J. Indian bot. Soc. Vol. 90 (3&4) 2011 : 200-206

PART III: AN ACCOUNT OF HETEROCYSTOUS STIGONEMATALEAN CYANOBACTERIAL BIODIVERSITY OF NORTH EAST REGION OF INDIA

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The present study deals with biodiversity of stigonematalean cyanobacteria encountered from the states of North East India falling under Indo-Burma biodiversity hotspots. During monsoon and post monsoon period of 2004-2011 algal and soil samples were collected for purification and identification of cyanobacteria. Total 23 stigonematales cultures were isolated which belong to 5 genera viz. *Nostochopsis* (2), Mastigocladopsis (1), *Hapalosiphon* (9), *Westiellopsis* (10) and *Stigonema* (1). All these isolates were deposited to the Freshwater Cyanobacterial and Microalgal Repository at IBSD, Imphal, Manipur, a creation of national facility by DBT, Govt. of India (Ref. No.: BT/PR11323/PBD/26/171/2008 dated 31st March, 2009) for maintenance and preservation after allotment of accession numbers.

Keywords: Biodiversity, Cyanobacteria, Indo-Burma biodiversity hotspots, Repository

North East India (Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Sikkim and Tripura) forms a part of the Indo-Burma diversity 'hotspot' which represents the transitional zone between the Indian, Indo-Malayan and Indo-Chinese bio-geographic regions making it the geographical 'gateway' for much of India's flora and fauna. As a consequence, the area is one of the richest in biological values, high in endemism and holds a large number of rare species. The forests in the region are extremely diverse in structure and composition and combine tropical and temperate forest types, alpine meadows and cold deserts. Cyanobacteria are oxygenic photosynthetic prokaryotes possessing the ability to synthesize chlorophyll-a and they occur on all the different climates prevalent in the NE region of India. A heterocyst is a differentiated cyanobacterial cell that carries out nitrogen fixation under aerobic conditions. They are formed in response to a lack of fixed nitrogen (NH_4 or NO_3). The mature heterocysts contain no functional photosystem II and cannot produce oxygen. Instead, they contain only photosystem I, which enables them to carry out cyclic photophosphorylation and ATP regeneration. These changes provide the appropriate conditions for the functioning of the oxygen-sensitive nitrogenase. Heterocyst-differentiating cyanobacteria correspond to a monophyletic lineage (Giovannoni *et al*. 1988, Wilmotte and Herdman 2001) in which two subgroups are traditionally distinguished. The first subgroup consists of filamentous heterocystous cyanobacteria dividing always in a plane at right angles to the long axis of the trichome and therefore uniseriate and lacking true branchings. They are grouped in Subsection IV according to the proposed bacteriological classification (Rippka *et al*. 1979, Castenholz 2001) and in the order Nostocales in the traditional classification system (Komarek and Anagnostidis 1989). The second subgroup consists of filamentous

Received on September 14, 2011 Accepted on December 3, 2011

heterocystous cyanobacteria in which longitudinal and oblique cell divisions occur in addition to transverse cell divisions resulting in periodic true branching in all genera and in multiseriate trichomes (two or more rows of cells) in some genera. They are classified in Subsection V (formerly order Stigonematales) (Rippka *et al*. 1979, Anagnostidis and Komarek 1990; Castenholz 2001). Analysis of new Stigonematales strains show that the true branching cyanobacteria are polyphyletic and should be separated into at least two major groups based on the branching. The first group is characterized by T-branching and the second group by Y-branching (Anagnostidis and Komarek 1990, Gugger and Hoffmann 2004).

MATERIALS AND METHODS

More than 200 algal and soil samples were collected from different ecological habitats of Assam, Arunachal Pradesh, Manipur, Meghalaya and Mizoram states of NE region of India during 2004-2011. GPS data were recorded of each and every sample with the help of Garmin eTrex Vista, Global Positioning System (GPS). Samples were raised in BG-11 medium (Stanier *et al*. 1971) in absence of $NaNO_3$ as the present study was based on nitrogen fixer. pH determination of the soil were done by adding 1:2 distilled water with soil and stirred for 30 mins with the help of Sartorius digital pH meter. Isolation were carried out by inoculating 1g of soil samples to 100 ml of sterile medium in 250 ml conical flask and incubated for 30 days at 28 ±2°C under 4-5 K lux light intensity from white fluorescence lamp. The mix colonies in the flask were isolated and purified by using standard streaking techniques. The microscopic observation was made with the aid of Nikon Model 80*i* and Carl Zeiss Model Axio Scope A1 Trinocular Microscope with phase contrast and Fluorescence attachments. The taxonomic identification of unialgal cultures was done by using the available monograph (Desikachary 1959). Total 23 isolates which belong to 5 genera of order stigonematales viz. *Nostochopsis* (2), Mastigocladopsis (1)**,** *Hapalosiphon (9), Westiellopsis* (10) and *Stigonema* (1) were isolated and maintained in (N) BG-11 medium at 28 ±2°C under 4-5 K lux light intensity in a temperature regulated room with light and dark phase maintained at 14/10 hrs. The fully grown unialgal uncontaminated cultures were deposited in the Fresh water Cyanobacterial and Microalgal Repository at IBSD, Imphal, Manipur after allotment of accession numbers.

RESULTS AND DISCUSSION

The stigonematales encountered from different NE region of India are shown in details below.

1. *Nostochopsis*: 1 species from 2 locations were encountered and is described below:
A. *Nostochopsis lobatus* Wood em. Geitler: This species were collected from 2 NE states of India:

a) BTA 323 from potamoplankton, Moreh, Chandel, Manipur, India; DOC: 16th April, 2008; GPS: Altitude: 213m; Latitude/ Longitude: N24°15'11.1"/ E094°17'50.5"

b) BTA 782 from moist soil, University Campus, Itanagar, Arunachal Pradesh, India; DOC: 22nd Dec., 2009; GPS: Altitude: 201m; Latitude/ Longitude: N27°08'52.4"/ E093°45'53.9"

Thallus and filament behaviour: Yellowish green, thallus spherical or sub-spherical, more or less irregularly lobed; trichomes end tapering or slightly pointed; cell barrel shaped 4-5.5 µm broad; heterocyst mostly lateral or terminal ellipsoidal mostly 6-9 µm broad, mucilage homogenous; thin and firm colourless sheath present in the branching zone, diffluent at the apices of branches. (Plate-1 Fig. 7, 17)

2. *Mastigocladopsis:* 1 species from 1 location was encountered and is described below:
A. *Mastigocladopsis laminosus* Cohn: This

species was collected from 1 NE state of India:
a) BTA 656 from damp area, Doimukh, Nirjuli, Arunachal Pradesh, India; DOC: 22nd June, 2009; GPS: Altitude: 201m; Latitude/ Longitude: N27º08'52.4"/E093º 45'53.9"

Thallus and filament behaviour: Blue or olive green, thallus membranous, spongy, sheath distinct; cells in the main filament barrel shaped to short cylindrical, those of side branches long cylindrical; heterocyst intercalary spherical or ellipsoidal, 6.5 µm broad. (Plate-1 Fig. 16)

3. *Hapalosiphon:* 2 species from 5 locations were encountered and is described below:
A. *Hapalosiphon welwitschii* W. et G.S. West: This species was collected from 3 NE states of India of different habitats and is described below:

a) BTA 214 from lithophilic, Wangkhei, Imphal East, Manipur, India**;** DOC: 2nd July, 2007; GPS: Altitude 772m; Latitude/Longitude: N24°48'54.2"/ E093°56'13.1"

b) BTA 232 from lithophilic, Cherrapunjee, Meghalaya, India**;** DOC: 14th June, 2007; GPS: Altitude: 1462m; Latitude/Longitude: N25º17'08.9"/ E091º42'59.8"

c) BTA 502 from foot hill, Moreh, Chandel, Manipur, India; DOC: 24th Oct., 2009; GPS: Altitude: 205m; Latitude/ Longitude: N24°15'08.1"/ E094°17'59.9"

d) BTA 745 from grassfoot, Sivasagar, Assam, India; DOC: 26th July, 2010; GPS: Altitude: 83m; Latitude/Longitude: N26°59'20.4"/ E094°37'57.3"

e) BTA 769 from epilithic, temple floor, Sivasagar, Assam, India; DOC: 26th July, 2010; GPS: Altitude: 83m; Latitude/ Longitude: N26°59'20.3"/ E094°37'59.1"

Thallus and filament behaviour: Dark green, filaments closely entangled with sheath thin and hyaline, cells spherical, quadrate or longer than broad 3-7.5 µm broad, branches short and narrower than the main axis; heterocyst rare, intercalary, quadrate rounded or long cylindrical 4.5-7 µm broad and 7.5-10 µm long. (Plate-1 Fig. 8-12)

B) *Hapalosiphon stuhlmannii* Hieron: This species was encountered from 1 NE state of India:

a) BTA 935 from dry soil, Chandel, Moreh, Manipur, India; DOC: 14th March, 2011; GPS: Altitude: 227m; Latitude/ Longitude: N24°15'11.6"/ E094°17'55.8"

Thallus and filament behaviour: Thallus floccose, blue-green; richly branched; cells mostly one or seldom in two rows; sheath seemingly thick; lateral branches erect, 5-8 µm broad; repeatedly branch, with long cylindrical cells; heterocyst spherical or oblong. (Plate-1 Fig. 20)

C. *Hapalosiphon* sp.: This species was collected from 3 NE states of India of different habitats and is described below:

a) BTA 681 from attached on tank wall, Moreh, Chandel, Manipur, India; DOC: 3rd July, 2010; GPS: Altitude: 215m; Latitude/ Longitude: N24°15'11.1"/ E094°17'50.5"

b) BTA 723 from water logged, Doimukh, Nirjuli, Arunachal Pradesh, India**;** DOC: 22nd Dec., 2010; GPS: Altitude: 201m Latitude/ Longitude: N27º08'52.4"/ E093º45'53.9"

c) BTA 810 from river bank, Tamdil, Aizawl, Mizoram, India**;** DOC: 4th Oct., 2010; GPS: Altitude: 791m; Latitude/ Longitude: N23°44'26.3"/ E092°57'04.4"

Thallus and filament behaviour: Blue green, mostly with two or three rows; cells quadrate to spherical, mostly as long as broad; lateral branches erect, cells transverse with longitudinal and transverse section. (Plate-1 Fig. 13-15)

4. *Westiellopsis*: 1 species from 10 locations were encountered and described below:

A. *Westiellopsis prolifica* Janet. This species was collected from 4 North East states of India of different ecological habitats:

a) BTA 55 from Loktak lake, Sendra, Bishnupur, Manipur, India; DOC: 29th May, 2004; GPS: Altitude: 772m; Latitude/

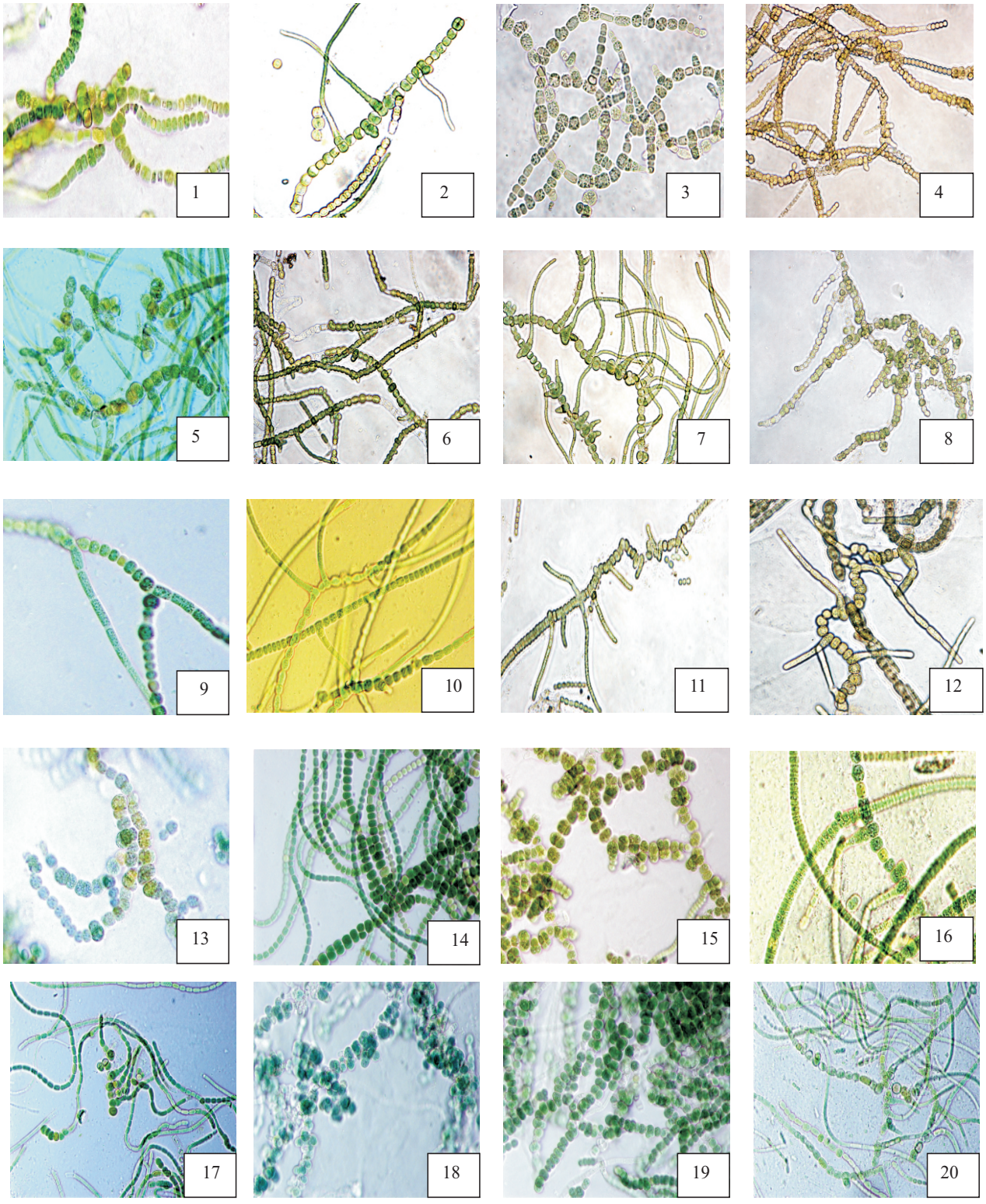

Plate 1:Figures 1-20 Heterocystous stigonematalean cyanobacteria of NE India

1-6. *Westiellopsis prolifica,* 7. *Nostochopsis lobatus,* 8-12. *Hapalosiphon welwitschii,* 13-15.*Hapalosiphon sp.,* 16. *Mastigocladopsis laminosus,*17**.** *Nostochopsis lobatus,* 18. *Stigonema dendroideum,* 19. *Westiellopsis prolifica,* 20. *Hapalosiphon stuhlmannii.*

Longitude: N24°30'51.8"/ E093°47'36.0"
b) BTA 141 from moist soil, Takyelpat, Imphal West, Manipur, India; DOC: 19th Oct., 2006; GPS: Altitude: 782m; Latitude/ Longitude: N24º48'14.3"/ E093º54'18.3"
c) BTA 211 from lithophilic, Wangkhei, Imphal East, Manipur, India; DOC: 2nd July, 2007; GPS: Altitude: 772m; Latitude/Longitude: N24°48'54.2"/ E093°56'13.1"
d) BTA 253 from moist soil, Shillong, Meghalaya, India; DOC: 13th June, 2007; GPS: Altitude: 1396m; Latitude/Longitude: N25°36'45.5"/ E091°54'05.35"
e) BTA 303 from lithophilic, Moreh, Chandel, Manipur, India; DOC: 16th April, 2008; GPS: Altitude: 213m; Latitude/Longitude: N24°15'11.1"/ E094°17'50.5"
f) BTA 645 from paddy field, Tangkham, Imphal East, Manipur, India; DOC: 9th Sep., 2009;GPS: Altitude: 780m; Latitude/ Longitude: N24°53'54.9"/ E093°59'03.8"
g) BTA 686 from damp soil, Moreh, Chandel, Manipur, India; DOC: 3rd July, 2010; GPS: Altitude: 215m; Latitude/Longitude: N24°15'11.1"/ E094°17'50.5"
h) BTA 707 from moist soil, University campus, Arunachal Pradesh, India; DOC: 23rd Dec., 2010; GPS: Altitude: 201m; Latitude/ Longitude: N27°08'52.4"/ E093°45'53.9"
i) BTA 748 from water logged soil, Moreh, Chandel, Manipur, India; DOC: 3rd July, 2010; GPS: Altitude: 215m; Latitude/Longitude: N24°15'11.1"/ E094°17'50.5"
j) BTA 812 from river bank, Tuirini, Aizawl, Mizoram, India; DOC: 4th Oct., 2010; GPS: Altitude: 342m; Latitude/ Longitude: N23°41'17.0"/ E092°52'50.9"

Thallus and filament behaviour: Dark green, thallus filamentous, true branching with thick torulose primary branch and thin erect secondary filaments; sheathless consisting of single row of cells; heterocyst oblong-cylindrical intercalary, 5.5-6 µm broad and 10.5-22 µm long; barrel shaped cells, 8-12 µm broad; gonidia formed singly; secondary filaments showing multiseriation (Plate-1 Fig. 1-6)

5. *Stigonema*: 1 species from 1 location was encountered and is described below:
A. *Stigonema dendroideum* Fremy: This species was collected from 1 NE state of India:
a) BTA 801 from hill slope, Chatlang, Aizawl, Mizoram, India; DOC: 2nd Dec., 2010; GPS: Altitude: 1170m; Latitude/ Longitude: N23°46'14.2"/ E092°44'17.8"

Thallus and filament behaviour: Brownish black, filaments creeping; rich true branching; secondary branches mostly attenuated; sheath inside colourless; not lamellated; trichomes in the main filament 2-3 rows of cells having 14-20 µm broad; heterocyst sparse, intercalary or lateral; sheath colourless, not lamellated. (Plate-1 Fig. 18)

Total 5 genera viz. *Nostochopsis* Wood em. Geitler, Mastigocladopsis Iyenger et Desikachary, *Hapalosiphon* Nag., *Westiellopsis* Janet and *Stigonema* Ag. belonging to Stigonematales were encountered from 5 states of NE region of India viz. Arunachal Pradesh, Assam, Manipur, Meghalaya and Mizoram from different geographical, climatic and seasonal periods ranging from plane areas to mountain region; from moderate rainfall to high rainfall areas and high humid temperature to low temperature were observed. Genus *Westiellopsis* Janet with 1 species viz. *Westiellopsis prolifica* Janet was found abundant in Manipur (7 isolates) and 1 isolate each was encountered from Arunachal Pradesh, Meghalaya and Mizoram. Genus *Hapalosiphon* Nag. was encountered from 5 NE states; Arunachal Pradesh (1), Assam (2), Manipur (3), Meghalaya (1) and Mizoram (1) of different ecological habitats like river bank, hill foot, stone surface and water logged areas and was found growing during monsoon and

winter season. The altitude observed for the genera growing was from 83m to 1462m. *Nostochopsis* Wood em. Geitler of 1 species viz. *Nostochopsis lobatus* Wood em. Geitler was encountered from 2 NE states viz. Manipur (1) and Arunachal Pradesh (1) during spring and winter season in streams and moist soil at altitude 201 to 214m. Least genera encountered were *Mastigocladopsis Iyenger* et Desikachary *and Stigonema* Ag. isolated from Arunachal Pradesh (1) and Mizoram (1) respectively. They were observed in monsoon and winter season with altitude 201m and 1170m. From this study, we observed that genus *Westiellopsis* Janet are found abundantly than other genera but diversity was found more in the genus *Hapalosiphon* Nag. All the 23 isolates of 5 genera are preserved and maintained in the Fresh water Cyanobacterial and Microalgal Repository at IBSD, Imphal, Manipur, India.

A large number of poorly studied stigonematalean cyanobacteria are known from freshwater and subaerial biotopes, such as slightly acidic, oligotrophic lakes or caves. Recently, 4 isolates (*Stigonema* sp., *Fischerella muscicola, Hapalosiphon fontinalis, Westiellopsis* sp.) of order Stigonematales from different locations of Garhwal Himalaya were reported on the basis of morphotypic characteristics (Pandey 2010). *Hapalosiphon welwitschii* was reported from Kakoijana reserve forest, Assam, India (Saha *et al.* 2007). Mastigocladus laminosus was isolated and reported from hot springs in Japan and Myanmar and were analyzed to determine taxonomy and biogeography *(*Soe 2011). *Westiellopsis* sp. and *Fischerella muscicola* strains, a ubiquitous distribution throughout tropical and subtropical regions strains were isolated from geothermal sites and hot springs of Costa Rica (Finsinger *et al*. 2008). This study may lead in the further understanding of eco-diversity of stigonematales in fresh water habitats.

*Abbreviations: DOC- Date of collection; NE- North East

Authors are thankful to the Department of Biotechnology, Govt. of India for providing financial assistance and we are thankful to the Director, IBSD, Imphal, Manipur for laboratory facility. Also Mr. Oinam Avijeet Singh & Ms. Gunapati Oinam are thankful to Ms. Laxmipriya, Ms. Indrama & Mr. Ojit for all support during isolation, identification and characterization of stigonematalean cyanobacteria and preparation of present manuscript.

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