



## Morpho-taxonomic identification of mangrove-associated coccoid cyanobacteria from the southwest coast of India

Arun T Ram<sup>1\*</sup>, Tessy Paul P<sup>2</sup>

<sup>1</sup> Department of Botany, M.E.S. Asmabi College, (Affiliated to University of Calicut), P Vemballur, Kodungallur, Thrissur District, Kerala, India

<sup>2</sup> Department of Botany, Christ College (Autonomous), (Affiliated to University of Calicut), Irinjalakuda, Thrissur District, Kerala, India

### Abstract

A total of 10 coccoid cyanobacterial species belonging to 5 genera were reported from the different study areas of mangrove ecosystems of the southwest coast of India from July 2015 to June 2020. All the collected cyanobacterial samples were identified up to species level. The study recorded 5 epipsammic (*Aphanothece granulosa* (N.L.Gardner) Komárek & Komáková-Legnerova 2007, *Aphanothece stagnina* (Sprengel) A. Braun in Rabenhort 1863, *Gloeothece samoensis* Wille 1913, *Gloeothece rupestris* (Lyngbye) Borneo in Wittrock & Nordstedt 1880, *Chroococcus pallidus* Nageli 1849), 2 benthic (*Gloeothece ustulata* Beck-Mannagetta 1929, *Anathece bacteriosa* (Komarek & Komarkova-Legnrova) Komarek, Kastovsky & Jezberova 2011), 1 epiphytic (*Chroococcus indicus* Zeller 1873), 1 planktic (*Aphanothece bullosa* (Meneghini) Rabenhorst 1865), while *Aphanocapsa grevillei* (Berkeley) Rabenhorst 1865) was occurred from epiphytic and benthic habitat.

**Keywords:** coccoid cyanobacteria, mangrove environment, diversity, taxonomy

### Introduction

Cyanobacteria are one of the pioneer species of earth and research has been conducted to better understand their evolutionary, ecological, biochemical, and taxonomic difficulties (Alvarenga *et al.*, 2017) [2]. Through the ability to fix atmospheric nitrogen, cyanobacteria are the only prokaryotes that can significantly contribute to oxygen-evolving photosynthesis and the nitrogen economy of aquatic and terrestrial environments (Kirsch *et al.*, 2019; Fay, 1981) [9, 6]. For the research in the taxonomy of cyanobacteria, microscopy has always been vital, and light microscopy has been the foundation of such research (Stolz, 2000) [20]. The identification of cyanobacteria based on the morphology requires visible characteristics that can be recognised even at low magnifications using light microscopy. This can be problematic, especially for groups (eg. Chroococcales and Synechococcales) that lack such characteristics and have small cell sizes (Albrecht *et al.*, 2017) [1].

Biodiversity research on mangrove-dwelling coccoid cyanobacteria began in 1980 with a focus on cyanobacterial distribution, zonation, and taxonomic diversity (Potts, 1980) [15]. Coccoid cyanobacteria in mangrove habitats are rarely reported from the southwest coast of India and the majority of the species were referred to in the literature of Joseph and Saramma (2016) [8] and Ramamurthy and Abhinand (2016) [16]. This paper aims to extend our knowledge of the coccoid cyanobacteria from the mangrove environments of southern Kerala.

### Materials and Methods

#### Study area

The study was conducted through extensive field visits for five years, from July 2015 to June 2020, in the mangrove

areas of southern Kerala, distributed along the South West Coast of India. The sampling sites of cyanobacterial samples were provided in Table 1.

**Table 1:** Sampling sites of cyanobacterial samples

No.	Study Area	Latitude	Longitude
1.	Kadinamkulam	8° 37' 15.01" N	76° 48' 43.81" E
2.	Pathiramanal	9° 37' 09.78" N	76° 23' 06.43" E
3.	Ezhupunna	9° 49' 24.43" N	76° 18' 39.54" E
4.	Kumarakom	9° 36' 59.46" N	76° 25' 21.46" E
5.	Mekkara	9° 48' 54.31" N	76° 21' 57.54" E
6.	Vypin	9° 58' 26.46" N	76° 14' 38.14" E

### Collection, storage and identification of samples

Cyanobacterial samples were collected from the water bodies, soil, bark and pneumatophores of mangrove plants. Sterile plastic bottles were used for the collection of cyanobacterial samples. With the help of sterile blades, the samples were scraped, transferred into sterile plastic bottles and transported to the Department of Botany, MES Asmabi College, Kodungallur, Kerala, for taxonomic and culture studies. Bottles were labelled according to date, sampling sites and voucher numbers. In the laboratory, within 6-8 hours one set of samples were preserved in 4% formaldehyde solution. The other set of live samples were observed under a compound microscope and then transferred to BG11 medium (Rippka *et al.*, 1979) [17] for culture, and they were maintained in the culture room under a white fluorescent lamp ( $\pm 3000$  lux), 14"10 L/day at  $\pm 25^{\circ}\text{C}$ .

Microscopic analysis was done for the identification of cyanobacteria. Photomicrographs were taken using a Leica DM 1000 LED compound microscope. A Leica DM 1000 microscope system can incorporate a digital camera, image

processing workstation and sophisticated software for image organization and archiving. Measurements were taken by using Leica Application Suite (LAS version 5.0.2). The cyanobacterial species were identified using the monographs and standard works of literature (Desikachary, 1959; Komarek and Anagnostidis, 1998; Komarek and Komarkova-Legnerova, 2007; Komarek *et al.*, 2011)<sup>[5, 11, 12, 13]</sup>. Using all available information, morpho-taxonomic identification was done up to the species level.

## Result and Discussion

A total of 10 cyanobacterial species belonging to five genera were reported from different study areas of mangrove environments in southern Kerala. All the cyanobacterial samples were identified taxonomically up to species level. Classification of cyanobacteria is based on the revised classification system of Komarek *et al.* (2014)<sup>[10]</sup> and (<http://www.cyanodb.cz/>) Hauer and Komarek, (2021)<sup>[7]</sup>. The details were as follows;

**Phylum/Class:** Cyanobacteria

**Order:** Chroococcales

**Family:** Aphanothecaceae

**Genus:** *Aphanothece* Nägeli 1849

**Species:** *A. bullosa* (Meneghini) Rabenhorst 1865

*A. granulosa* (N.L.Gardner) Komárek & Komáková-Legnerova 2007

*A. stagnina* (Sprengel) A. Braun in Rabenhorst 1863

**Family:** Chroococcaceae

**Genus:** *Chroococcus* Nägeli 1849

**Species:** *C. indicus* Zeller 1873 *C. pallidus* Nageli 1849

**Genus:** *Gloethece* Nägeli 1849

**Species:** *G. rupestris* (Lyngbye) Bornet in Wittrock & Nordstedt 1880 *G. samoensis* Wille 1913 *G. ustulata* Beck-Mannagetta 1929

**Order:** Synechococcales

**Family:** Merismopediaceae

**Genus:** *Aphanocapsa* Nägeli 1849

**Species:** *A. grevillei* (Berkeley) Rabenhorst 1865

**Family:** Synechococcaceae

**Genus:** *Anathece* Komárek *et al.* 2011.

**Species:** *A. bacteriosa* (Komarek et Komarkova-Legnerova) Komarek, Kastovsky et Jezberova 2011.

## 1. Aphanothecaceae

*Aphanothece bullosa* (Meneghini) Rabenhorst 1865

**Description:** Colonies macroscopic, mucilaginous, more or less spherical. Cells oval to elongate cylindrical with rounded ends, with more or less homologous content, sometimes finely granular, pale blue-green, without individual envelopes, 2.52-4.51 µm broad, 5.23-7.11 µm long.

**Habitat:** Planktic form appeared as green in colour.

Specimen Examined: INDIA: Kerala; Ernakulam district, Vypin, 9° 58' 26.46" N, 76° 14' 38.14" E, 16.07.2015, MES 13761. Kottayam district, Mekkara, 9° 48' 54.31" N 76° 21' 57.54" E. 18.09.2016, MES 13786. (Fig. 1. A).

*Aphanothece granulosa* (N.L.Gardner) Komárek & Komáková-Legnerova 2007

**Description:** Colonies microscopic, cells loosely arranged, in older colonies densely agglomerated cells in fine, colourless mucilage, usually well-defined at the margin and overlapping the cells. Cells oval, granulated, 4.62 – 5.27 µm broad, 7.43 – 12.52 µm long.

**Habitat:** On mangrove-soil appeared as dark brownish/black coloured cyanobacterial mat

Specimen Examined: INDIA: Kerala; Ernakulam district, Vypin, 9° 58' 26.46" N, 76° 14' 38.14" E, 24.09.2017, MES 13774. (Fig. 1. B).

*Aphanothece stagnina* (Sprengel) A. Braun in Rabenhorst 1863

**Description:** Thallus gelatinous, spherical, ellipsoidal, brownish or violet colour; cells oblong, more or less ovoid or widely cylindrical with rounded ends, 3.6 µm to 6.2 µm broad, 6.7 µm to 12.3 µm long, densely or sparsely arranged, without individual envelopes, homogeneous mucilage.

**Habitat and Ecology:** Dark blackish or brownish mat collected from the mangrove soil surface.

Specimen Examined: INDIA: Kerala; Alappuzha district, Pathiramanal, 9° 37' 09.78" N, 76° 23' 06.43" E, 28.09.2016. MES 13716. Kottayam district, Mekkara, 12.08.2016. MES 13752. (Fig. 1. C).

## 2. Chroococcaceae

*Chroococcus indicus* Zeller 1873

**Description:** Thallus gelatinous, thin, pale brownish; cells single, oblong to sub-spherical, 3.5 µm to 7.5 µm broad, 3.4 µm to 10.8 µm long, olive-green; sheath hyaline, conspicuous, contents granular.

**Distribution:** This species was reported as epiphytic, edaphic (Bano and Siddiqui, 2007)<sup>[3]</sup>, planktonic form (Sahoo *et al.*, 2020)<sup>[18]</sup>.

**Habitat:** Appeared as dark brownish coloured cyanobacterial mass on the pneumatophores of *Avicennia officinalis* L.

Specimen Examined: INDIA: Kerala; Alappuzha district, Ezhupunna, 9° 49' 24.43" N, 76° 18' 39.54" E, 24.11.2019. MES 13707. Thiruvananthapuram district, Kadinamkulam, 8° 37' 15.01" N 76° 48' 43.81" E, 14.07.2016. MES 13751. (Fig. 1. D).

*Chroococcus pallidus* Nageli 1849

**Description:** Colonies usually microscopic, gelatinous, amorphous. Cells single or in 2-3, without sheath 4.82 – 7.22 µm broad, with sheath 9.64- 13.92 µm broad, elliptic-oblong colonies, cells arranged in a common sheath, common sheath more or less delimited, inside confluent, colourless, structureless. Around the cells special colourless, more or less rounded, not lamellate envelopes present.

**Habitat:** On soil surface which is appeared as green coloured cyanobacterial mat

Specimen Examined: INDIA: Kerala; Thiruvananthapuram district, Kadinamkulam; 14.07.2016. MES 13750. Kottayam district, Kumarakom, 9° 36' 59.46" N 76° 25' 21.46" E, 13.11.2018. MES 13793. (Fig. 1. E).

*Gloethece rupestris* (Lyngbye) Bornet in Wittrock & Nordstedt 1880

**Description:** Colonies small, enveloped by small sheaths, with solitary sheathed cells. Gelatinous envelopes around the cell. Cells ellipsoidal to cylindrical, without envelope 4.13- 5.80 µm broad, 7.58- 8.74 µm long, contents pale blue-green, with sheath 8.76 µm broad, 10.75 µm long.

**Habitat:** On soil surface which is appeared as green coloured cyanobacterial mat

Specimen Examined: INDIA: Kerala; Thiruvananthapuram district, Kadinamkulam, 9° 49' 24.43" N, 76° 18' 39.54" E, 14.07.2016. MES 13750. Kottayam district, Kumarakom, 9°

36°59.46" N 76° 25' 21.46" E, 13.11.2018. MES 13793. (Fig. 1. F).

*Gloeothece samoensis* Wille 1913

**Description:** Round, oblong to elliptical colonies, with sheath 9.85-20.43 µm diam., with 2 cells. Sheath firm to diffuent, hyaline, conspicuous, smooth. Cells oblong to ellipsoid, 3.33-5.82 µm broad, 5.26-8.42 µm long. Cell content granulated, blue-green to olive green.

**Habitat:** On soil surface which is appeared as green coloured cyanobacterial mat

Specimen Examined: INDIA: Kerala; Alappuzha district, Pathiramanal; 28.09.2016. MES 13775. Kottayam district, Kumarakom, 9° 36'59.46" N 76° 25' 21.46" E, 17.09.2018, MES 13792, 05.06.2020, MES 13768. (Fig. 1. G).

*Gloeothece ustulata* Beck-Mannagetta 1929

**Description:** Colonies yellowish, irregularly spherically elongate, envelopes around cells hyaline, not lamellated, colourless or slightly bluish coloured. Cells broadly elliptical, with rounded, almost twice approaching or longer than wide, with homogeneous, slightly granulate content, 7.52 – 8.63 µm broad, 15.5 µm long.

**Habitat:** Appeared as dark brownish coloured benthic cyanobacterial mat

Specimen Examined: INDIA: Kerala; Ernakulam district, Vypin, 9° 58' 26.46" N, 76° 14' 38.14" E, 18.08.2016, MES 13764. 24.09.2017, MES 13776. (Fig. 1. H).

### 3. Merismopediaceae

*Aphanocapsa grevillei* (Berkeley) Rabenhorst 1865

**Description:** Thallus or colonies more or less spherical or hemispherical, greenish, mucilaginous, with irregularly and densely aggregated cells. Cells spherical or oval, 3.72 – 4.75 µm diameter, contents finely granular, individual envelopes not distinct.

**Habitat:** Found attached to the pneumatophores of *Avicennia officinalis* L. and as benthic form from the mangrove environment.

Specimen Examined: INDIA: Kerala; Kottayam district, Mekkara, 9° 48'54.31" N 76° 21' 57.54" E. 07.04.2015, MES 13770. 18.09.2016, MES 13787. (Fig. 1. I).

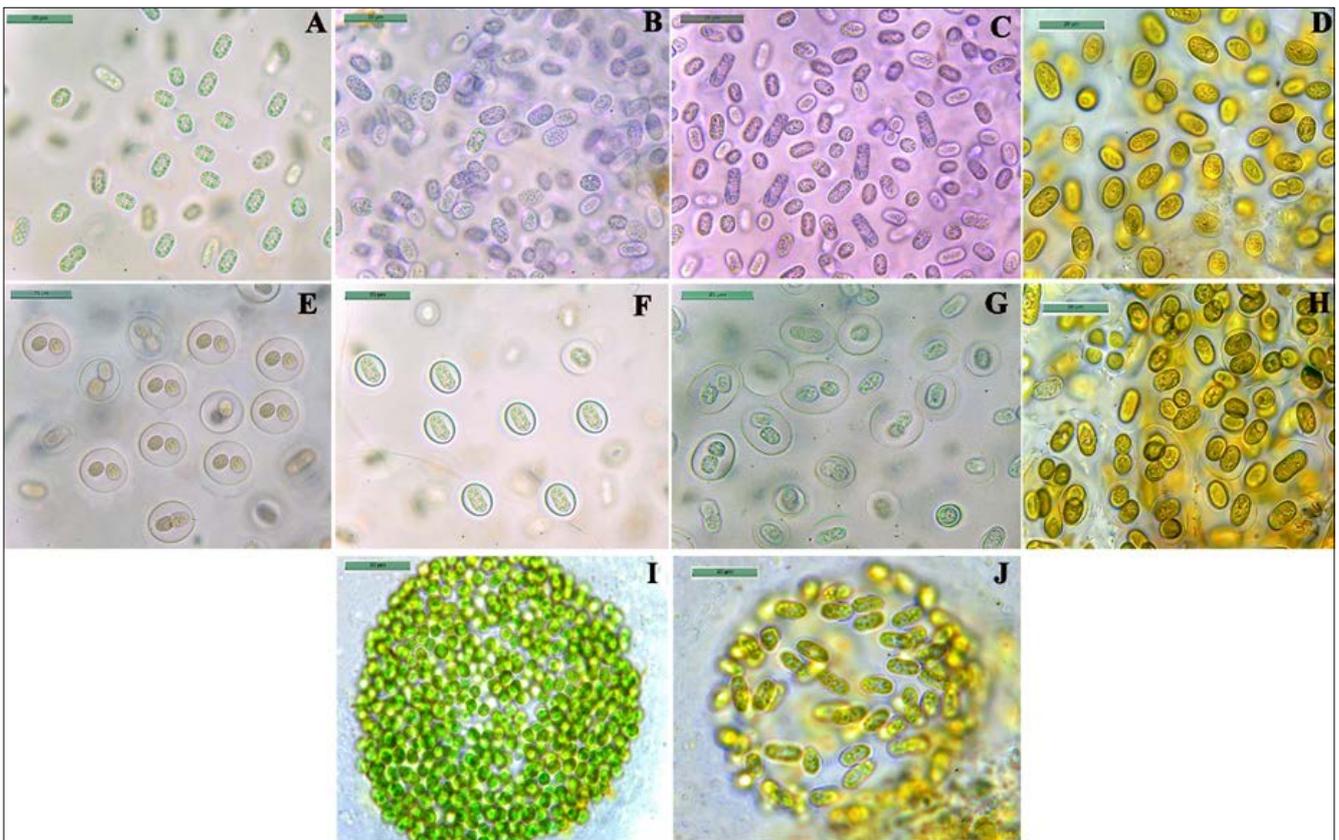
### 4. Synechococcaceae

*Anathece bacteriosa* (Komarek & Komarkova-Legnrova) Komarek, Kastovsky & Jezberova 2011.

**Description:** Thallus gelatinous, colonies macroscopic, irregular, mucilaginous, diffuent or with delimited margin, olive green, with irregularly agglomerated cells. Cells shortly rod-like, 1.32- 2.6 x 0.83-1.21 µm diameter.

**Habitat:** Dark green coloured benthic cyanobacterial mat.

Specimen Examined: INDIA: Kerala; Kottayam district, Mekkara, 9° 48'54.31" N 76° 21' 57.54" E. 14.03.2016, MES 13738. 14.07.2019, MES 13788. (Fig. 1. J).



**Fig 1:** A) *Aphanothece bullosa* (Meneghini) Rabenhorst 1865; B) *Aphanothece granulosa* (N.L.Gardner) Komárek & Komáková-Legnerova 2007; C) *Aphanothece stagnina* (Sprengel) A. Braun in Rabenhort 1863; D) *Chroococcus indicus* Zeller 1873; E) *Chroococcus pallidus* Nageli 1849; F) *Gloeothece rupestris* (Lyngbye) Borner in Wittrock & Nordstedt 1880; G) *Gloeothece samoensis* Wille 1913; H) *Gloeothece ustulata* Beck- Mannagetta 1929; I) *Aphanocapsa grevillei* (Berkeley) Rabenhorst 1865; J) *Anathece bacteriosa* (Komarek & Komarkova-Legnrova) Komarek, Kastovsky & Jezberova 2011.

The majority of the species were recorded from the mangrove soil surface (*Aphanothece granulosa* (N.L.Gardner) Komárek & Komáková-Legnerova 2007, *Aphanothece stagnina* (Sprengel) A. Braun in Rabenhort

1863, *Gloeothece samoensis* Wille 1913, *Gloeothece rupestris* (Lyngbye) Borner in Wittrock & Nordstedt 1880, *Chroococcus pallidus* Nageli 1849), two species were reported as benthic forms (*Gloeothece ustulata* Beck-

Mannagetta 1929, *Anathece bacteriosa* (Komarek & Komarkova-Legnerova) Komarek, Kastovsky & Jezberova 2011), 1 planktic (*Aphanothece bullosa* (Meneghini) Rabenhorst 1865), *Chroococcus indicus* Zeller 1873 occurred from the pneumatophores of *Avicennia officinalis* L., while *Aphanocapsa grevillei* (Berkeley) Rabenhorst 1865) was occurred from the pneumatophores of *Avicennia officinalis* L. and benthic habitat.

Regarding the taxonomy of mangrove-associated cyanobacteria, there were no exclusive studies reported from southern Kerala. The occurrence of *Aphanothece stagnina* (Sprengel) A. Braun in Rabenhorst 1863, *Gloeothece rupestris* (Lyngbye) Bornet in Wittrock & Nordstedt 1880, *Chroococcus pallidus* Nageli 1849 and *Chroococcus indicus* Zeller 1873 have been reported from the mangrove environments (Silva, 1991; Branco *et al.*, 1996; Zaib-Un-Nisa, 2000; Nogueira and Ferreira-Correia, 2001; Bano and Siddiqui, 2007; Joseph and Saramma, 2016) [19, 4, 21, 14, 3, 8].

During this research, *Aphanothece bullosa* (Meneghini) Rabenhorst 1865, *Aphanothece granulosa* (N.L.Gardner) Komárek & Komáková-Legnerova 2007, *Gloeothece samoensis* Wille 1913, *Gloeothece ustulata* Beck-Mannagetta 1929, *Aphanocapsa grevillei* (Berkeley) Rabenhorst 1865 and *Anathece bacteriosa* (Komarek & Komarkova-Legnerova) Komarek, Kastovsky & Jezberova 2011 were recorded for the first time from the mangrove environments of the southwest coast of India.

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