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DIVERSITY AND DISTRIBUTION PATTERN OF THE GENUS *CALOTHRIX* AGARDH EX BORNET ET FLAHAULT: A HETEROPOLAR CYANOPROKARYOTE FROM TRIPURA, INDIA

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ABSTRACT

The present communication deals with the biodiversity and distribution pattern of unbranched, filamentous, heterocystous, diazotrophic, heteropolar and uniseriate blue green algal genus *Calothrix* Agardh ex Bornet et Flahault from Tripura. In the present paper, we reported total 19 species of the genus *Calothrix* including one forma and one var. viz. *C. aeruginosa*, *C. breviararticulata*, *C. clavata*, *C. clavatooides*, *C. columbiana*, *C. conica*, *C. cylindrica*, *C. fusca* f. *durabilis*, *C. fusca*, *C. geitleri*, *C. geitonos*, *C. gloeocola*, *C. gracilis*, *C. javanica*, *C. linearis*, *C. marchica* v. *intermedia*, *C. parva*, *C. scopulorum* and *C. subsimplex* from rice fields and sub-aerial habitats of Kumarghat and Kailashahar area of Unakoti district of Tripura, India. All the strains are new addition to the flora of Tripura.

Keywords : Cyanobacteria, diazotroph, heterocystous, diversity, *Calothrix*, Tripura.

Introduction

The Blue-green algae (Cyanobacteria/cyanoprokaryotes) are primary colonizers and photosynthetic prokaryotes found in almost every environment on Earth. Traditionally, they are classified on the basis morphological parameters (Geitler 1932, Desikachary, 1959) but later on a new taxa-rich classification system was developed on the basis of molecular methods, ultra-structure, morphological diversity and ecophysiological characters in Bergey's Manual of Systematic Bacteriology (Castenholz, 2001). Based on polyphasic approach and molecular data, Komárek (2014) divided Nostocales into 12 families including Rivulariaceae Kützing ex Bornet et Flahault. The members of the family are characterized by heterocystous, obligately heteropolar and uniseriate filamentous. The genus *Calothrix* Agardh (Bornet and Flahault, 1886) is a filamentous, heterocystous and diazotrophic Blue-green Alga usually found creeping or attached to the base of the substrate with basal heterocysts. The genus *Calothrix* commonly occurs in both terrestrial and aquatic environments including moist soils, sub-aerial habitats i.e. tree barks, rocks, walls and exposed roof surfaces of monuments and buildings (Whitton and Potts, 2000). Colonies of *Calothrix* form wide range of characteristics size, shape and colours from dark green to black, yellow-green to red-brown. All the species of *Calothrix* can be used as natural source of nitrogen fertilizers in rice fields due to their diazotrophic potentiality to naturally fix atmospheric nitrogen and regain soil health (De, 1939). The genus *Calothrix* is distinguished from its close relative *Microchaete*, *Fortiea* and *Gloeotrichia* on the basis of akinetes and narrowed hairy trichome.

The main aim of the present investigation was to study the diversity of *Calothrix* species from Tripura, India and to compare their occurrence and distribution pattern in five different types of habitats including 1. Uncultivated land soils (UL), 2. Rice fields (RF), 3. Building walls (BW), 4. Roof tops (RT) and 5. Tree barks (TB). In the present investigation we are reporting nineteen strains belonging nineteen species of *Calothrix* including one forma and one var. viz. *C. aeruginosa*, *C. breviararticulata*, *C. clavata*, *C. clavatooides*, *C. columbiana*, *C. conica*, *C. cylindrica*, *C. fusca* f. *durabilis*, *C. fusca*, *C. geitleri*, *C. geitonos*, *C. gloeocola*, *C. gracilis*, *C. javanica*, *C. linearis*, *C. marchica* v. *intermedia*, *C. parva*, *C. scopulorum* and *C. subsimplex* from different habitats of Tripura, India.

Materials and Methods

Total 80 samples containing visible growth of blue green algae of dark blue-green, brown and dark brownish colored were collected from five different types of habitats including 1. Uncultivated land soils (UL), 2. Rice fields (RF), 3. Building walls (BW), 4. Roof tops (RT) and 5. Tree barks (TB) were collected randomly from Unakoti districts of Tripura during October-November 2017. Out of total 80 samples, 40 samples were from five selected rice fields (eight samples from each rice field) of Kumarghat and 40 samples (eight samples from each) were from rest four other habitats of Kailashahar, Unakoti, Tripura. All the collected samples were dried at 40°C by hot air oven for four hours and powdered by mortar pestle and 1 mg from each sample were inoculated into sterilized petridishes (Borosil) containing sterilized nitrogen deficient BG-11 liquid medium (Stainer

et al., 1971). All the samples were incubated for one week under controlled culture conditions (Temperature 28±2°C; light-4000-6000Lux, 14:10 h light:dark cycle) and total 260 enriched culture were raised, out of that 80 strains were of *Calothrix*. All the strains were raised as unialgal cultures following the method described by Kant *et al.* (2005a). Out of total, 80 strains belong to nineteen species of *Calothrix*. Details of occurrence and distribution pattern of nineteen species are given in Table-1. The morphological details of all the strains of the *Calothrix* were observed with the help of Microscope (Trinocular, Olympus, CH20i) and digital camera (Magnus, Magcam DC10) and their morpho-metric parameters were recorded. All the isolated strains of *Calothrix* were identified up to the species level with the help of available literatures (Geitler 1932; Desikachary 1959, Komárek, 2013). Morphological details of nineteen strains, one strain from each species of *Calothrix* are being described in the present paper following the Nostocales (Komárek, 2013).

Table 1 : Showing occurrence and distribution of *Calothrix* species in different biotopes

Species	RF	UL	RT	BW	TB
<i>C. aeruginosa</i>	+	-	-	-	+
<i>C. breviararticulata</i>	-	+	+	+	+
<i>C. clavata</i>	-	+	-	-	
<i>C. clavatooides</i>	+	-	-	+	+
<i>C. columbiana</i>	-	+	-	+	-
<i>C. conica</i>	+	+	-	-	-
<i>C. cylindrica</i>	-	-	-	+	+
<i>C. fusca</i> f. <i>durabilis</i>	-	-	+	-	-
<i>C. fusca</i>	+	+	-	-	-
<i>C. geitleri</i>	+	+	-	-	-
<i>C. gelatinosa</i>	+	+	-	-	-
<i>C. gloeocola</i>		+	-	+	-
<i>C. gracilis</i>	+		-	-	-
<i>C. javanica</i>	+	+	-	-	+
<i>C. linearis</i>	+			-	+
<i>C. marchica</i> v. <i>intermedia</i>	+	+	+	-	+
<i>C. parva</i>	+	+	-	-	+
<i>C. scopulorum</i>	+	+	+	-	+
<i>C. subsimplex</i>	+	-	-	+	-

RF=Rice Field, UL= Uncultivated Land, RF=Roof Top, BW=Building Wall, TB=Tree Bark

Morphological Observation

Description of *Calothrix* species:

Calothrix aeruginosa Woronichin 1923 (Fig. A17)

Woronichin, 1923

Filaments solitary or in small groups, connected by gelatinous bases up to 150 µm long, 9-12 µm wide near bases, slightly narrowed towards ends. Sheaths relatively wide, hyaline colourless, open at the apex. Trichomes continually attenuated towards end, 8.5 µm wide near the base, constricted at cross walls, not narrowed into a hair. Cells bright blue-green always distinctly shorter than wide,

apical cells rounded conical. Heterocysts basal, spherical, 8-9 µm wide.

Calothrix breviararticulata West et West 1897 (Fig. A19)

West and West, 1897

Filaments solitary or in irregular groups, up to 380 µm long 11-16 µm wide near the base, 10-12.5 µm in the middle, gradually narrowed towards the ends. Sheaths thick, laminated brown to blackish. Trichomes 7-11.5 µm wide at the base, 5-7.5 µm wide in the middle, gradually narrowed towards end, ending in a hair. Cells short with length usually less than width. Only in the apical region of the trichome the cells are little longer and distinctly longer only in the hair. Heterocysts basal, solitary, hemispherical.

Calothrix clavata West 1914a (Fig. A1)

West, 1914a

Colonies microscopic, gelatinous, in small groups, forming light blue-green or yellow-green mats. Filaments solitary or aggregated, up to 100 µm long and about 7-10 µm wide at the base, slightly flexuous up to almost straight, at the base swollen, then gradually and distinctly attenuated to a long hair. Sheaths attached to trichomes, thin, colourless. Trichomes 5-5.5 µm wide near the base, about 2.5 µm in the middle, pale blue-green, slightly constricted at cross walls, not constricted at narrowed portions and in hairs. Cells shorter than wide and barrel shaped near the base, in higher portions cylindrical up to 3 times longer than wide. Heterocysts solitary, basal, hemispherical or conical rounded.

Calothrix clavatooides Ghose 1927 (Fig. A2)

Ghose, 1927

Colonies microscopic, solitary or in small groups, up to 200 (300) µm long, straight or curved, swollen at the base, where they are 10-14 µm wide. Sheaths thin, colourless, attached to the trichomes. Trichomes gradually narrowed towards ends, constricted at cross walls, narrowed to a long hair. Cells shortly barrel shaped, along the whole length shorter than wide, (8.5) 10-13 µm wide near the base, later elongated, from the middle up to isodiametric and 2-3 times longer than wide (about 3-4 µm wide), in the hair long cylindrical (6-9µm wide and 2 µm long). Heterocysts basal, solitary, spherical, 3.8-6.4 µm in diameter.

Calothrix columbiana West 1914b (Fig. A3)

West, 1914b

Colonies usually microscopic, mucilaginous, pale blue-green, flat mats. Filaments in groups, epiphytic, up to 350 µm long, almost straight or slightly flexuous, often curved at the bases, gradually attenuated towards ends. Sheaths hyaline, colourless, slightly mucous, not lamellated. Trichomes gradually attenuated towards ends, not constricted at cross walls, 6-8 µm wide near the bases, 4-5 µm in the middle. Cells quadratic near the bases, later up to 2-3 times longer than wide, cylindrical. Heterocysts basal, solitary, sub-spherical, 6 µm in diameter. Akinetes solitary at Heterocysts, ellipsoid, truncate and thickened towards vegetative cells, with smooth and colourless episore, 12-14.5µm wide and 10 µm long.

***Calothrix conica* Gardner 1927 (Fig. A4)**

Gardner, 1927

Colonies usually microscopic, gelatinous, light blue-green or olive green forming mats. Filaments slightly swollen at the base, with a very sparse false branching, up to 125 μm long, but usually shorter, 6.8-7.5 μm wide at the base, straight or curved. Sheaths hyaline, thin and close-fitting, ephemeral. Trichomes 4.8-5.6 μm wide, with 1-3 larger basal cells (but not widened), in basal parts not, in upper parts distinctly constricted, narrowed but without hairs. Cells cylindrical or isodiametric in basal parts of filaments, in higher parts shorter than wide, sometimes with 1-3 cells attenuated at the ends, blue-green; apical cells conical. Heterocysts spherical to sub-spherical, single, basal, closed in the sheath.

***Calothrix cylindrica* Frémy 1924 (Fig. A5)**

Frémy, 1924

Colonies microscopic, mucilaginous, widened, thin, dark blue-green. Filaments irregularly organized, cylindrical, 14-18 μm wide at the base. Cells gelatinous, enveloping only the lower half of a trichome, cylindrical (not attenuated towards ends), open at the end, usually 2-layered, with outer layer smooth and colourless, with inner layer perpendicularly constricted, colourless to yellow. Trichomes greyish blue-green, 10-12 μm wide, constricted at cross walls, elongated into a relatively short, thick and usually bent hair. Cells barrel shaped, usually shorter than wide in the basal parts, 4-6 μm long, in the upper part cylindrical up to slightly longer than wide in hairs. Heterocysts basal, hemispherical or kidney-shaped.

***Calothrix fusca* f. *durabilis* Starmach 1958 (Fig. A7)**

Starmach, 1958

Filaments solitary or in groups, straight or flexuous, sometimes with laterally small false branches up to 120 μm long, 7-16 μm wide near the base (trichome 4.5-11 μm), in the middle 5-10 μm wide (trichome 3.5-7 μm). Sheaths lamellated, thick yellow-brown, funnel like widened at the ends. Heterocysts hemispherical, commonly rounded-conical.

***Calothrix fusca* Bornet et Flahault 1886 (Fig. A6)**

Bornet and Flahault, 1886

Filaments solitary or in irregular groups, typically unbranched, straight or irregularly coiled, short, usually only 0.3mm long, near the base onion-like widened and 14-15 μm wide, above the widening 9-14 μm wide. Sheaths thin to thick, lamellated, colourless, at the ends sometimes funnel-like widened. Trichomes narrowed towards the ends usually 7-8 μm wide in the middle, not or slightly constricted cross-walls, ending in a thin, not very long, cellular hair present in some trichomes is fragile. Cells near the base are shorter, 7-8 μm wide, blue-green or dirty green. Heterocysts basal, hemispherical or rounded and shortened, usually narrower than the basal cells.

***Calothrix geitleri* Copeland 1936 (Fig. A8)**

Copeland, 1936

Filaments single or in tangled clumps, up to 800 μm long, flexuous and contorted, slightly swollen at the base, gradually narrowed up to a colourless hairs, 9 μm wide at the widened base, above the base 6-8.5 μm . Sheaths close, thin, 1

μm colourless, homogenous, gelatinous. Trichomes slightly constricted at cross walls in basal parts. Cells blue-green, 4-6 μm wide and 4.8-8 μm long at the base, 1.5 μm wide and 6 μm long in hairs. Heterocysts basal within sheaths, usually single or 2-3 in a row, mostly ellipsoidal 5-9 μm wide and 6-7 μm long, sometimes the most basal heterocysts are conical rounded.

***Calothrix geitonos* Skuja 1949 (Fig. A9)**

Skuja, 1949

Filaments aggregated in small groups, rarely solitary. Trichomes solitary or flexuous, up to 1 mm long, continually attenuated into long, hyaline, multi-cellular hairs, constricted or not constricted at cross-walls. Sheaths up to 2.5 μm wide, with parallel lamination, colourless to yellow-brown, colourless and mucilaginous in external parts. Cells in the base up to 8 μm , 5-6 μm in central parts, 1.5 μm wide in hairs. Heterocysts basal, solitary, spherical, sub-spherical or oval, 8-14 μm wide and 6.8-11 μm long.

***Calothrix gloeocola* Skuja 1949 (Fig. A10)**

Skuja, 1949

Colonies microscopic, olive-green or blue-green. Filaments solitary, simple, flexuous, in mucilage of other colonial cyanobacteria, 8.5-10.2 μm wide in basal parts. Trichomes continually narrowed towards ends, up to 650 μm long, very slightly widened at the base, slightly constricted or not constricted at the cross-walls. Sheaths thin, 1.5-5 μm wide, colourless, homogenous, often diffluent. Cells (4.6) 5-6.5 (7.1) μm wide at the base, in middle part 3 μm , in hairs 0.5-0.7 μm wide. Heterocysts only basal, solitary or geminate, globose or slightly truncate, hemispherical or oval, (5.8) 7-11.7 μm wide 4.6-7.1 μm long.

***Calothrix gracilis* Fritsch 1912 (Fig. A11)**

Fritsch, 1912

Filaments straight or slightly coiled, solitary or in groups, up to 6 μm wide or sometimes slightly swollen near the bases, 9-10 μm wide, up to 400 μm long. Sheaths thin, colourless, narrow, rarely slightly widened near the base. Trichomes continually narrowed towards end 5-9 μm wide near the base, about 3 μm wide in the middle not ended by hairs, distinctly constricted at cross walls. Cells near the base barrel shaped and shorter than wide, higher more cylindrical and elongated. Heterocysts basal, usually single, closed in the sheaths, 5.5 μm in diameter.

***Calothrix javanica* De-Wildeman 1897 (Fig. A13)**

De Wildeman, 1897

Mucilaginous colony with solitary filaments, slightly widened at the base 6-8 μm wide, narrowed towards end. Sheaths indistinct, colourless not structured. Trichomes 4-6 μm wide, continually narrowed towards end. Slightly or not constricted at the cross walls, elongated into a thin hair. Cells usually longer than wide or isodiametric. Heterocysts basal 4-5 μm wide, 9-12 μm long. Akinetes solitary or in pairs above heterocysts, 4-6 μm wide and 6-10 μm long.

***Calothrix linearis* Gardner 1926 (Fig. A14)**

Gardner, 1926

Colonies microscopic with filaments among other cyanoprokaryotes and algae, up to 800 (1500) μm long,

slightly swollen (widened) at the base, in middle part cylindrical, slightly falsely branched. Sheaths thin, colourless, not lamellated. Trichomes mostly cylindrical with few (3-5) cells widened and 6 μm wide at the base (these cells change into akinetes) in upper part 2.5 μm wide, elongated in a hair. Cells in lower portions distinctly shorter than wide, near the ends up to 3 times longer than wide. Heterocysts hemispherical, basal and perhaps also intercalary.

***Calothrix marchica* v. *intermedia* Rao 1937 (Fig. A12)**

Rao, 1937

Colonies microscopic, epiphytic on other algae, placed singly or in groups of two or three, 6-8 μm broad and up to 350 μm long, with straight attenuation, without a terminal hair. Sheath thin, firm, hyaline. Trichomes 5.8-7.8 μm broad, constricted at the cross walls. Cells quadratic, shorter or longer than broad, 3.2-8 μm long, end cells rounded. Heterocysts single, basal, usually spherical, 5.6-7.8 μm broad.

***Calothrix parva* Ercegović 1925 (Fig. A18)**

Ercegović, 1925

Colonies microscopic, yellow-green or yellow-brown mats. Filaments solitary, in irregular groups or mixed with other algae, never branched, rarely and very slightly widened, (6) 9-16 μm wide at the base, (5) 7-13 μm wide in the middle, up to 95 μm long. Sheaths firm, thick, slightly lamellated, funnel like widened. Trichomes at bases 6.7-11.2 μm , slightly widened, in the middle 4.6-8.4 μm wide, not or slightly and indistinctly constricted at cross walls, ending in a short hair. Cells in lower parts short, about $\frac{1}{3}$ or $\frac{1}{4}$ of the width. Heterocysts solitary, basal, spherical or hemispherical, 6-9 μm wide or 4-7.2 μm long.

***Calothrix scopulorum* [Weber et Mohr] Agardh ex Bornet et Flahault 1886 (Fig. A15)**

Algarum and Bárbara, 1886

Colonies microscopic, dark green, olive green up to blackish, composed of bush like irregular fascicles, mostly on stony substrates. Filaments irregularly coiled and flexuous, up to 1 mm long, 10-18 μm wide and slightly widened at the base. Sheaths colourless to brown often composed of funnel-like layers, lamellated. Trichomes slightly widened near the base, 8-15 μm wide, later cylindrical for a long part, at the end narrowed to a long hair, sometimes scarcely falsely branched in lower parts slightly constricted at cross wall, later unconstricted. Cells blue-green or olive-green, shorter than wide up to isodiametric. Heterocysts basal, sometimes a few in a row, hemispherical to cylindrical, very rarely intercalary.

***Calothrix subsimplex* Jao 1939 (Fig. A16)**

Jao, 1939

Colonies usually microscopic, solitary or in groups, up to 1 mm long, erect or creeping, at bases not widened and not branched, 11-17 μm wide. Sheaths firm, colourless, not lamellated. Trichomes blue-green, not constricted at cross walls or slightly constricted, gradually attenuated into a long

hair, 9-13.5 μm wide at the base. Cells usually shorter than wide, rarely up to isodiametric, 4.5-9 μm long, slightly elongated in upper part of trichome, long cylindrical in hairs. Heterocysts basal, solitary, hemispherical or slightly conical-rounded, 7.2-12.6 μm wide and 9.9-14.4 μm long.

Discussion

The role of blue green algae in agriculture has been realized for a long time (De 1939, Tiwari, 1972; Whitton and Potts, 2000), but its real potential in the form of real field's application needs more attention (Whitton, 2008). The taxonomy of the genus *Calothrix* is very complicated and contains many morphotypes and ecotypes. The genus *Calothrix* is a heteropolar, filamentous and heterocystous Blue-green Alga and its trichomes are differentiated into vegetative cells, heterocysts and akinetes (Castenholz 1989; Komárek, 2013). After independence, blue-green algae of India have been explored by a large number of researchers (Mitra, 1951; Singh, 1961; Pandey, 1965; Pandey and Mitra, 1965; Tiwari, 1972; Laloraya and Mitra, 1973; Tiwari and Pandey, 1976; Jeeji-Bai, 1977; Anand, 1989; Nayak *et al.*, 1996; Kant *et al.*, 2004a-c, 2005a&b, 2006a&b; Tiwari *et al.*, 2005; Prasanna *et al.*, 2006; Sihvonen *et al.*, 2007; Tiwari *et al.*, 2007; Kesharwani *et al.*, 2008; Tandon *et al.*, 2014; 2021, Singh *et al.*, 2021; 2022) due to their diazotrophic potential but they could not make serious attempt to explore the Tripura.

Desikacharya (1959) reported 39 species including six var. of *Calothrix*. Komárek (2013) reported 131 species of *Calothrix* of which 15 species are with akinetes, 41 species without akinetes, 17 species without terminal hairs and 25 species are unclear and needs revision. Guiry and Guiry (2021) listed total 222 species names in the database for the genus *Calothrix*, out of which 141 species have been accepted taxonomically.

In India, *Calothrix indica* was reported first time by Montagne (1849) from Assam a North East state but Tripura remained unexplored for long time after independence. Later on Singh *et al.* (1997); Das *et al.* (2010); Kant (2012); Ghosh *et al.* (2019); Kant *et al.* (2020a-b, 2021a-b, 2022); Sarma *et al.* (2020) and Bharati *et al.* (2020) tried to explore Tripura state for the occurrence of algal biodiversity. Singh *et al.* (1997) reported only a single species of *Calothrix* viz. *C. elenkinii* from Tripura. In our present study we are reporting 19 taxa including one forma. and one var. of genus *Calothrix* viz. *C. aeruginosa*, *C. breviararticulata*, *C. clavata*, *C. clavatooides*, *C. columbiana*, *C. conica*, *C. cylindrica*, *C. fusca* f. *durabilis*, *C. fusca*, *C. geitleri*, *C. geitonos*, *C. gloeocola*, *C. gracilis*, *C. javanica*, *C. linearis*, *C. marchica* v. *intermedia*, *C. parva*, *C. scopulorum* and *C. subsimplex*. All the nineteen species reported in our present investigation are new to the flora of Tripura.

Conclusion

On the basis of the present study, it could be concluded that the species of *Calothrix* grow in mixed form in almost all types of biotopes viz. aquatic, terrestrial and sub-aerial habitats but their growth cannot be identified easily in nature because the form very tiny microscopic colonies.

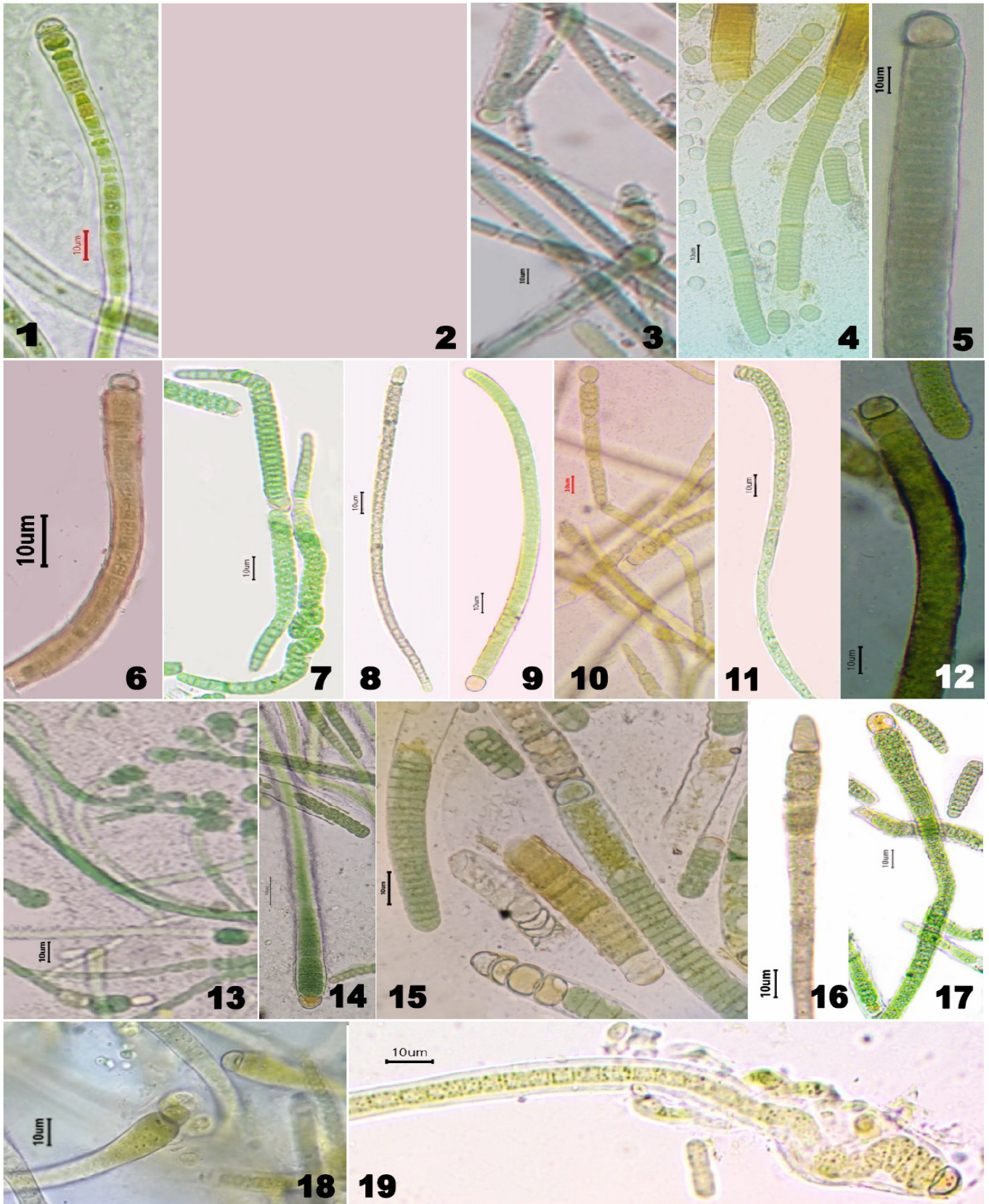


Figure-A: Description of *Calothrix* species (Figures A1-A19)

- | | | |
|--|---|-------------------------------|
| 1. <i>C. clavata</i> | 8. <i>C. geitleri</i> | 15. <i>C. scopulorum</i> |
| 2. <i>C. clavatooides</i> | 9. <i>C. geitonos</i> | 16. <i>C. subsimplex</i> |
| 3. <i>C. columbiana</i> | 10. <i>C. gloeocola</i> | 17. <i>C. aeruginosa</i> |
| 4. <i>C. conica</i> | 11. <i>C. gracilis</i> | 18. <i>C. parva</i> |
| 5. <i>C. cylindrica</i> | 12. <i>C. marchica</i> v. <i>intermedia</i> | 19. <i>C. breviarticulata</i> |
| 6. <i>C. fusca</i> | | |
| 7. <i>C. fusca</i> f. <i>durabilis</i> | 13. <i>C. javanica</i> | |
| | 14. <i>C. linearis</i> | |

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