Figure 2. (A-I) Trichomes of *Anabaena iyengarii* showing heterocytes with flanking akinetes of different shapes ranging from spherical to ellipsoidal; (J-M) *Anabaena torulosa* trichomes with more than one akinetes flanking the heterocytes; (N) Trichome of *Anabaena iyengarii* showing conical apical cell. Scale bar 5 μm.

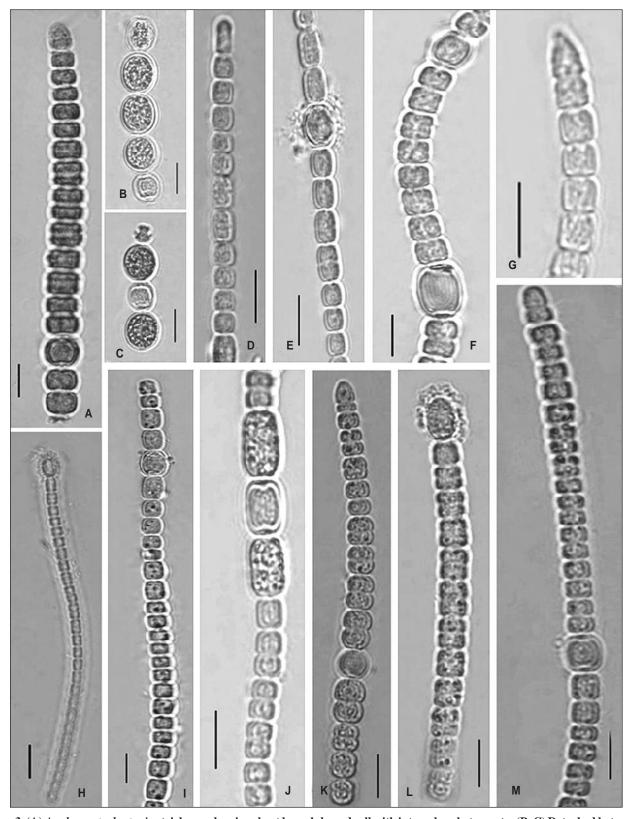


Figure 3. (A) Anabaena turkestanica trichome showing short barrel shaped cell with intercalary heterocyte; (B, C) Detached heterocyte of Anabaena turkestanica with more than one akinete; (D-E) Trichomes of Anabaena iyengarii with elongated cells; (F, G) Trichomes of Anabaena iyengarii with barrel shaped cells; (H) Anabaenopsis hispanica trichome showing terminal heterocyte; (I, J) Anabaena torulosa trichome; (K) Johanseninema constrictum trichome showing heterocyte, akinetes were not observed; (L) Cylindrospermum gregarium trichome showing terminal heterocyte; (M) Johanseninema constrictum trichome. Scale bar 5 µm.

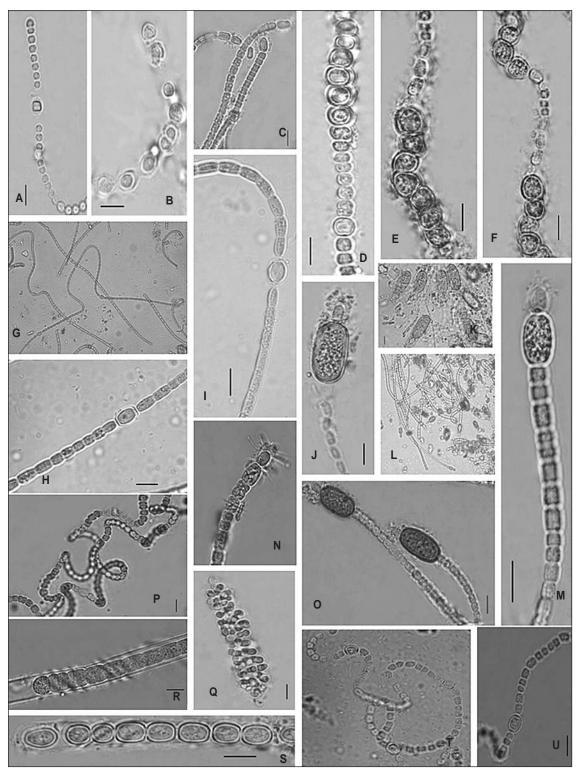


Figure 4. (A) Nostoc calcicola trichome with intercalary heterocyte; (B) Nostoc calcicola trichome with rows of akinetes; (C) Cylindrospermum skuje trichomes with terminal ellipsoidal heterocyte; (D) Trichormus rotundosporus with rows of akinetes located distantly from intercalary heterocyte; (E-F) Isocystis infusiona trichomes with rows of akinetes having thick yellowish exospores; (G) Association of Nostoc carneum trichomes with both intercalary and terminal heterocytes; (H, I) Nostoc carneum trichomes with intercalary heterocyte; (J, K)Trichomes of Cylindrospermum stagnale; (L) Cluster of Cylindrospermum michailovskoense trichomes; (M) Single trichome of Cylindrospermum michailovskoense; (N, O) Cylindrospermum skuje; (P, Q) Flexuous and coiled trichome showing terminal heterocytes in Nostoc linckia; (R) Aulosira thermalis filament; (S) Series of akinetes formed in filament of Aulosira thermalis; (T, U) Trichomes of Nostoc linckia. Scale bar 5 µm.

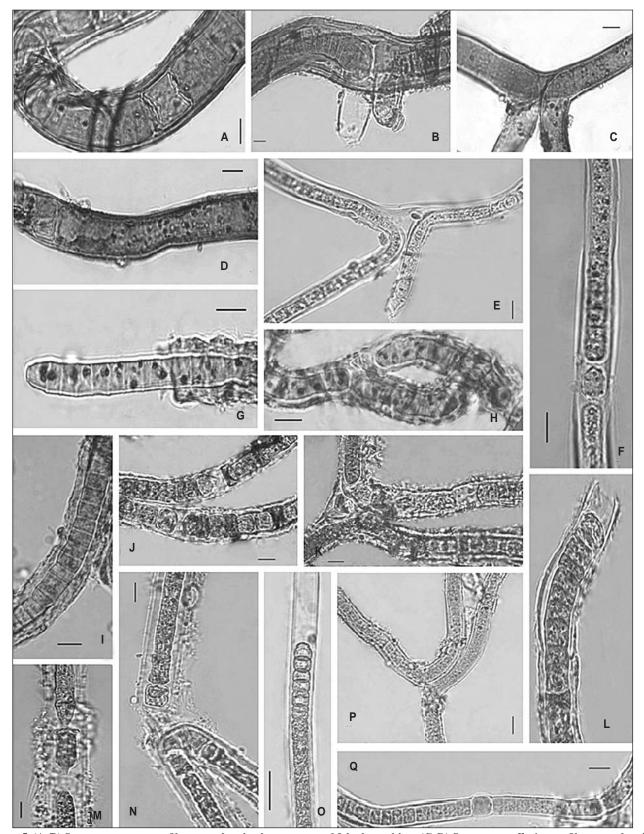


Figure 5. (A, B) Scytonema stuposum filaments showing heterocyte and false branching; (C, D) Scytonema zellerianum filaments showing heterocyte and false branching; (E, F) Scytonema drilosiphon filaments showing heterocyte and false branching; (G-I) Scytonema malaviyanense; (J-L) Scytonema twymanianum; (M-O) Tolypothrix willei; (P) Scytonema hofmanii; (Q) Scytonema ocellatum. Scale bar 5 µm.

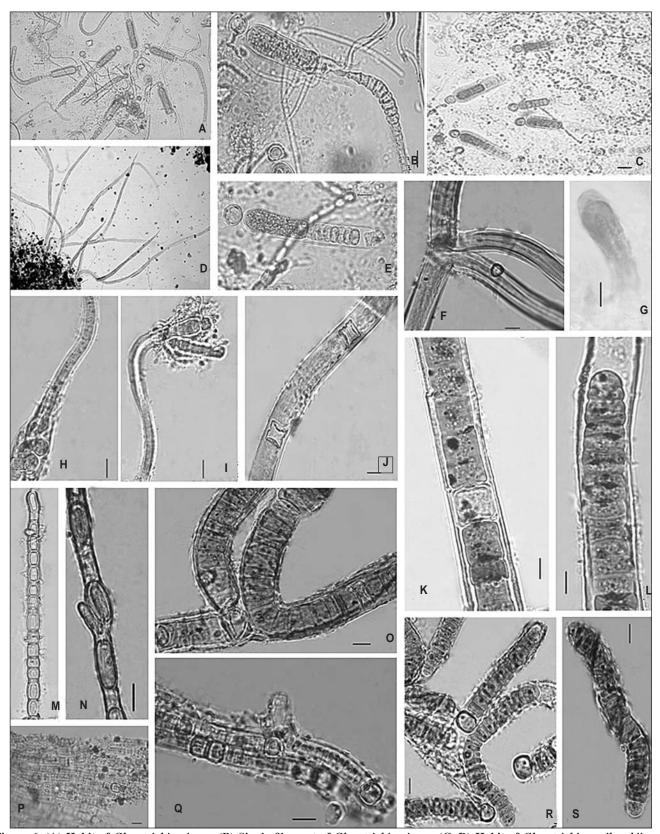


Figure 6. (A) Habit of Gloeotrichia pisum; (B) Single filament of Gloeotrichia pisum; (C, D) Habit of Gloeotrichia raciborskii and Dichothrix baueriana; (E) Single filament of Gloeotrichia raciborskii; (F, G) Dichothrix baueriana; (H, I) Calothrix fusca; (J) Filament showing heterocyte in Dichothrix baueriana; (K, L) Brasilonema octagenarum; (M, N) Aulosira doliispora; (O) Scytonema millei; (P, Q) Coleodesmium wrangelii; (R, S) Hassallia boutellei Scale bar 5 µm.

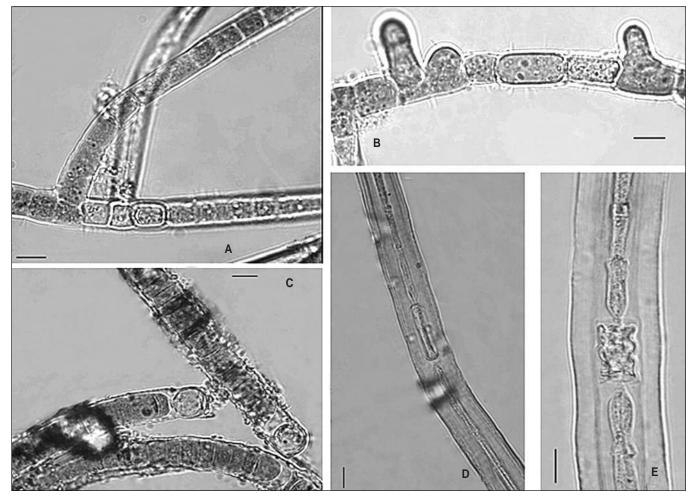


Figure 7. Filaments of (A, B) Hapalosiphon pumilus; (C) Ophiothrix epidendron; (D, E) Scytonema pratii. Scale bar 5 µm.

SEM observations (Fig. 8N-R): Cells barrel shaped with intercalary as well as terminal heterocytes. Intercalary heterocytes showed distinct thickening of wall which differ from the wall thickening of *Anabaena* sp.

Place of collection – Gabgachhi (Malda), Bongabari (Purulia)

Habitat - Rice field, grass lands

Voucher No. CUH/AL/FW/CYANO-165,238

Nostoc carneum C.Agardh ex Bornet & Flahault [Fig. 4G-I, Fig. 8J-L]

Reference – Komarek 2013, p.968, fig.1261

Lectotype – *Nostoc commune* Vaucher ex Bornet & Flahault.

Description—Colonies irregularly clustering, gelatinous, fine, macroscopic, free floating, brownish. Filaments flexuous, freely entangled; sheaths unclear, colourless. Cells long, usually cylindrical, 5-8×3-4 μm. Heterocytes

oval, slightly elongated, 4-6 µm wide. Akinetes were not observed.

SEM Observations (Fig. 8J-L): Cells elongated and connected with mucilaginous connections. Heterocytes were elongated and with thickened wall but no pattern of thickening was observed. Akinetes were not detected in light micrographs whereas in SEM, spheroidal akinetes (diameter 5-6 µm) were clearly observed flanking the heterocyte.

Place of collection - Gabgachhi (Malda)

Habitat - Stagnant water

Voucher No. CUH/AL/FW/CYANO-169

Isocystis infusiona Borzì ex Bornet & Flahault [Fig. 4E, F]

Reference - Komarek 2013, p.922, fig.1198

Lectotype – *Isocystis messanensis Borzì* ex *Bornet* & *Flahault*.

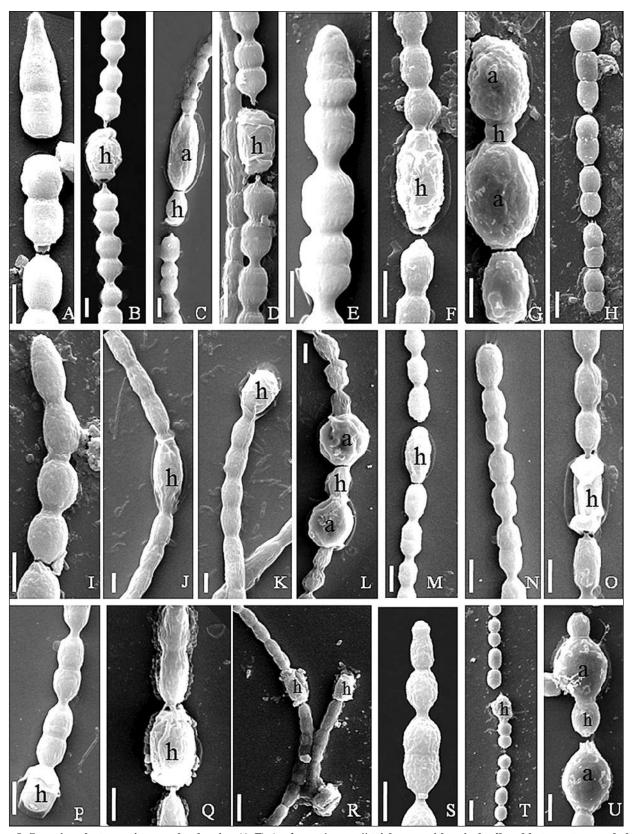


Figure 8. Scanning electron micrographs showing (A-F) *Anabaena iyengarii* trichomes with apical cell and heterocyte morphology; (G-I) Trichome and heterocytes with flanking akinetes of *Anabaena turkestanica*; (J-L) Trichome and heterocytes with flanking akinetes of *Nostoc carneum*; (M) Trichome of *Anabaena iyengarii*; (N-R) Trichomes of *Nostoc linckia*; (S-U) Trichomes of *Anabaena iyengarii*. Scale bar 2 µm. [h Heterocyte; a Akinete.]

Heterotypic Synonym – Anabaena infusionum Kützing

Description – Filaments solitary; trichomes constricted at the cross walls. Cells oval, pale blue green, 1.8- $2.2\times1-1.5~\mu m$. Akinetes spherical, 4- $5\times4.8-5.4~\mu m$, larger than vegetative cells with smooth brown wall.

Place of collection – Telberia (Bankura)

Habitat – Water reservoir

Voucher No. CUH /AL/FW/CYANO-40

Anabaenopsis hispanica P. González [Fig. 3H]

Reference - Komarek 2013, p.787, fig. 986B

Lectotype – Anabaenopsis elenkinii V.V.Miller

Description – Trichomes straight, constricted at cross walls. Cells of different sizes, 1.5-7.3×2-4.4 μm. Heterocytes ellipsoidal 2.5-11.6×2-7.3 μm. In the present study akinetes not found.

Place of collection – Saheb bandh (Purulia)

Habitat – Planktonic

Voucher No. CUH/AL/FW/CYANO-117

Aulosira thermalis G.S. West [Fig. 4R,S]

Reference - Komarek 2013, p. 855, fig.1090

Lectotype – *Aulosira laxa* O.Kirchner ex Bornet & Flahault.

Description – Filaments form olive green to brown thallus, flexuous, 3-6 μm wide. Trichomes simple, constricted at cross walls. Sheaths firm, colourless, homogeneous in vegetative filaments. Cells $2.4\text{-}4.5\times$ 5-6 μm; apical cells conical rounded. Akinetes serially upto 10-15 in a row, usually oval in shape with thick lamellated cell wall, $3.6\text{-}10\times3.6\text{-}6$ μm.

Place of collection – Serampore (Hoogly)

Habitat – Stagnant water mixed with other filamentous cyanobacteria

Voucher No. CUH/AL/FW/CYANO-97

Aulosira doliispora Komárek et al. [Fig. 6M, N]

Reference – Komarek 2013, p.861, fig.1101

Lectotype – *Aulosira laxa* O.Kirchner ex Bornet & Flahault.

Description – Colonies macroscopic, membranaceous. Sheath thin but firm, colourless, not lamellated, at the ends of filaments narrowed into a thin hair like long process. Tolypotrichoid false branching. Trichomes cylindrical, not constricted at cross walls. Cells 4.8-5×3-4.5 μm, cylindrical. Heterocytes intercalary

cylindrical, $5\text{-}23.5\times3.5\text{-}6~\mu m$. Akinetes develop from intercalary cells in long rows, $10\text{-}18.2\times7\text{-}9~\mu m$, with smooth brownish exospore.

Place of collection – Lower dam (Purulia)

Habitat - Stagnant small water body

Voucher No. CUH/AL/FW/CYANO-118

Cylindrospermum gregarium (Zakrzewski) Elenkin [Fig. 3L]

Reference - Komarek 2013, p.897, fig.1165

Lectotype – *Cylindrospermum majus* Kützing ex Bornet & Flahault.

Description – Filaments straight. Cells quadratic, 2.8-6.5×2-4 μm. Heterocytes ellipsoidal, 5-8×3-5 μm. Akinetes unobserved.

Place of collection – Gazole (Malda)

Habitat - Clear stagnant water in grass land

Voucher No. CUH/AL/FW/CYANO-147

Cylindrospermum michailovskoense Elenkin [Fig. 4L, M]

References – Komarek 2013, p. 885, fig.1140; Desikachary 1959, p. 368, pl. 65, fig. 1

Lectotype – *Cylindrospermum majus* Kützing ex Bornet & Flahault.

Description – Thallus mucilaginous, blue green with densely aggregated filaments. Trichomes flexuous constricted at the cross walls. Cells pale blue green, slightly barrel shaped, 2.5-7.2×2-5 μm; end cells rounded. Heterocytes are a little elongated, 3-12×2-7 μm. Akinetes solitary, oval 7-32.6×4-13.2 μm with smooth colourless exospore.

Place of collection – Gazole (Malda)

Habitat – Clear stagnant water in grass land Voucher No. CUH/AL/FW/CYANO-148

Cylindrospermum skuje Komárek [Fig. 4C, N, O]

Reference - Komarek 2013, p. 885, fig. 1138

Lectotype – *Cylindrospermum majus* Kützing ex Bornet & Flahault

Description – Mats blue green, trichomes flexuous, constricted at cross walls. Cells \pm cylindrical, 1.6-5×2.2-3 μm. Heterocytes ovoid, 2-8×1.5-4.5 μm. Akinetes oval-cylindrical, solitary, 12-36×7-11.5 μm with smooth brownish exospores.

Place of collection – Adina (Malda)

Habitat – Small pool

Voucher No. CUH/AL/FW/CYANO-163

Cylindrospermum stagnale Bornet & Flahault [Fig. 4J, K]

References – Komarek 2013, p. 878, fig. 1129; Desikachary 1959, p. 363, pl. 65, fig. 9

Lectotype – *Cylindrospermum majus* Kützing ex Bornet & Flahault.

Description – Mats mucilaginous, blue green attached to submersed substrates, mostly water plants, later coming free and flowing near the water surface. Trichomes flexuous, distinctly constricted at the cross walls. Cells 2 × longer than wide, 1.5-6 μm wide. Heterocytes oval with rounded ends $3.4\text{-}16\times2.6\text{-}7$ μm. Akinetes solitary, cylindrical or cylindrical-oval and rounded at the ends $15\text{-}40\times8\text{-}16$ μm with smooth yellow brown exospores.

Place of collection – Gabgachhi (Malda)

Habitat – Clear stagnant water in grass land

Voucher No. CUH/AL/FW/CYANO-168

Trichormus rotundosporus (Hollerbach) Komárek & Anagnostidis [Fig. 4D]

Reference – Komarek 2013, p. 933, fig. 1213

Holotype – *Trichormus variabilis* (Kützing ex Bornet & Flahault) Komárek & Anagnostidis.

Homotypic Synonym – Anabaena variabilis var. rotundospora Hollerbach

Description–Mats blue green with irregularly entangled trichomes without sheaths. Trichomes cylindrical not attenuated towards ends constricted at cross walls. Cells barrel shaped 1.5-2.5×3-4.5 μm. Heterocytes \pm spherical 4-8×4-7 μm. Akinetes in rows arising distant from heterocytes \pm spherical, 4.5-10.8 μm in diameter.

Place of collection - Gabgachhi (Malda)

Habitat – Clear stagnant water in grass land

Voucher No. CUH/AL/FW/CYANO-166

FAMILY – SCYTONEMATACEAE

Scytonema stuposum Bornet ex Bornet & Flahault [Fig. 5A, B]

References – Komarek 2013, p. 76, fig. 39; Desikachary 1959, p. 459, pl. 93, fig. 4

Lectotype – *Scytonema hofmanii* C.Agardh ex Bornet & Flahault

Description – Thallus prostrate, wooly, brownish red in colour. Filaments entangled, coiled, 18-21 μm wide with geminate false branches. Sheath thick, gelatinous, yellowish in colour. Trichomes cylindrical, distinctly constricted at cross walls, blue green in colour. Cells shorter than wide, $10.2\text{-}17.5\times4\text{-}5$ μm, terminal cells rounded. Heterocytes solitary, quadratic, yellowish, $8\text{-}10\times10\text{-}15$ μm.

Place of collection - Sumendu Lake (Darjeeling)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-12

Scytonema zellerianum Brühl & Biswas [Fig. 5C, D]

Reference - Komarek 2013, p. 105, fig. 84

Lectotype – *Scytonema hofmanii* C.Agardh ex Bornet & Flahault.

Description – Trichomes 7-20 μ m wide, not constricted at cross walls, with geminate false branching. Sheath thin, narrow, smooth. Cells barrel shaped, shorter than wide, 1-2×4-5 μ m yellowish green in colour. Heterocytes quadrate, 4-5×6-7 μ m.

Place of collection - Siliguri (Jalpaiguri)

Habitat – Stone surface

Voucher No. CUH/AL/AE/CYANO-52

Scytonema drilosiphon Elenkin [Yelenkin] & V.I. Polyansky [Polyanski] [Fig. 5E, F]

Reference – Komarek 2013, p. 73, fig. 33

Lectotype – *Scytonema hofmanii* C. Agardh ex Bornet & Flahault.

Description – Thallus woolly, filaments 7-13 μm wide, with single geminate false branching. Sheath firm, not lamellated, colourless to slight yellowish. Trichomes blue green, cylindrical, very slightly constricted at cross walls, 5.5-10 μm wide. Cells cylindrical, $2.5-12\times3-5$ μm. Heterocytes quadratic $5-12\times4-5$ μm.

Place of collection - Baruipur (South 24 Parganas)

Habitat - Rock surface

Voucher No. CUH/AL/AE/CYANO-38

Scytonema malaviyanense Bharadwaja [Fig. 5G-I]

References – Komarek 2013, p.94, fig.86; Desikachary 1959, p. 483, pl. 86, fig. l

Lectotype – *Scytonema hofmanii* C.Agardh ex Bornet & Flahault.

Description – Thallus thick, spongy. Filaments flexuous, interwoven 8-10 μ m wide, sheaths thick firm, yellowish. Cells 2.5-7×4-6 μ m. Heterocytes not present in the filaments. False branches are same as mother filament.

Place of collection - Siliguri (New Jalpaiguri)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-54

Scytonema twymanianum Welsh [Fig. 5J-L]

Reference - Komarek 2013, p. 101, fig. 83

Lectotype – *Scytonema hofmanii* C.Agardh ex Bornet & Flahault.

Description – Thallus macroscopic, very dark olive green. Filaments interwoven, bluish green, 10-17 μm wide with false branching, branches geminate. Sheaths firm, colourless 1-2 μm thick. Cells $5-6\times7-8$ μm in dimension. Heterocytes sub-quadrate, solitary, intercalary, generally shorter than long, numerous short hormogonia.

Place of collection - Kandi (Murshidabad)

Habitat – Surface of a fungal fruiting body belonging to Polyporaceae.

Voucher No. CUH/AL/AE/CYANO-82

Scytonema ocellatum Lyngbye ex Bornet & Flahault [Fig. 5Q]

References – Komarek 2013, p. 75, fig. 37; Desikachary 1959, p. 467, pl. 92, fig. 3

Lectotype - *Scytonema hofmanii* C.Agardh ex Bornet & Flahault

Description – Thallus bluish. Filaments 6-18 μm wide, false branched. Sheaths firm, on outside rough, colourless to yellowish. Trichomes cylindrical not constricted at cross walls, not attenuated at the ends. Cells $2.5\text{-}4\times5\text{-}14$ μm. Heterocytes short barrel shaped $5\text{-}9\times5\text{-}7$ μm, yellowish some times.

Place of collection – Raghunathpur (Purulia)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-243

Scytonema hofmanii C.Agardh ex Bornet & Flahault [Fig. 5P]

References – Komarek 2013, p.71, fig.32; Desikachary 1959, p.476, pl. 91, fig. 2

Lectotype – *Scytonema hofmanii* C.Agardh ex Bornet & Flahault.

Description – Thallus woolly. Filaments in irregular fascicles, 5-10.5 μm wide with geminate false branching. Sheaths narrow thin colourless to yellow. Trichomes cylindrical, not constricted at cross walls. Cells 2.5- $4\times4.6-9.5$ μm, very slightly widened at the ends. Heterocytes solitary, cylindrical 4.6-10×4.6-5 μm.

Place of collection - Kolkata

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-249

Brasilonema octagenarum R. Anguiar, M.F. Fiore, M.W. Franco, M.C. Ventrella, A.S. Lorenzi, C.A. Vanetti & A.C. Alfenas [Fig. 6K,L]

Reference - Komarek 2013, p. 109, fig. 90

Holotype – *Brasilonema bromeliae* Fiore, Sant-Anna, de Paiva Azevedo, Komarek, Kastovsky, Sulek & Lorenzi.

Description – Thallus macroscopic composed of irregularly fasciculated filaments. Filaments cylindrical 9-18.5 μm wide. Sheaths thin firm, colourless. Cells ± isodiametric with brownish violet cell content, not constricted at cell wall, 1.5-13.3×5-9 μm. Heterocytes cylindrical, intercalary 5.4-15.6×10-17 μm.

Place of collection – Kharagpur (Midnapore)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-116

Scytonema millei Bornet ex Bornet & Flahault [Fig. 6O]

References – Komarek 2013, p.94, fig.68; Desikachary 1959, p. 460, pl. 93, fig.2, 3

Lectotype – *Scytonema hofmanii* C.Agardh ex Bornet & Flahault.

Description – Thallus woolly, brownish. Filaments 15-21 μm wide, false branches same as main filaments. Sheath firm, brown, yellowish. Trichomes constricted at cross walls, cells $4-5\times10-15$ μm. Heterocytes intercalary solitary dimension similar to vegetative cells.

Place of collection – Duff Street (Kolkata)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-256

Scytonema pratii Komárek [Fig. 7D, E]

Reference - Komarek 2013, p. 120, fig. 104

Lectotype – *Scytonema hofmanii* C.Agardh ex Bornet & Flahault.

Description – Thallus in form of cottony mats, filaments

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coiled, 13.4-15.2 μm wide, sheaths thick, about 3 μm wide, initially colourless and indistinctly lamellated, later yellowish. Cells cylindrical 10-20×1.2-5 μm . Heterocytes intercalary, wider than vegetative cells, cylindrical, 12-20.5×5-9.6 μm .

Place of collection – Siliguri (New Jalpaiguri)

Habitat - Rock surface

Voucher No. CUH/AL/AE/CYANO-70

FAMILY - RIVULARIACEAE

Gloeotrichia pisum Thuret ex Bornet & Flahault [Fig. 6A, B]

References – Komarek 2013, p. 358, fig. 422; Desikachary 1959, p. 556, pl. 116, fig. 4, 5

Holotype – *Gloeotrichia pisum* Thuret ex Bornet & Flahault.

Heterotypic Synonyms – Rivularia pisum C. Agardh 1824; *Physactis pisum* (C. Agardh) Kützing 1849: Rivularia durissima Rabenhorst 1865.

Description – Colonies hemispherical, brownish. Filaments radially arranged. Sheaths attached to trichomes, colourless. Trichomes constricted at cross walls. Cells 3-20×4-10 μm wide. Only 2-3 μm wide near the end. Heterocytes basal, spherical, 5-15 μm in diameter. Akinetes solitary, cylindrical, 20-400×9-10 μm.

Place of collection – Gabgachhi (Malda)

Habitat – Stagnant water

Voucher No. CUH/AL/FW/CYANO-169

Gloeotrichia raciborskii Woloszynska [Fig. 6C, E]

References – Komarek 2013, p.373, fig. 440; Desikachary 1959, p. 562, pl. 118, fig. 14

Holotype – *Gloeotrichia pisum* Thuret ex Bornet & Flahault.

Description – Colonies spherical, brownish. Trichomes clearly constricted at cross walls. Cells barrel shaped, bright blue green, $3-4\times6-9$ μm near basal parts, towards ends narrowed into long hairs. Heterocytes spherical, 5-14μm in diameter. Akinetes cylindrical $20-55\times7-25$ μm with colourless exospores.

Place of collection – Gabgachhi (Malda)

Habitat – Clear stagnant water in grass land

Voucher No. CUH/AL/FW/CYANO-166

Dichothrix baueriana Bornet & Flahault [Fig. 6D, F, G, J]

References – Komarek 2013, p. 323, fig. 373; Desikachary 1959, p. 546, pl. 111, fig. 1, 3, 4

Holotype – *Dichothrix penicillata* Zanardini ex Bornet & Flahault.

Description–Colonies fasciculate, bush like, brownish. Trichomes 1.5-7.5 μm wide, continually narrowed towards ends, terminated by long hair, constricted at cross walls, distinctly widened and bulbous at the base. Cells shorter than wide. Heterocytes basal \pm hemispherical, 2-3×3-4 μm.

Place of collection – Serampore (Hoogly)

Habitat - Stagnant water

Voucher No. CUH/AL/FW/CYANO-30

Calothrix fusca f. durabilis Starmach [Fig. 6H, I]

References-Komarek 2013, p. 228, fig. 227; Desikachary 1959, p. 527, pl. 107, fig. 10

Lectotype – *Calothrix confervicola* C.Agardh ex Bornet & Flahault.

Heterotypic Synonym – *Mastichothrix fusca* Kützing 1843

Description – Filaments flexuous with lateral false branches, 4-16 μm wide near bases, in the middle 2.5-5 μm wide. Heterocytes rounded conical in shape.

Place of collection – Rasikbill (Koochbihar)

Habitat – Wetted soil surface

Voucher No. CUH/AL/AE/CYANO-67

FAMILY - MICROCHAETACEAE

Tolypothrix willei N.L. Gardner [Fig. 5M-O]

Reference - Komarek 2013, p. 453, fig. 554

Lectotype – *Tolypothrix distorta* Kützing ex Bornet & Flahault.

Description – Filaments grow solitary among other algae, 7-10 μm wide, sparsely branched. Trichomes slightly narrowed in old parts and widened towards end. Cells 5-7×4-5 μm in middle parts when mature. Apical cells spherical or sub spherical to rounded. Heterocytes 6.5-7.2×4.5-5 μm, intercalary and the base of false branches.

Place of collection – Nil nirjon (Birbhum)

Habitat – Lake

Voucher No. CUH/AL/FW/CYANO-101

Coleodesmium wrangelii Borzì ex Geitler [Fig. 6P, Q]

Reference – Komarek 2013, p. 467, fig. 571

Holotype – Coleodesmium wrangelii Borzì ex Geitler

Heterotypic Synonym – Coleodesmiopsis fremyi Dutt, Datta & Gupta 1982

Description – Thallus heteropolar, composed of richly false branched, brush like filaments, with 2-10 trichomes in a sheath; branches remained joined parallel to the mother filament and are diverged only in the upper part. Trichomes cylindrical, 4-12 μm wide. Cells 2-5×4-5 μm. Heterocytes mostly basal in trichomes and at the bases of branches, sometimes in pairs, shortly cylindrical, $5-6\times5-6$ μm.

Place of collection – Kandi (Murshidabad)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-80

Hassallia boutellei Bornet et Flahault 1888 [Fig. 6R, S]

Reference – Komarek 2013, p. 476, fig. 581

Lectotype – *Hassallia byssoidea* Hassall ex Bornet & Flahault

Description—Filaments 5-7μm wide with short, arcuated false branches. Sheaths thin, narrow, colourless up to golden yellow. Trichomes constricted at cross walls, not attenuated at the ends. Cells shortly barrel shaped, $2\text{-}3\times4\text{-}5$ μm, apical cells rounded. Heterocytes solitary basal on trichomes, $3\text{-}5\times4\text{-}5$ μm.

Place of collection – Jadavpur (Kolkata)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-257

Ophiothrix epidendron Sant'anna, Azevedo, J. Kastovský & J. Komárek [Fig. 7C]

Reference - Komarek 2013, p. 425, fig. 520

Holotype – *Ophiothrix epidendron* Sant'anna, Azevedo, J. Kastovský & J. Komárek

Description – Filaments solitary, not false branched, flexuous, 7-13 μm wide. Sheaths attached to trichomes at ends. Trichomes not constricted at cross walls, not attenuated at ends. Cells olive green very short 1-3×7-9 μm .

Place of collection—Barasat (North 24 Parganas)

Habitat – Tree bark

Voucher No. CUH/AL/AE/CYANO-154

FAMILY - HAPALOSIPHONACEAE

Hapalosiphon pumilus Kirchner ex Bornet & Flahault [Fig. 7A, B]

Reference - Komarek 2013, p. 517, fig. 629-634

Lectotype – *Hapalosiphon pumilus* Kirchner ex Bornet & Flahault.

Description – Colonies tufty, blue green. Filaments densely entangled 7-25 μm wide. Branches vertically divaricated, sometimes erect slightly narrower than main filaments and 6-12 μm wide. Sheath colourless. Cells barrel shaped 7-10×4-5 μm. Heterocyte dimensions similar to vegetative cells.

Place of collection - Chakvrigu (Uttar Dinajpur)

Habitat – Rice field

Voucher No. CUH/AL/FW/CYANO-153

We found that in the major genera some are largely aquatic – Anabaena, Nostoc, Gloeotrichia, Dichothrix, Tolypothrix, Cylindrospermum, Trichormus, Isocystis, Anabaenopsis and Hapalosiphon, whereas others are largely terrestrial – Scytonema, Calothrix, Ophiothrix and Hassallia.

Discussion

In the present investigation, heterocytous morphotypes of cyanobacteria from West Bengal have been surveyed. Several research studies have been carried out on heterocytous cyanobacteria regarding their distributional specifications and their utility in enriching soil fertility by capturing atmospheric nitrogen (Roger & Reynaud 1980; Shubert & Starks 1980; Issa et al. 2007). Cyanobacteria are widely distributed in aquatic, semi-dry and dry habitats.

Among nitrogen fixing cyanobacteria, Anabaena species are mainly reported from paddy fields (Tirkey & Adhikary, 2005). In the present study, all species of Anabaena were reported from aquatic habitats including rice fields as well as wetlands. In Satara district in Maharastra, paddy field soil samples were studied and Anabaena was found to be the most dominant taxa (Ghadage & Karande, 2019). As we report here, other aquatic genera include Nostoc, Gloeotrichia, Dichothrix, Tolypothrix, Cylindrospermum, Trichormus, Isocystis, Anabaenopsis and Hapalosiphon. Similar findings were reported in Assam, where Nostoc and Anabaena (in addition to other genera) were dominant in rice field ecosystem (Dasgupta & Ahmed, 2013). In the present investigation, Nostoc calcicola was found in

sub-aerophytic habitats and *N. carneum* was collected from aquatic habitats in paddy fields. Similar findings were reported by Venkataraman et al. (1974) where *Nostoc* and *Cylindrospermum* were reported from both aquatic and terrestrial habitats. *Aulosira* have been considered as dominant component of rice fields but in the present study, *Aulosira* have been collected from stagnant water bodies.

We show that the following genera are largely terrestrial – *Scytonema*, *Calothrix*, *Ophiothrix* and *Hassallia*. Similarly, Venkataraman et al. (1974) showed that soil inhabiting N₂ fixing cyanobacteria mainly include *Scytonema*, *Calothrix* and *Westiellopsis*. Several samples from rivers, lakes, puddles and pools of Ambegaon Tehsil in Maharashtra were surveyed by Namdeo (2018) and *Anabaena*, *Scytonema*, *Calothrix* were found to be more dominant than *Nodularia*, *Aulosira* and *Homeothrix*.

In heterocytous cyanobacteria, akinetes and heterocytes are specialised structures which need to be characterized for their surface details. Light photomicrographs may not always resolve the morphological details. From SEM images, the exact shape of apical cells and surface topography of heterocytes were clearly observed and light microscopic observations were confirmed. Besides exploring diversity of heterocytous forms, this study has also shown the use of electron micrographs for more precise characterization of morphology of heterocytous cyanobacteria.

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Declarations

Authors declare no conflicts of interest.

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