Original Research Article

Diversity of family Scytonemataceae (Cyanobacteria) from the Wayanad district, Western Ghats regions of Kerala, India.

ABSTRACT

The Scytonemataceae is the only traditional cyanobacterial family that exhibits the false branching phenotype and includes ecologically significant species, making it particularly interesting from an ecologic as well as economic perspective. This family is characterized by its taxonomic diversity and widespread distribution. The current study focuses on the diversity of the Scytonemataceae family in the Wayanad district, located in the Western Ghats of Kerala. The Western Ghats is notable for its exceptionally high level of biological diversity and endemism. Additionally, this area is ecologically sensitive and serves as a site for the systematic elucidation of many species. The research found that Scytonemataceae are commonly found on rock surfaces in the Wayanad district. These species display a color range that varies from pale or olive green to light brownish-black. The species were characterized and identified based on various morphological features, including cell dimensions, color, shape, type of branching, sheath characteristics, and cell contents. A total of 19 species were recorded, with the dominant genus being *Scytonema* with 15 species. *Brasilonema* follows with 3 species, and *Petalonema* contributes to one species, all belonging to the order Nostocales. Notably, among the 19 species identified, 11 are new records for the cyanobacterial flora of the Western Ghats in India.

Keywords: *Cyanobacteria, Nostocales, Brasilonema, Scytonema, Petalonema.*

INTRODUCTION

The Cyanobacteria are the largest, most diverse, and widely distributed group of photosynthetic prokaryotes. They are recognized as the first microorganisms on Earth capable of producing oxygen through photosynthesis (Sukenik et al. 2009; Rasmussen et al. 2008). These organisms possess the unique ability to perform mutually compatible functions such as nitrogen fixation and photosynthesis. They can thrive in various environments, including extreme climatic conditions and in both oxic and anoxic environments (Whitton and Potts, 2000; Thajuddin and Subramanian, 2005). Among them, Scytonemataceae populations are particularly prominent and are commonly found on rock surfaces. Their occurrence, growth pattern, and distribution vary significantly with changing environmental conditions. This diverse group has various applications in biotechnology, pharmacology, and agriculture.

Cyanobacteria have several advantages as hosts for biotechnological applications owing to minimal growth requirements, easy genetic manipulation, and appealing platforms that enable carbon-neutral production processes (Lau et al. 2015; Kumar et al. 2016). The Scytonemataceae family has significant potential applications in agriculture due to its capacity for nitrogen fixation, making it a promising contributor to this field. Specifically, *Scytonema varium* has demonstrated significant antiviral activity against the human immunodeficiency virus (HIV), supporting its potential use in AIDS therapy (Boyd et al. 1997; Loya et al. 1998; Bokesch et al. 2003). Scytonemin, primarily found in *Scytonema javanicum*, is a UV-A protective pigment known for absorbing harmful ultraviolet radiation (UV-C, UV-B, and UV-A), making it a suitable ingredient for sunscreens. Additionally, it inhibits the proliferation of human fibroblasts, endothelial cells, and tumor cells, indicating its potential as an anti-inflammatory and antiproliferative agent (Stevenson et al. 2002). Furthermore, *Scytonema* species have demonstrated antibacterial properties against *Pseudomonas striata*, *Bacillus subtilis*, and *Escherichia coli* (Tyagi et al. 2014). *Scytonema bilaspurense* has demonstrated cytotoxic effects against human cancer cell lines (Ngo et al. 2022). Certain Scytonema species, including *S. foetidum,* are recognized for producing geosmin, the compound responsible for the earthy aroma in soil (Tawong et al. 2022). Petalonema species play a vital role in enhancing soil fertility through nitrogen fixation. These microorganisms can be used as biofertilizers in agricultural practices to improve soil quality and increase crop yields (Abed et al. 2010). Additionally, Brasilonema species are valuable as a source of high-quality protein and essential nutrients. The biomass of Brasilonema species can serve as a raw material in various industrial sectors, including food production (Menescal et al. 2020).

The genus *Scytonema* is an important member of the Cyanophycean family, characterized by false branching and isopolar filaments. These filaments often appear entangled or irregularly coiled and are typically covered with colored or colorless sheaths. The trichomes are mainly olive green, blue-green, brownish, or yellowish (Komarek, 2013). In contrast, the genus *Brasilonema* features isopolar filaments that are densely packed and exhibit rare instances of false branching (Fiore et al. 2007). *Brasilonema* is characterized by its macroscopic filaments, heterocysts, and cell division occurring in a single plane. Additionally, *Brasilonema* can be distinguished from other taxa by its erect, fasciculate thalli and the presence of vacuole-like spaces in the center of the cytoplasm. While both genera share similar morphological traits, the primary distinction lies in the shape of their young trichomes, which are 'J' or 'C' shaped in *Brasilonema*, unlike those in *Scytonema* (Fiore et al. 2007; Sant' Anna et al. 2011). However, the diversity and taxonomic classification of these genera remain poorly understood (Komarek et al. 2013). The genus *Petalonema* is a filamentous cyanobacterium characterized by false branching and the presence of heterocytes. *Petalonema* species primarily thrive in terrestrial environments, commonly found on calcareous wet rocks, stones, and dripping walls. Morphologically, *Petalonema* resembles the genus *Scytonema*, but it has distinct characteristics that set it apart. These include irregular false branching, very broad and thick lamellated sheaths surrounding the trichomes, and sheaths that are several times broader than the trichomes themselves, with funnel-like divergent ends (Komarek et al. 2013).

Kerala is one of the least explored areas in the field of cyanobacteria. The Western Ghats region falling in the eastern side of the state is renowned for its rich ecology and biodiversity, especially due to its diverse habitats. The Western Ghats is characterized by high biological diversity and endemism. Also, the area is ecologically sensitive and constitutes sites for the systematic elucidation of many species. Nevertheless, little research has been attempted on cyanobacteria, particularly the family Scytonemataceae in this region. In this perspective, the study has been attempted with the concept that it will provide comprehensive information on cyanobacterial diversity in this region, that too for the first time.

Referring to the literature on Cyanobacterial taxa concerning Kerala, research on the diversity in the Scytonemataceae family is scarce. Also, the Western Ghats region of Kerala, which contributes to a spectrum of microhabitats, has not been extensively studied for Cyanobacterial diversity, especially for the family Scytonemataceae. Initial investigations of the present study, on Kerala's Western Ghats produced astounding findings, indicating that the cyanobacteria in these regions are extremely diversified. These microorganisms show great promise, and gaining a comprehensive understanding of the distribution of the Scytonemataceae family in the Western Ghats region requires a detailed ecological study. Hence the present study has been undertaken on the diversity of family Scytonemataceae in the Wayanad district of Kerala, which geographically falls fully in the Western Ghats mountain chain.

MATERIALS AND METHODS:

Study area

The Wayanad district is located in the northeastern state of Kerala, India. It is situated within the Western Ghats and lies at the southernmost extremity of the Deccan Plateau. The district shares its western border with Kozhikode and is predominantly covered by dense forests typical of the Western Ghats. The Western Ghats are a mountain range that runs parallel to the western coast of the Indian peninsula. The range begins in the state of Gujarat and extends down to Kerala. It separates the Deccan Plateau from the two narrow coastal strips known as the Konkan and Malabar coasts. Covering an area of nearly 140000 square kilometers, the Western Ghats are considered one of the world's eight "hottest hotspots" of biological diversity (Indian culture, 2024; Balasubramanian and Sangha 2023).

Wayanad has a pleasant climate, with an average annual rainfall of 2,786 mm. The areas of Lakkidi, Vythiri, and Meppady receive particularly high levels of rainfall. The southern, southwestern, and northeastern parts of the district typically receive over 3,000 mm of rainfall each year, while the eastern and northeastern regions receive comparatively less, averaging less than 1,500 mm. (Department of Mining and Geology, 2016)

Sl.No	Sampling location	Location Code	Elevation (m)	Coordinates
1	Edakkal	W1a	933	N11°37'47.52"-E076°13'44.12"
2	Edakkal	W1b	913	N11°37'47.79"-E076°13'40.58"
3	Edakkal	W1c	915	N11°37'20.12"-E076°13'42.71"
4	Edakkal	W1d	921	N11°37'18.97"-E076°13'44.92"
5	Phantom rock	W2a	924	N11°38'08.55"-E076°12'11.61"
6	Phantom rock	W2b	911	N11°38'09.96"-E076°12'14.27"
7	Govindamoola chira	W3	937	N11°37'41.96"-E076°14'30.07"
8	Ambukuthi 19	W4	921	N11°37'01.80"-E076°14'06.19"
9	Meppady	W5	855	N11°33'28.3" E076°07'55.2"
10	Rippon	W6	844	N11°32'25.02"-E076°08'10.94"
11	Thirunelli	W7a	851	N11°30'41.79"-E076°14'30.21"
12	Thirunelli	W7b	879	N11°54'49.33"-E075°59'39.29"
13	Kanthanpara	W8	867	N11°31'41.35"-E076°09'25.75"

Table 1. Coordinates and elevation of sampling locations

Sampling

Specimens were collected seasonally (pre-monsoon, monsoon, and post-monsoon) from 13 sites of the Western Ghats, specifically falling in the Wayanad district (Fig.1.). The coordinates and elevation of sampling sites were recorded using an Etrex 20X Garmin GPS(Table 1). Cyanobacterial patches were collected from various rock surfaces at different sampling sites by scraping them with sterile scalpels and placing them in collection bottles. In total, 33 samples were collected from 13 sampling locations. Microclimatic parameters associated with the habitat, such as atmospheric temperature (Divinext digital Thermometer), surface temperature (Metravi AVM-08 IR thermometer), humidity (Divinext digital hygrometer), and wind speed (Prova AVM -01 anemometer) were recorded. Data concerning diurnal temperature and relative humidity were acquired from Google Earth Pro (2022). Other habit and habitat characteristics of patches were identified and recorded. Each specimen was assigned a unique voucher number for identification purposes.

Culturing and Identification

Two sets of cyanobacterial specimens were collected and brought to the laboratory for further study. To remove any debris and impurities, the samples were first rinsed with distilled water. They were then cultured in a BG-11 medium (Rippka et al. 1979), which is a nitrogen-free medium prepared by omitting sodium nitrate. The specimens were maintained at a temperature of 25 ± 2°C and illuminated with 3000 lx, following a light/dark cycle of 16:8 hours. The other set of samples was preserved in 4% (v/v) formaldehyde and deposited in the germplasm collection of cyanobacteria at the Division of Environmental Science, Department of Botany, University of Calicut.

For the identification of cyanobacteria, microscopic analysis was conducted on live specimens. The collected samples were examined, and photomicrographs were taken using a Leica DM6B compound microscope. Morphometric analyses were performed based on key morphological features, including cell dimensions, colour, shape, type of branching, sheath characteristics, and cell contents. These features were essential for the identification and classification of cyanobacteria, following the methods outlined by Komarek and Anagnostidis (1998, 2005) and Komarek (2013). Standard taxonomic manuals by Desikachary (1959) and Komarek (2013) were used for identifying cyanobacterial species. Furthermore, the identity of the taxonomically accepted species was verified as per Algaebase (https://www.algaebase.org/) (Guiry and Guiry 2024).

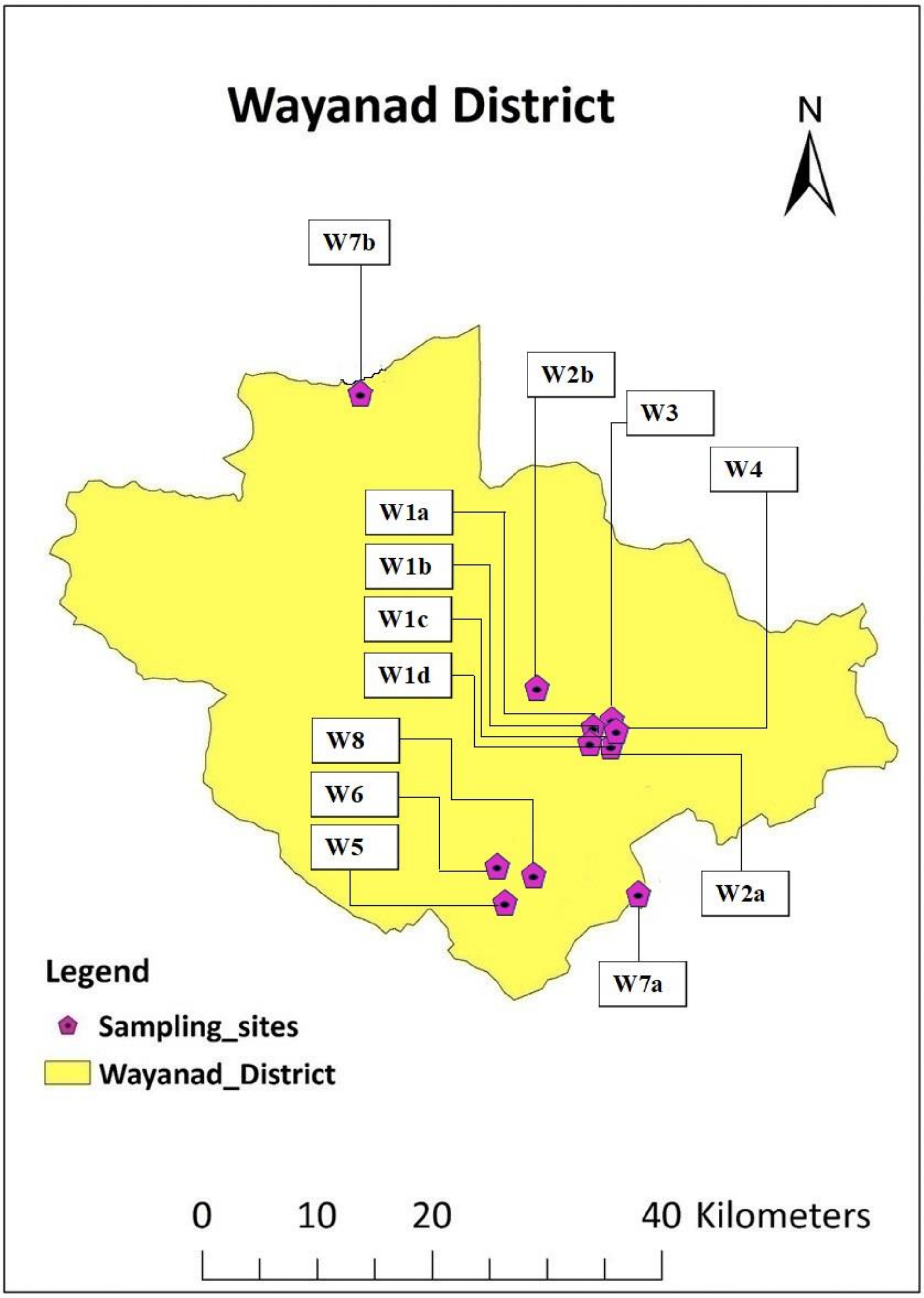

Fig.1. Map showing the study areas

RESULTS AND DISCUSSION

The study revealed a total of 19 species of cyanobacteria belonging to the order Nostocales. Among these, the genus Scytonema, represented by 15 species, showed the highest dominance.The present study identified 19 species from the Western Ghats region of

Kerala. Among these, 11 species are new to India: *Scytonema spirulinoides, S. masonianum, S. variabile, S. bivaginatum, S. arcangelii, S. tenellum, S. brunneum, S. polycystum, Brasilonema bromeliae, B. terrestre*, and *B. epidendron.* In total, 14 species are newly recorded in Kerala, which includes the 11 species mentioned above along with three additional species: S*cytonema malaviyaensis, S. drilosiphon, and Petalonema crustaceum.*

Family Scytonemataceae Rabenhorst ex Bornet et Flahault 1887

Heterocytous, uniseriate filamentous cyanobacteria with obligate false branching.Thallus usually prostrate, in the form of flat or woolly mats, less frequently forming erect fascicles. Filaments isopolar, branching starts usually in trichomes between vegetative cells between two slightly distant heterocytes.Trichomes always monoseriate, isopolar, cylindrical or widened or narrowed at both ends. Sheaths usually thick, firm or gelatinizing, often lamellated, colourless or coloured with sheath-pigments. Heterocytes initially sometimes basal, intercalary, mostly single.Reproduction by disintegration of the thallus or by hormogonia.

Key to the genera

1. Filaments free from thallus ……………………………………………………………………… 2

1. Filaments joined into thallus …………………………………………………..Brasilonema

2. Sheaths lamellated and coloured ………………………………………………. Petalonema

2. Sheaths usually colourless, rarely lamellated …………………………….…….. Scytonema

1 Brasilonema Fiore et al. 2007

Filaments joined into a thallus, attached to solid substrates , forming macroscopic, velvet-like strata or mats, usually composed from densely arranged, erect fascicles; filaments ensheathed, intensely and ± parallely fasciculated, long, mostly (7) 10-26 µm wide, cylindrical, of the same width along the whole length, usually with false branching, with branches single or in pairs, mainly in upper parts of filaments. Branches of the same width and morphology as the main filaments. Sheaths thin or slightly thickened, sometimes slightly lamellated, colourless or yellow brown. Trichomes cylindrical, not or slightly constricted at cross-walls, not attenuated or widened towards ends. Cells usually cylindrical, with blue-green or sightly violet content; apical cells morphologically identical with other vegetative cells. Heterocytes solitary, intercalary, discoid up to cylindrical and longer than wide. Akinetes absent. Reproduction by hormogonia.

Key to the Species

1. Thallus macroscopic, trichomes constricted at the cross walls ………………..………..…… 2

1. Thallus macroscopic, trichomes not constricted at the cross walls ....…..….***B. terrestre***

2. Thallus parallelly fasciculate ……………………………………………..…… ***B. epidendron***

2. Thallus irregularly fasciculate …………………………………………….….. ***B. bromeliae***

Brasilonema bromeliae Fiore et al. 2007

Komarek, 2013, vol.19, pp. 107-108, fig.88.

Description: Thallus is irregularly fasciculated. Filaments joined to flat macroscopic, velvet, blackish green to blackish violet mats, densely fasciculate, more or less creeping on the substrate, slightly irregularly coiled, 10-21µm wide, later joined in dense, erect fascicles. Branches rare. Sheaths firm, thin, colorless to yellow-brown. Trichomes are cylindrical, 8-18 µm wide, and not or very slightly constricted at cross-walls. Cells 1.8-16 µm long greyish-green, olive-green, brownish violet, Heterocyst solitary, intercalary, discoid or cylindrical 4-19×15-16.8 µm.

Specimen Examined: Edakkal, N11°37'20.12"-E076°13'42.71", Environmental Science Division, University of Calicut, CU No:173146. (Fig. 2, E).

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Brasilonema terrestre Sant' Anna et al. 2011

Komarek, 2013, vol.19, pp. 113, fig.94.

Description: Thallus macroscopic, composed of very irregularly fasciculated, partly erect filaments. Filaments cylindrical, 12-17µm wide. Sheaths are thin, firm, colourless to yellow-brown. Trichomes are cylindrical, 9-15µm wide, not constricted at cross walls. Cells ± isodiametric, shorter towards the ends, greyish-green or blue-green. Heterocytes are short barrel-shaped to cylindrical, 6-17×13-14 µm.

Specimen Examined: Edakkal, N11°37'47.52"-E076°13'44.12", Environmental Science Division, University of Calicut, CU No: 173144. (Fig. 3, I)

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Brasilonema epidendron Sant'Anna et al. 2011

Komarek, 2013, vol.19, pp. 108, fig.89.

Description: Thallus macroscopic, composed from parallelly fasciculated, erect filaments, irregularly arranged, dark green to blackish. Filaments 7-10.9×12 (14) µm wide, very rarely branched. Sheaths are thin, and firm. Trichomes 5.5-8.2-10 (11) µm wide, cylindrical not constricted at cross-walls, not attenuated towards ends. Cells are cylindrical or shortened. Heterocytes barrel-shaped to cylindrical, (7)8-10 (11.5) ×7-9µm.

Habitat: Epilithic.

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Specimen Examined: Phantom rock, N11°38'09.96"-E076°12'14.27", Environmental Science Division, University of Calicut, CU No: 163859. (Fig. 2, F).

2 Petalonema Berkeley ex Correns 1889

Filaments free, in clusters or irregular mats, irregularly coiled, creeping or erect, at first heteropolar with apical growth, subapical meristematic zones, falsely branched with single or branches in pairs, later becoming isopolar. Trichomes usually uniseriate, cylindrical, sometimes widened at the ends and narrowed in the middle parts. Constricted or unconstricted at the cross walls. Sheaths prominent, firm, delimited, extremely wide, mostly highly lamellated and funnel like divergent at the ends. Usually coloured, yellow, yellowish brown in colour; cells cylindrical, barrel shaped or spherical in shape. Heterocysts intercalary, sometimes at the bases of branches; usually branches arise between two heterocysts; spherical, oval, barrel shaped up to cylindrical, usually found as solitary, rarely in pairs. Akinetes unknown. Reproduction by hormogonia and disintegration of filaments.

Key to the species

1. Filaments 18-22 µm wide, blackish brown colour ……………………….. ***Petalonema crustraceum***

Petalonema crustaceum (Thuret ex Bornet & Flahault) Kirchner 1900

Komarek, 2013, vol.19, pp. 150, fig.144.

Description: Thallus crusty, blackish, or dark brown. Filaments (13) 18-22 µm wide, partly up to 30 µm wide, sometimes densely entangled, richly and repeatedly branched. Branches common, usually in pairs near the base of the thallus, in erect parts mainly solitary: paired branches usually grow in parallel at the point of branching, later divergent. Sheaths yellow-brown to dark brown, with fine or oblique lamellation. Trichomes are cylindrical, not constricted at the cross-walls. Cells are mostly shorter than wide, 4.5-7× (6-8)-12µm, rarely up to quadratic cylindrical. Heterocytes flattened spherical to barrel-shaped, 5.7-8 µm in diameter, rarely elliptical - cylindrical.

Habitat: Epilithic.

Comments: New record to the Western Ghats Cyanobacterial flora of Kerala.

Specimen Examined: Thirunelli, N11°30'41.79"-E076°14'30.21", Environmental Science Division, University of Calicut, CU No: 163852. (Fig.3, E).

3. **Scytonema** Agardh ex Bornet et Flahault 1887

Filaments solitary branched filaments or mats on the substrate. Filaments free or in fascicles, sometimes densely coiled, creeping on the substrate or with erected branches, commonly falsely branched, with one or (obligatory) two lateral branches. Filaments are enveloped always with firm, colourless or coloured sheaths; layers of sheaths have parallel lamina or are slightly divergent towards ends Branching initiates after trichome disintegration by help of necridic cells between two heterocytes, usually not at heterocytes; both branches grow parallelly aside or in crossing position; the filaments make sometimes typical loop-like lateral formations before branching, in which tops of the trichomes later divide. Trichomes solitary in sheaths, only single in filaments, isopolar, cylindrical, not diversified in basal and apical parts, uniseriate, usually with solitary heterocytes, constricted (rarely not constricted) at cross walls; terminal parts of branches cylindrical or slightly widened, with rounded apical cell; middle parts of trichomes sometimes with elongated, cylindrical cells. Cells distinctly shorter than wide, or isodiametric or slightly shorter or longer than wide, pale or olive-green, usually with solitary, irregularly disposed granules or with granular content, rarely yellowish or pinkish coloured; apical cells sometimes with large vacuoles. Heterocytes intercalary, solitary, rarely in pairs, cylindrical or barrel-shaped.

Key to the species

Thallus macroscopic, end filaments widened……………………………………………………5

1. Thallus macroscopic, end filaments not widened…………………………………………2

2. Filament width more than 12 µm ……………………………………………………… 3

2. Filament width less than 12 µm …………………………………… *Scytonema drilosiphon*

3. Filaments up to 30 µm wide …… 4

3. Filaments more than 30 µm wide …… *S. brunneum*

4. Trichomes narrow, constricted at the cross walls …… *S. variabile*

4. Trichomes wide, not constricted at the cross walls …… *S. myochrous*

5. Heterocyst cylindrical in shape …… 6

5. Heterocyst not cylindrical in shape …… 14

6. Thallus woolly …… 7

6. Thallus fruticose …… 13

7. Sheaths lamellated …… 8

7. Sheaths unlamellated …… 12

8. Filament width up to 16 µm …… 9

8. filament width more than 16 µm …… 10

9. Filaments coiled …… *S. tolypothrichoides*

9. Filaments straight …… *S. caldarium*

10. Trichomes constricted at the cross walls …… *S. malaviyanense*

10. Trichomes not constricted at the cross walls …… 11

11. Branches usually single …… *S. masonianum*

11. Branches usually geminate …… *S. pseudoguyanense*

12. Sheaths pigmented, brown in colour …… *S. spirulinoides*

12. Sheaths not pigmented …… *S. arcangeli*

13. Filaments with thin sheath …… *S. tenellum*

13. Filaments with thick sheath …… *S. polycystum*

14. Heterocyst ellipsoidal …… *S. crispum*

14. Heterocyst sub-rectangular …… *S. bivaginatum*

Scytonema arcangelii Bornet et Flahault 1886

Komarek, 2013, vol.19, pp. 83, fig.49.

Description: Thallus pillow-like, olive green. Filaments 12-17µm wide. False branching usually geminate, branches long and bent of the same morphology as the main filaments. Sheaths are distinct, thin, firm, colourless. Trichomes green, cylindrical along the whole length, not attenuated or widened at the ends, 9.5-14.5 µm wide, not constricted at cross walls. Cells in the middle of trichomes are cylindrical, quadratic, and rarely indistinctly barrel-shaped. Heterocytes are quadratic or slightly longer or shorter than wide, 6-14.5 µm long.

Habitat: Epilithic.

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Specimen Examined: Ambukuthi 19, N11°37'01.80"-E076°14'06.19", Environmental Science Division, University of Calicut, CU No: 163819, Meppadi, N11°33'28.3" E076°07'55.23", Environmental Science Division, University of Calicut, CU No: 163810. (Fig.2, D).

Scytonema brunneum Schmidle 1901

Komarek, 2013, vol.19, pp. 116, fig.95.

Description: Thallus flat, brownish. Filaments straight, 14-24 µm wide, in lower parts creeping on the substrate. False branching is rare, usually solitary, and rarely branches in pairs. Sheaths wide, obliquely lamellated, yellow-brown. Trichomes brown, at the ends rarely blue-green, cylindrical, in middle parts without constrictions, towards ends widened and constricted at cross-walls. Cells in the middle of trichomes are longer than wide, cylindrical, near the ends barrel-shaped, isodiametric up to distinctly shorter than wide. Heterocytes are usually cylindrical, elliptical, and solitary.

Habitat: Epilithic.

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Specimen Examined: Rippon, N11°32'25.02"-E076°08'10.94", Environmental Science Division, University of Calicut, CU No: 163854.

(Fig. 2, B).

Scytonema bivaginatum Welsh 1965

Komarek, 2013, vol.19, pp. 85, fig.53.

Description: Thallus woolly, dark brown. The main filaments are up to 30 µm wide, rarely geminate, and false branches are about 12 µm wide. Sheaths are firm, smooth, and usually hyaline with a thin, about 2-3 µm thick, yellow layer next to the trichome and a wider, colorless outerpart, about 10 µm thick. In young branches, sheaths are only homogeneous and hyaline. Trichomes in main filaments 6-7 µm wide, in branches 3.5-5 µm wide, cylindrical, not constricted at cross-walls, sometimes with granular content. Cells 3-8×6-7 µm in old parts,10-15 ×± 3.5µm in branches. Heterocytes intercalary, sub-rectangular, ±12×8.5 µm.

Specimen Examined: Edakkal, N11°37'18.97"-E076°13'44.92", Environmental Science Division, University of Calicut, CU No: 163818. (Fig. 2, C).

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Scytonema caldarium Setchell 1899

Komarek, 2013, vol.19, pp. 125, fig.107.

Description: Thallus expanded, flocculent, Filaments 12-16 µm in diameter, with paired or single false branches. Sheaths firm, Parallelly lamellated, in young parts colourless, in old filaments golden-brown. Trichomes are cylindrical and narrow with long cells, not constricted cross-walls, olive-green or yellowish. Cells are usually longer than wide, 6.9-18.4 µm long, 4-9 µm wide, in meristematic regions shorter; terminal cells rounded. Heterocytes are cylindrical, longer than wide.

Habitat: Epilithic.

Specimen Examined: Phantom rock, N11°38'08.55"-E076°12'11.61", Environmental Science Division, University of Calicut, CU No: 173139. (Fig. 3, G).

Scytonema crispum Bornet ex Toni 1907

Komarek, 2013, vol.19, pp. 82-83, fig.48.

(This name is currently regarded as a synonym of *Heteroscytonema crispum* (Bornet ex De Toni) G.B.McGregor & Sendall 2018)

Description: Thallus caespitose, fasciculated, woolly, green, olive green, blue-green to brownish. Filaments entangled, coiled, (14) 18-30 (36) µm wide. Branches of the same width as the main filaments. Sheaths firm, colorless, or slightly yellowish brown. Trichomes constricted at cross-walls. Cells 4.6-11.5× 12-32 µm, in old parts of trichomes up to isodiametric, olive-green, blue-green, or yellowish brown, usually with granular content. Heterocytes ellipsoidal, rounded, solitary.

Habitat: Epilithic.

Specimen Examined: Govindamoolachira, N11°37'41.96"-E076°14'30.07", Environmental Science Division, University of Calicut, CU No: 163820. (Fig. 3, C).

Scytonema drilosiphon [Kutzing] Elenkin et Poljanskij 1922

Komarek, 2013, vol.19, pp. 73, fig.33.

Description: Thallus woolly, up to 1-3 mm thick, grey or pale greyish blue-green, sometimes slightly up to densely covered by $CaCO_3$- precipitates. Filaments intertwined ± in bundles, 7-13µm wide, with sparse geminate or single false branching. Sheaths are firm, thin, not lamellated, colourless, or slightly yellowish. Trichomes are blue-green, cylindrical, at the ends sometimes slightly widened, not constricted at cross-walls, 5.5-10 µm wide. Cells cylindrical, in the middle of trichomes isodiametric, 2.5-12 µm long; terminal cells rounded. Heterocytes elliptical to quadratic, 8-12 (14) × 7-9µm.

Specimen Examined: Thirunelli, N11°30'41.79"-E076°14'30.21", Environmental Science Division, University of Calicut, CU No:163831. (Fig. 3, B).

Comments: New record to the Western Ghats Cyanobacterial flora of Kerala.

Scytonema malaviyanense Bharadwaja 1930

Desikachary, 1959, pp. 483; Komarek, 2013, vol.19, pp. 94, fig.86.

Description: Thallus thick, spongy, bluish-green. Filaments flexuous, interwoven, young 8-10 µm, old 16.8-20.2 µm wide, up to 4mm long. False branches only geminate,6.5 -8.5 µm wide, up to 14-16 µm wide in older parts. Sheaths firm, at first thin, hyaline or slightly yellow, later thick, lamellated and yellowish-brown, brown to dark brown,1.4-4 µm thick. Trichomes bluish-green, with older trichomes slightly constricted at cross-walls. Cells isodiametric. Heterocytes are absent in young filaments, but present in old ones, intercalary, quadratic, 11.2-15.4 × 9.8-15.4 µm.

Specimen Examined: Kanthanpara, N11°31'41.35"-E076°09'25.75", Environmental Science Division, University of Calicut, CU No:173138. (Fig.3, A).

Comments: New record to the Western Ghats Cyanobacterial flora of Kerala.

Scytonema masonianum Welsh 1963

Komarek, 2013, vol.19, pp. 94-95, fig.67.

Description: Thallus floccose, dark green to brownish. Old filaments intricately intertwined, about 20 µm wide, younger parts of filaments and branches about 13 µm wide; filaments false branched, branches infrequent, V-formed or lateral, commonly single, rarely geminate. Sheaths are well developed, straight, firm, parallelly and faintly, but distinctly lamellated or diffluent, especially in older parts, 2-4 µm thick, greenish-yellow towards ends hyaline. Trichomes brownish-yellow, not constricted at cross-walls. Cells 4-12 µm long. Heterocytes are not numerous, intercalary, or rectangular.

Specimen Examined: Phantom rock, N11°38'09.96"-E076°12'14.27", Environmental Science Division, University of Calicut, CU No: 173142. (Fig. 3, F).

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Scytonema myochrous C.A. Agardh ex Bornet et Flahault 1886

Desikachary,1959, pp. 487, pl. 90, fig.3; Komarek, 2013, vol.19, pp. 119, figs.99,100.

Description: Thallus flat-hemispherical to expanded, leathery, crusty, brown-black or blackish-green. Filaments entangled, (10-14) 18-36 (40) µm wide, with usually common false branching; branches usually in pairs, rarely solitary, long. Sheaths are dark yellow, yellow-brown, rarely pale yellow, with distinctly divergent layers. Trichomes are cylindrical, sometimes slightly wider at the ends, not constricted at cross-walls, only slightly constricted at the ends of trichomes in branches. Cells cylindrical, blue-green or olive-green, in older parts of trichomes quadratic or slightly longer or shorter than wide (4-14 µm long), rarely up to 2× longer than wide, 6-12 µm (rarely up to 23 µm) wide, towards ends sometimes shortened and up to slightly short barrel-shaped (as short as 3.5 µm long); end cells rounded,12-13 µm wide. Heterocytes are cylindrical, rarely spherical or shortly obliquely elliptic, isodiametric-rounded or elongated elliptical, usually 8-10 µm wide.

Habitat: Epilithic.

Specimen Examined: Ambukuthi 19, N11°37'01.80"-E076°14'06.19", Environmental Science Division, University of Calicut, CU No: 163855. (Fig. 3, D).

Scytonema polycystum Bornet et Flahault 1887

Komarek 2013, vol.19, pp. 96, fig.70.

Description: Thallus cluster-like, greyish-green. Filaments entangled, 14-24 µm wide, scarcely falsely branched. Branches of the same morphology as the main filaments, geminate or single. Sheaths thin up to 3 µm thick, not lamellated. Trichomes are cylindrical, 10-15 (20) µm wide, not attenuated towards ends, not constricted at cross-walls. Cells cylindrical,3-6 µm long; terminal cells rounded. Heterocytes 7-21 µm long, cylindrical.

Habitat: Epilithic.

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Specimen Examined: Phantom rock, N11°38'09.96"-E076°12'14.27", Environmental Science Division, University of Calicut, CU No: 163813. (Fig. 3, H).

Scytonema pseudoguyanense Bharadwaja 1934

Desikachary, 1959, pp. 472, pl. 89, fig.2; Komarek, 2013, vol.19, pp. 132, fig.121.

Description: Thallus in the form of a thick cushion, with long erect threads, brownish green. Filaments are irregularly curved and densely entangled, 15.7-18.9 (23.1) µm wide, with numerous false branches which are invariably slightly narrower than the main filaments, forming secondary branches at an early stage; branching usually geminate and sometimes (young) arising in characteristic loops. Sheaths firm, pigmented with parallel stratification, 3.1 (4.5) µm wide, in old filaments up to 7.3 µm wide, in young branches only narrowed to 2.1 µm. Trichomes are cylindrical, not constricted at cross-walls, 8.4-9.5 (10.5) µm, in old parts only

5.2 µm wide. Cells almost quadratic, in old parts of filaments cylindrical, up to 4× longer than broad, at the ends shortened and flattened. Heterocytes are cylindrical, quadratic, or ellipsoidal, rarely longer or shorter than wide, 7.3-18.6 (21) × 10.5-12.6 µm.

Distribution: India (Gupta 2012), Jharkhand (Gupta 2021), China (Chu et al. 2007).

Habitat: Epilithic.

Specimen Examined: Meppadi, N 11° 30' 41.93" - E076° 0'56.8", Environmental Science Division, University of Calicut, CU No: 163857. (Fig. 2, G).

Scytonema spirulinoides Gardner 1927

Komarek 2013, vol.19, pp. 133, fig.125.

Description: Thallus flocculent. Filaments curved and spirally coiled, 15-20 µm wide, usually geminate branching, Sheaths thick, homogeneous, without lamellation, dark brown. Trichomes are cylindrical, not constricted at cross-walls, in old parts distinctly narrower than towards ends,10-12 µm wide. Cells cylindrical, isodiametric towards ends shortened. Heterocytes are cylindrical, of the same shape and size as the cells.

Habitat: Epilithic.

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Specimen Examined: Govindamoolachira, N11°37'41.96"-E076°14'30.07", Environmental Science Division, University of Calicut, CU
(Fig. 2, J).

Scytonema tenellum Gardner 1927

Komarek, 2013, vol.19, pp. 101-102, fig.82.

Filaments flexuous or densely intertwined, erect, 12-16µm wide. Branches geminate, branches perpendicular to gently curved. Sheaths are thin, smooth, lamellated. Trichomes cylindrical, 10-14 µm wide, pale olive or blue-green. Heterocytes are numerous, and golden yellow.

Specimen Examined: Thirunelli, N11°54'49.33"-E075°59'39.29", Environmental Science Division, University of Calicut, CU No: 163832. (Fig.2, I).

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Scytonema tolypothrichoides Kutzing ex Bornet et Flahault 1886

Desikachary, 1959, pp. 479, pl. 91, fig. 1; Komarek, 2013, vol.19, pp. 75, fig.36.

Description: Thallus caespitose, floating, flake or tuft-like, green or brownish, up to 3cm in diameter, with radially arranged, coiled filaments. Filaments 10-17 µm wide, 5-6mm long. The sheath is initially colorless, later yellow or orange-brown, sometimes slightly parallelly striated with divergent layers towards ends; sometimes with slight development of ferric precipitates. Trichomes are cylindrical, not constricted at the cross walls, and sometimes narrowed or widened towards ends in narrowed or widened sheaths. Heterocytes are barrel-shaped, almost spherical up to elongated cylindrical, 6-16 µm long.

Habitat: Epilithic.

Specimen Examined: Edakkal, N11°37'47.79"-E076°13'40.58", Environmental Science Division, University of Calicut, CU No: 173145. (Fig. 2, H).

Scytonema variabile Gardner 1927

Komarek, 2013, vol.19, pp. 133, fig.128.

Description: Thallus in the form of a thick layer**.** Filaments are mostly straight, sparsely branched with single or geminate branches; main filaments are 34-42 µm wide, branches thinner, and all filaments very variable in width in different parts. Sheaths hyaline to yellowish-brown, 6-12 µm thick, homogeneous or with a few divergent layers, subgelatinous. Trichomes are very variable in width, distinctly narrower in older parts and slightly constricted at cross walls, widened toward ends up to 25 µm and constricted distinctly. Cells cylindrical, bright blue-green, in older segments of trichomes long cylindrical and up to 2x longer than wide, towards ends widened, short barrel-shaped; terminal cells widely rounded. Heterocytes are cylindrical, of the same size and shape as the vegetative cells.

Habitat: Epilithic.

Comments: New record to the Western Ghats Cyanobacterial flora of India.

Specimen Examined: Edakkal, N11°37'18.97"-E076°13'44.92", Environmental Science Division, University of Calicut, CU No: 173147. (Fig. 2, A).

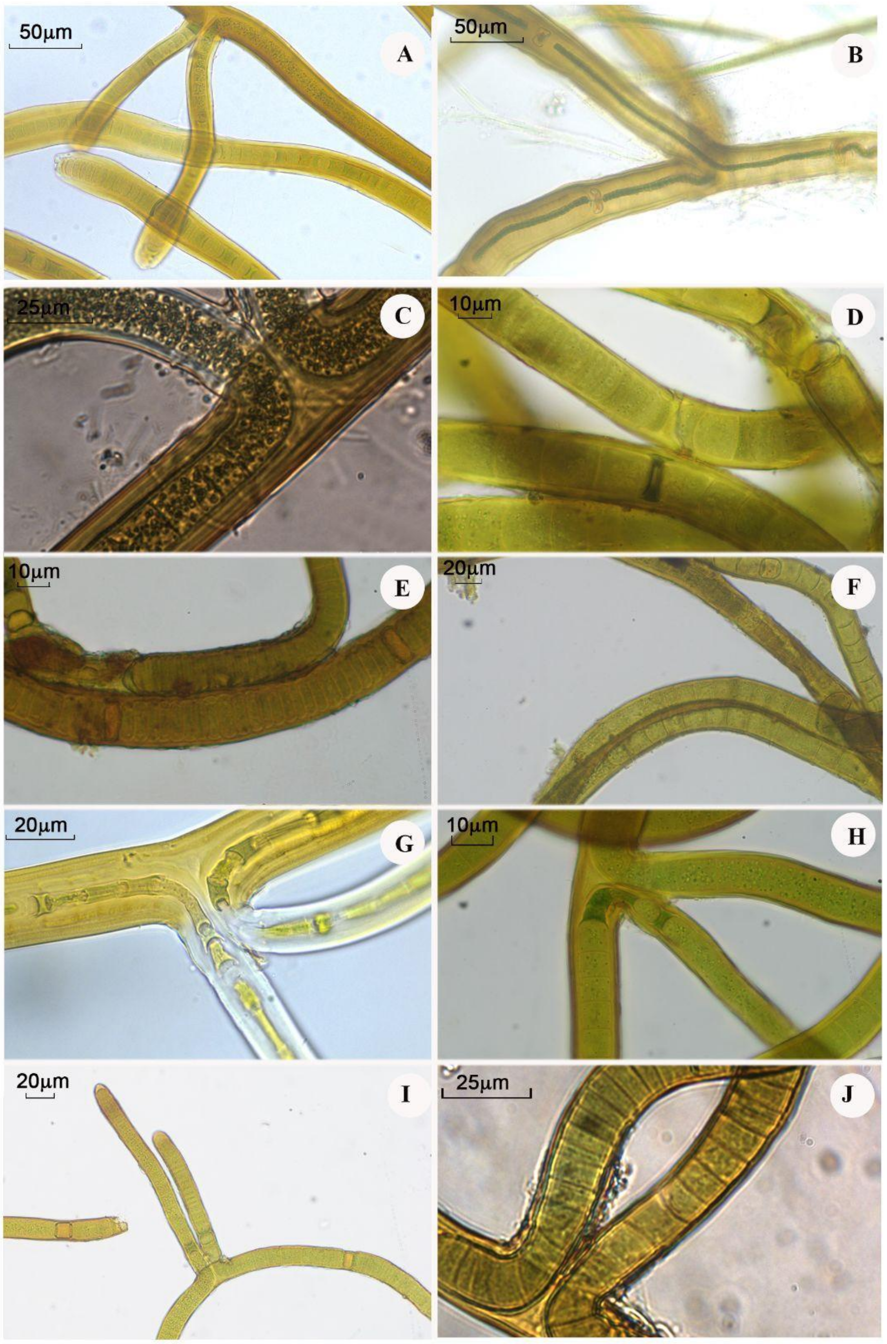

Fig. 2. A. *Scytonema variabile*, B. *S. brunneum*, C. *S. bivaginatum*, D. *S. arcangelii*, E. *Brasilonema bromeliae*, F. *B. epidendron*, G. *Scytonema pseudoguyanense*, H. *S. tolypothrichoides*, I. *S. tenellum,* J. *S. spirulinoides.*

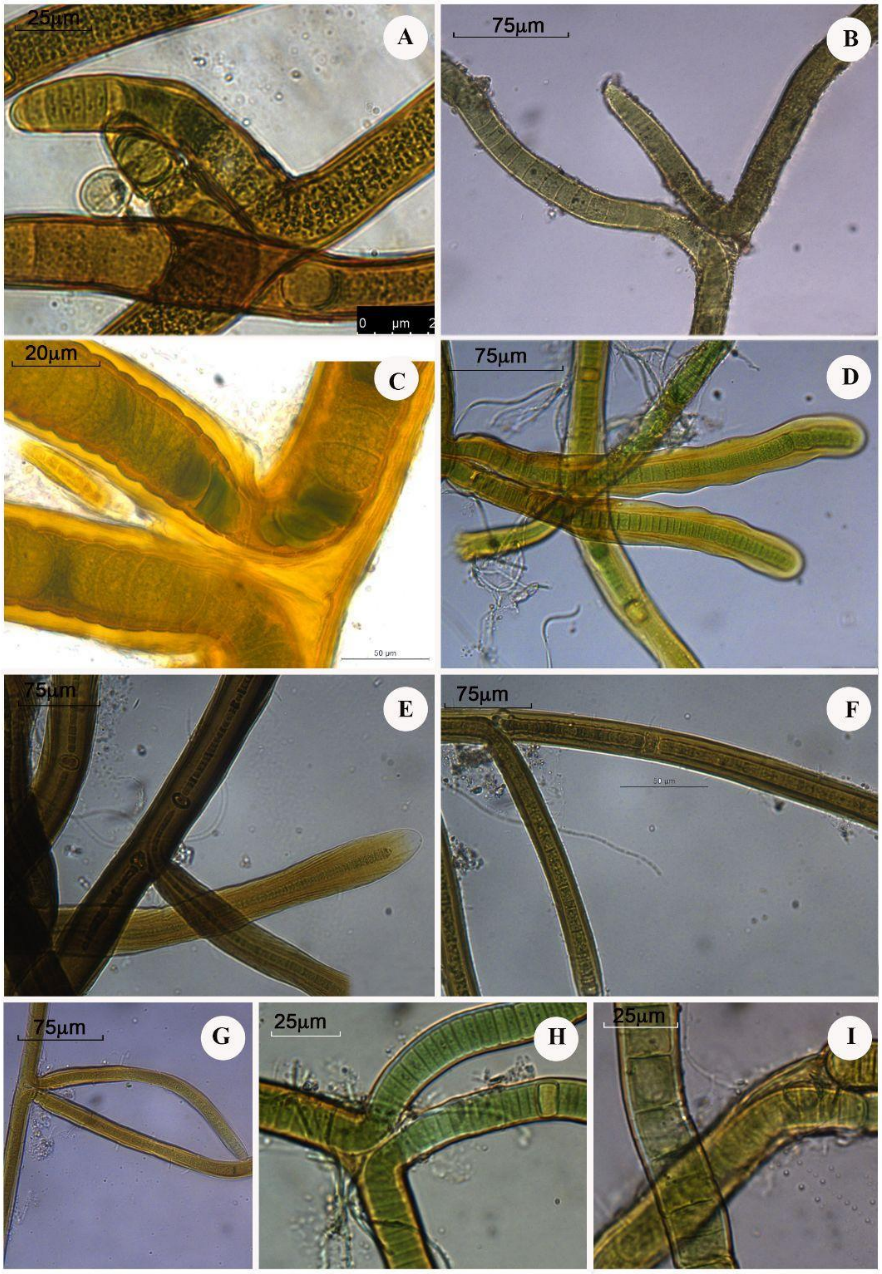

Fig. 3. A. *Scytonema malaviyanense*, B. *S. drilosiphon*, C. *S. crispum*, D. *S. myochrous*, E. *Petalonema crustaceum*, F. *Scytonema masonianum*, G. *S. caldarium*, H. *S. polycystum*, I. *Brasilonema terrestre*.

The Present study observed that the members of this family are abundantly seen on rock surfaces. The rock surfaces provide a suitable substrate for the significant growth of *Scytonema*, *Brasilonema*, and *Petalonema* species, often forming mats. Cyanobacteria adhere to rock surfaces by producing and secreting mucilaginous substances known as extracellular polymeric substances. The protective sheath layers of cyanobacteria consist of polysaccharides that retain moisture, allowing them to endure severe drought conditions (Keshari and Adhikary 2013; Rossi et al. 2012; Cappitelli et al. 2012). Additionally, rocky environments shield cyanobacterial species from various environmental stresses, such as high UV radiation, extreme temperatures, desiccation, and physical removal by wind (Hughes and Lawley 2003).

The study investigated the diversity and species-level identification of members of the Scytonemataceae family. A total of 19 species were identified from the Western Ghats region of Kerala, including *Scytonema drilosiphon, S. bivaginatum, S. malaviyanense, S. masonianum, S. tenellum, S. spirulinoides, S. caldarium, S. variabile, S. tolypothrichoides, S. crispum, S. pseudoguyanense, S. myochrous, S. arcangelii, S. brunneum, S. polycystum, Brasilonema terrestre, B. epidendron, B. bromeliae, and Petalonema crustaceum*. Among these species, 11 are reported as new to India, specifically *Scytonema spirulinoides, S. masonianum, S. variabile, S. bivaginatum, S. arcangelii, S. tenellum, S. brunneum, S. polycystum, Brasilonema bromeliae, B. terrestre, and B. epidendron.* In total, 14 species are new to Kerala, including the aforementioned 11 and three additional species: *Scytonema malaviyaensis, S. drilosiphon*, and *Petalonema crustaceum.*

In Indian ecosystems, cyanobacteria from the Scytonemataceae family are mainly represented by species of the genus Scytonema (Maltsev et al. 2021). Among the representatives of the genus, the species *Scytonema pseudoguyanense* Bharadwaja (1934) has been reported in various locations. Adhikary et al. (2015) observed them in stone temples of Bhubaneswar, India. Samad and Adhikary (2008) confirmed their presence on the walls of Parasurameswar temple, Bhubaneswar, Orissa. Mahanandia and Singh (2023) documented their existence on monuments and exterior surfaces of building facades in Odisha state, India. Dasgupta et al. (2017) reported them from suburban waterbodies in Lucknow, Uttar Pradesh. Philip (2021) recorded from Karapuzha, Wayanad, Kerala. In the current study, the same species were noted on the moist rock in Meppadi of Wayanad district.

Fiore et al. (2007) isolated *Brasilonema bromeliae* from sub-aerophytic environments in tropical and subtropical Brazil. Sant' Anna et al. (2011) reported the presence of *Brasilonema epidendron* and *Brasilonema terrestre* also from Brazil. The current investigation reported *Brasilonema terrestre* in Edakkal, Wayanad district of the Western Ghats region of Kerala. Additionally, *Brasilonema epidendron* was recorded at Phantom Rock, Wayanad. This study demonstrates the occurrence of *Brasilonema bromeliae* from Edakkal, Wayanad district, within the Western Ghats region of Kerala. Biswas (1934) described *Petalonema crustaceum* from Cherrapunji, Meghalaya. Singh and Singh (2019) also described *Petalonema crustaceum* from Sirsi taluk, Uttara Kannada, Karnataka. In the current investigation, the occurrence of this species was observed in Thirunelli, Wayanad district in the Western Ghats region of Kerala.

The occurrence of *Scytonema myochrous* was documented on rock surfaces in Ambukuthi 19, Wayanad districts. This observation is consistent with findings by various researchers. Suresh et al. (2012) recorded *Scytonema myochrous* from the Western Ghats and Eastern Ghats of India. In tropical environments, Budel (1999) observed on moist rock, and Popovic et al. (2018) confirmed its existence on selected monuments in Serbia. Philip (2021) recorded its presence in Eravikulam National Park, Idukki, Kerala. Sabere et al. (2021) reported *Scytonema myochrous*, a cyanobacterium, is a new record for the Egyptian algal inventory.

Philip (2021) recorded *Scytonema caldarium* from Karappuzha, Wayanad, Kerala. The current research recorded its presence in Phantom rock, Wayanad district in the Western Ghats region of Kerala. *Scytonema malaviyanense* was observed on the bark of trees. Banerjee et al. (2020) reported it from West Bengal, India. The current investigation reported its presence from Kanthanpara, Wayanad district in the Western Ghats region of Kerala. *S. tolypothrichoides* was previously reported by Nikam et al. (2010) from the Western Ghats region of Maharashtra state. Deb et al. (2015) isolated from acidic rice field soil from the Dholai region of the Cachar district in Assam, North-East India. Kumar et al. (2016) recorded from biological soil crust in the eastern region of India. Ram (2022) observed from Sakthikulangara, Kollam. The present study confirmed its presence in Edakkal, Wayanad district of the Western Ghats region of Kerala.

Nowicka-Krawczyk et al. (2014) reported *Scytonema drilosiphon* from historical buildings in the former Auschwitz II-Birkenau concentration camp, in Poland. Banerjee et al. (2020) reported *Scytonema drilosiphon* from West Bengal, India. The current study demonstrates the presence of this species from Thirunelli, Wayanad district in the Western Ghats region of Kerala. Komarek et al. (2013) reported *Scytonema crispum* from Atlantic Rainforest in Southeast Brazil. Philip (2021) recorded from Pazhassi Dam, Kannur, Kerala. In the current investigation, this species was observed in Govindamoolachira, Wayanad, in the Western Ghats region of Kerala.

Sarma et al. (2023) reported *Scytonema bivaginatum* from Tripura, India, and the species were new additions to the flora of Tripura, India. The present work confirmed their presence in Edakkal, Wayanad district. Komarek et al. (2013) reported *Scytonema arcangelii* from Atlantic Rainforest in Southeast Brazil. Aguiar Caires et al. (2019), recorded from tropical marine environments of Bahia State, Northeastern Brazil. The present study documented its existence on rocks in Ambukuthi 19, Wayanad district, in the Western Ghats region of Kerala.

Cyanobacteria have evolved several adaptations to survive, compete, and eventually dominate in a wide array of ecosystems (Rastogi and Madamwar 2015; Menamo and Wolde 2015). Due to their adaptive capacities, cyanobacterial diversity occupies a huge spectrum of habitats on the Earth. Their diversity is due to the cellular mechanisms that allow them to adapt in response to environmental changes and grow easily as a dense population with remarkable speed. Nevertheless, their rapid growth rate varies depending on biotic factors, nutrient levels, climate change, or the recent threats due to global warming (Sukenik et al. 2012; Paerl and Paul 2012).

CONCLUSION

The present study investigates the diversity of the family Scytonemataceae in Wayanad district, Western Ghats region of Kerala. This research provides comprehensive information on the cyanobacterial diversity in this area, marking it as the first time study. Species within the Scytonemataceae family are typically abundant in wet environments and often attach to rock surfaces. The present study recorded 19 species from the Western Ghats region of Kerala. Among these, 11 species are new to India, including *Scytonema spirulinoides, S. masonianum, S. variabile, S. bivaginatum, S. arcangelii, S. tenellum, S. brunneum, S. polycystum, Brasilonema bromeliae, B. terrestre, and B. epidendron*. In total, 14 species are new to Kerala, including the 11 species mentioned above and three other species: *Scytonema malaviyaensis, S. drilosiphon*, and *Petalonema crustaceum*.

Wayanad, situated in the Western Ghats, is a biodiversity hotspot known for its unique ecosystems. The area's rocky outcrops and shaded, humid environments provide an ideal habitat for *Scytonema* species belonging to the family Scytonemataceae. However, these populations face several threats. Habitat loss due to construction, quarrying, and deforestation disrupts the rocky habitats vital for Scytonemataceae family. Additionally, pollution and climate change, characterized by altered rainfall patterns, increased temperatures, and changes in water availability further threaten these habitats. Recognizing the ecological and economic value of cyanobacteria is essential for their conservation. By protecting their natural habitats and promoting sustainable practices, we can ensure that these microorganisms continue contributing to Earth's ecosystems and driving future innovations. The biotechnological potential of *Scytonema* species in Wayanad District emphasizes their importance for sustainable development and ecological health. Their ability to produce valuable compounds, such as scytonemin and geosmin, and their roles in nitrogen fixation and soil stabilization, underscores the need for effective conservation strategies. Protecting these microorganisms will preserve biodiversity and strengthen the resilience of the local ecosystems against environmental threats.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technology Grammarly has been used and NO text-to-image generators have been used during the writing or editing of this manuscript

COMPETING INTERESTS

Authors have declared that no competing interests exist

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