

SYSTEMATICS OF *CHROOCOCCUS* FROM PAKISTAN

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Abstract

The present paper reports taxonomic enumeration of genus *Chroococcus* belonging to the Chroococcaceae family from the Kallar Kahar Lake, Chakwal, Pakistan. In all, 18 species have been recorded from the study area. Of them, 5 species viz., *C. microscopicus*, *C. planctonicus*, *C. polyedrififormis*, *C. subtilissimus* and *C. vacuolatus* are new records for Pakistan and South Asia. Their taxonomic description and geographic distribution is provided and discussed in this paper.

Key words: *Chroococcus*, Brackish water, Microalgae, Cyanobacteria, Kallar Kahar Lake, Geographic distribution.

Introduction

The Coccoid genus *Chroococcus* Nageli belongs to phylum cyanobacteria, the most primitive group of organisms recorded in the Precambrian (Abed *et al.*, 2014; Cuzman *et al.*, 2010; Lee, 2008), containing 149 species (<http://www.algaebase.org>). It posses simple cell structure (Gama *et al.*, 2014) which can colonize bare areas, live on land and in water (Budel, 2010; Pridmore & Etheredge, 2010), produce extracellular polymeric substances (Song *et al.*, 2015) are good indicators of nutrient status of aquatic ecosystem such as *Chroococcus turgidus* (Willen, 2000). They have chlorophyll a and undergo oxygenic photosynthesis with two photosystems I and II (Mur *et al.*, 1999). Those cyanobacteria, which are not able to fix nitrogen, are important as food, feed, fuel, fertilizer, medicine and combating pollution (Jeyachitra *et al.*, 2013), similar to diatoms (Munir *et al.*, 2012). Besides, the species of *C. dispersus* also show antimicrobial activity (Ghasemi *et al.*, 2007) and contain more than two hundred type II restriction endonucleases (Lyra *et al.*, 2000).

There is great morphological diversity among the members of family Chroococcaceae. Their diversity and occurrence in the ponds, puddles, water channels from all provinces of Pakistan was studied (Ghose, 1919, 1924; Faridi, 1970, 1971; Ali & Sandhu, 1972; Masud-ul-Hassan, 1978a, b; Farzana & Nizamuddin, 1979; Leghari & Arbani, 1983, 1984; Shameel & Butt 1984; Leghari *et al.*, 2000, 2001; Naz *et al.*, 2003, 2004, Janjua *et al.*, 2009). However, members of Chroococcaceae were investigated rarely from the Pakistani Lakes, including Manchar Lake (Mahar *et al.*, 2009), Keenjhar Lake (Lashari *et al.*, 2014), Sonharo, Mehro Pateji and Cholari Lakes (Leghari *et al.*, 2000). All previous endeavors altogether have reported approximately 41 species of *Chroococcus* while this work contributes five new records to the existing list and also presents their geographical distribution.

Materials and Methods

The Kallar Kahar Lake (located between 32° 46' 30.31" North, 72° 42' 23.80" East in the District Chakwal, Punjab, Pakistan) was thoroughly explored in different seasons for the collection of *Chroococcus* species. Seven sites were selected to collect algal samples from surface

water as well as from different substrates present in the Lake. The opaque plastic bottles were used for sample collection, labeled and preserved with 3% formalin (Munir *et al.*, 2013). Each sample was homogenized using Homogenizer and one drop of sample was taken and placed on the glass slide, covered with glass cover slip and observed under Leica DMLB microscope®. Various objectives were used for the magnification of images. Microphotographs were captured at 100X objective and 10X eyepiece. The algal taxa were identified using algal floristic materials (Desikatcheri, 1959; Prescott, 1962; Komarek & Angnostidis, 1999).

Results and Discussion

Taxonomic evaluation of the collected material indicated the presence of 18 species of *Chroococcus*. Their taxonomic descriptions and systematic treatment is as follows:

Chroococcus Naegeli 1849

Single or colony of 2-32 cells, free floating or attached to various substrates, with a common mucilage, hyaline or colored; ovate, spherical or hemispherical; each cell has an individual envelope; cell content granular; vacuole absent.

1. *C. varius* A. Braun in Rabenhorst 1861, (Plate 1: Fig. P)

Source of identification: Komarek & Angnostidis, 1999, p. 293, Plate 1: Fig. 387

General characters: Cell diameter 2.7 µm; colonized with single cell or group of 2-4 cells with individual lamellate or homogenous envelope; cell pale blue green. Colonies microscopic or large, macroscopic, slimy or gelatinous, colorless and dirty olive-green or brownish.

Habitat/ Occurrence: It was collected from the Lake growing as metaphyton during October 2011.

Geographical distribution: Spain (Cuzman *et al.*, 2010: 92), China (Liu, 2008), India (Samad & Adhikary, 2008: 97), Nepal (Rai *et al.*, 2010: 337) and Pakistan (Barkatullah *et al.*, 2013: 331; Naz *et al.*, 2004: 256; Husna *et al.*, 2005: 146; Leghari *et al.*, 2005: 155).

Key to species

- 1a. Colonies macroscopic:
 2a. Envelop indistinctly lamellate or homogeneous 1. *C. varius*
 2b. Envelop not lamellate:
 31. Cells in 2-8-celled groups 2. *C. cohaerens*
 3b. Cells in 2-4-celled groups:
 41. Envelopes narrow, sometimes diffluent 3. *C. limneticus*
 4b. Envelopes firm, delimited 4. *C. lithophilus*
 1b. Colonies microscopic:
 5a. Cells 2-4 in colony:
 6a. Diameter 13-27 μm 5. *C. westii*
 6b. Diameter 0.8-1 μm 6. *C. subtilissimus*
 7a. Cells spherical or slightly elongate, or hemispherical 7. *C. planctonicus*
 7b. Cells triangular or variable in shape 8. *C. polyedrififormis*
 8a. Cells blue green 9. *C. schizodermaticus*
 8b. Cells pale blue-green 10. *C. minor*
 5b. Cells 4-16-32 in colony:
 9a. Cells irregularly, sparsely or almost densely arranged 11. *C. dispersus*
 9b. Cells more or less regularly arranged 12. *C. minimus*
 10a. Aerate present one large, more or less central 13. *C. vacuolatus*
 10b. Aerate absent 14. *C. microscopicus*
 11a. Cells arrangement in cubic or sarcinoid clusters 15. *C. prescottii*
 11b. Cells arrangement typical chroococcoid 16. *C. thermalis*
 12a. Cell solitary or in microscopic colony, more or less spherical or oval 17. *C. minutus*
 12b. Cells rarely solitary, spherical or widely oval 18. *C. turgidus*

2. *C. cohaerens* (Brebisson) Nageli 1849, (Plate 1: Fig. C)

Source of Identification: Komarek & Angnostidis, 1999, P. 294, **Plate 1: Fig. 385**

Synonym: *Chroococcus bituminosus* (Bory) Hansgirg 1892
General characters: Cell length 1.7 μm , diameter 2.5 μm ; cells hemispherical, in 2-8-celled groups or forming micro- or macroscopic colonies, which are gelatinous, amorphous, dirty green; mucilage colorless; envelope not lamellate.

Habitat/ Occurrence: Specimen was collected from water surface in January 2011.

Geographical distribution: Britain (John *et al.*, 2011), Romania (Caraus, 2012: 524), China (Chu & Tiffany, 1951: 712), Pakistan (Hussain *et al.*, 1984: 82; Anjum *et al.*, 1986: 120; Sarim *et al.*, 1998: 118; Naz *et al.*, 2004: 256; Husna *et al.*, 2005: 144; Leghari *et al.*, 2006: 34) Bangladesh (Hasan, 2012: 186) and India (Nandan & Ahuja, 2010)

3. *C. limneticus* Lemmermann 1898, (Plate 1: Fig. K)

Source of identification: Komarek & Angnostidis, 1999, P. 291, **Plate 1: Fig. 382 j**

Synonym: *Chroococcus limneticus* var. *carneus* (chodat) Lemmermann 1904; *Gleocapsa limnetica* (Lemmermann) Hollerbach in Elenkin 1938; *Anacystis limnetica* (Lemmermann) Drouet *et* Daily 1952; *Anacystis thermalis* f. *major* (Lagerheim) Drouet *et* Daily 1956.

General characters: Cell diameter 5.9 μm ; cells spherical or hemispherical; envelop not laminate, narrow sometimes diffluent and indistinct. Colonies free floating, mucilaginous, spherical or irregular with 2-4 cells.

Habitat/ Occurrence: This species was collected from the lake as metaphyton during spring and summer seasons.

Geographical distribution: Portugal (Figueiredo *et al.*, 2006: 149); Egypt (El-Karim, 2008: 347), Turkey (Verol & Sen, 2014), Pakistan (Barkatullah *et al.*, 2013: 331; Mehwish & Aliya, 2005: 118; Leghari *et al.*, 2005: 39; Leghari *et al.*, 2005: 155; Leghari *et al.*, 2006: 34; Hussain *et al.*, 2008: 150; Anjum *et al.*, 1986: 120), India (Nandan & Ahuja, 2010: 133), Nepal (Rai *et al.*, 2010: 337) and Sri Lanka (Silva *et al.*, 2013: 96).

4. *C. lithophilus* Ercegovic 1925, (Plate 1: Fig. F)

Source of Identification: Komarek & Anagnostidis, 1999, P. 301, **Plate 1: Fig. 397**

General characters: Cell diameter 5 μm , hemispherical; envelope firm, delimited, thin, not lamellate and colorless; colonies micro or macroscopic, free floating, blue green.

Habitat/ Occurrence: Free floating colonies were collected from the water surface in July 2011.

Geographical distribution: Rome (Bellinzoni *et al.*, 2003: 205), China (Chu & Tiffany, 1951: 712), Pakistan (Munir *et al.*, 2008: 217) and India (Samad & Adhikary, 2008: 97).

5. *C. westii* Boye-Petersen 1929, (Plate 1: Fig. I)

Source of identification: Komarek & Angnostidis, 1999, P. 304, **Plate 1: Fig. 403**

General characters: Cell diameter 12.9-27 μm ; colony with 2-4 cells with individual concentrically lamellate envelope; cell hemispherical, blue green with granular content.

Habitat/ Occurrence: Collected from wet rocks in the Lake in summer.

Geographical distribution: Romania (Caraus, 2012: 18), Oman (Abed *et al.*, 2013: 1438), China (Zhang *et al.*, 2010: 183; 2011: 283) and Pakistan (Leghari *et al.*, 2005: 169).

6. *C. subtilissimus* Skuja 1937, (Plate 1: Fig. Q)

Source of identification: Komarek & Anagnostidis, 1999, P. 294, **Plate 1: Fig. 386**

General characters: Cell diameter 0.8 µm; irregular colony of 2-4 closely packed cells, mucilaginous; cells blue green, discoid, spherical or hemispherical; envelope not lamellate.

Habitat/ Occurrence: *C. subtilissimus* was collected from the submerged rocks in the Lake.

Geographical distribution: Greece (Komarek & Anagnostidis, 1999: 293).

7. *C. planctonicus* Bethge 1935 (Plate 1: Fig. A)

Source of identification: Komarek & Anagnostidis, 1999, P. 285, **Plate 1: Fig. 372**

General characters: Cell diameter 4.4 µm, cells in group of 2 or 4, spherical or slightly elongate or hemispherical, round, pale blue green and with aerotopes; individual cell envelope absent.

Habitat/ Occurrence: *C. planctonicus* occurred as epiphyte, lithophytes and metaphyton during winter and summer.

Geographical distribution: Argentina: (Navarro & Modenutti, 2012: 192), Clipperton Island (Charpy *et al.*, 2010: 777).

8. *C. polyedrififormis* Schmidle 1902, (Plate 1: Fig. M)

Source of identification: Komarek & Anagnostidis, 1999, P. 309, **Plate 1: Fig. 411**

Synonym: *Chroococcus multicellularis* (Chu) Chu 1952

General characters: Cell length 4.2 µm, diameter 2.4 µm; colony microscopic, free floating, cell triangular or variable in shape enclosed in an envelope and blue green.

Habitat/ Occurrence: The cells of this Cynaobacterium were collected from lake among other filamentous algae as metaphyton during summer.

Distribution: Australia and New Zealand, (Bostock & Holland, 2010: 257), Mexico (Komarek & Novelo, 1994: 3).

9. *C. schizodermaticus* W. & G. S. West 1892, (Plate 1: Fig. R)

Source of identification: Komarek & Anagnostidis, 1999, P. 299, **Plate 1: Fig. 395**

General characters: Cell diameter 11.4 µm diameter; colony of 2-4 cells, gelatinous; each cell envelope lamellate; cell irregular round, spherical or hemispherical and blue green.

Habitat/ Occurrence: During the summer it was collected from the rocks forming a mat.

Geographical distribution: Caribbean (Rejmankov & Komarkov, 2000: 141), China (Chu & Tiffany, 1951: 712), East Nepal (Rai & Misra, 2010: 124), Nepal (Rai *et al.*, 2010: 337), India (Samad & Adhikary, 2008: 98), Azad Jammu & Kashmir (Leghari *et al.*, 2007: 31).

10. *C. minor* (Kützinger) Nageli 1849, (Plate 1: Fig. E)

Source of identification: Komarek & Anagnostidis, 1999, P. 294, **Plate 1: Fig. 388**

General characters: Cell diameter 4.1 µm; colony microscopic 2-4 celled, irregular, mucilage wide; Cells spherical or hemispherical and pale blue-green; envelop scarcely visible.

Habitat/ Occurrence: This metaphyton was collected during January.

Geographical distribution: Saudi Arabia (El-Naggar, 1994: 205), Korea (Park, 2012: 10), China (Chu & Tiffany, 1951: 712), India (Mongra, 2013: 926), Nepal (Rai *et al.*, 2010: 337), Pakistan (Barkatullah *et al.*, 2013: 331; Leghari *et al.*, 2005: 39; Leghari *et al.*, 2006: 34) and AJK (Khuhawar *et al.*, 2009: 1910).

11. *C. dispersus* (Keissl.) Lemmermann 1904, (Plate 1: Fig. D)

Source of identification: Komarek & Anagnostidis, 1999, P. 286, **Plate 1: Fig. 373**

Synonym: *Chroococcus limneticus* var. *subsalsus* Lemmermann 1901; *C. minor* var. *dispersus* Keissl. 1902; *Gleocapsa minor* f. *dispersa* (Keissler) Hollerbach in Elenkin 1938.

General characters: Cell diameter 3.1 µm, hemispherical; colony microscopic, free floating and 4 celled; common mucilage distinct, irregularly, sparsely or almost densely arranged.

Habitat/ Occurrence: Found as planktonic, lithophytes and epiphytic on *Cladophora* sp. during May 2011.

Geographical distribution: Europe, America (Munawar *et al.*, 2009: 1016), China (Shams *et al.*, 2012: 721; Chu and Tiffany, 1951: 712), Egypt (El-Sheekh *et al.*, 2010: 2626), Pakistan (Mehwish & Aliya, 2005: 118; Leghari *et al.*, 2005: 155; Leghari *et al.*, 2006: 34; Mehwish & Aliya, 2006: 150), Bangladesh (Khondker *et al.*, 2006) and Sri Lanka (Pathmalala & Piyasiri, 1995: 33).

12. *C. minimus* (Keissler) Lemmermann 1904, (Plate 1: Fig. L)

Source of identification: Komarek & Anagnostidis, 1999, P. 285, **Plate 1: Fig. 371**

Synonym: *Chroococcus minutus* var. *minimus* Keissler 1901; *C. dispersus* var. *minor* G. M. Smith 1920; *Gleocapsa minima* (Keissler) Hollerbach in Elenkin 1938; *Microcystis chroococcoidea* var. *minor* Nygaard 1949; *Gleocapsa minima* f. *smithii* Hollerbach *et al.*, 1953.

General characters: Cell diameter 2.8 µm, cell spherical or irregular, 2- many cells together; colony free floating irregular or spherical; aerotopes absent.

Habitat/ Occurrence: It was collected from stagnant water as epiphyte on *Cladophora* and metaphyton throughout the year.

Geographical distribution: Korea (Park, 2012), Romania (Caraus, 2012), India (Saha *et al.*, 2007: 219), Azad Jammu and Kashmir (Khuhawar *et al.*, 2009: 1910) and Pakistan (Leghari *et al.*, 2006: 34).

13. *C. vacuolatus* Skuja 1939, (Plate 1: Fig. S)

Source of identification: Komarek & Anagnostidis, 1999, P. 285, **Plate 1: Fig. 370**

Synonym: *Gleocapsa vacuolata* (Skuja) Hollerbach et al., 1953

General characters: Cell diameter 2.5 µm, spherical or hemispherical cells, yellow blue green; colony microscopic, irregular, mucilaginous with group of 4-16-32 cells; individual cell envelop present; aerotop present one large, more or less central.

Habitat/ Occurrence: *C. vacuolata* appeared in January as planktonic species in stagnant water along with *Phragmites karka*.

Geographical distribution: Korea (Park, 2012: 10), Romania (Caraus, 2012: 525).

14. *C. microscopicus* Komarkova-Legnerova et Cronberg, (Plate 1: Fig. J)

Source of identification: Komarek & Anagnostidis, 1999, P. 285, Plate 1: Fig. 367

General characters: Colonies microscopic, free floating irregular or spherical, pale blue green; Cell diameter 1 µm; cell spherical or irregular, 2-many cells together, sheath distinct, aerotopes absent; mucilage colorless and delicate.

Habitat/ Occurrence: *C. microscopicus* was found growing as metaphyton year round in the Lake.

Geographical distribution: Mostly distributed in the mesotrophic lakes, brackish bay and sea from Australia (Jahnert & Collins, 2011: 108), Korea (Park, 2012: 10), Romania (Caraus, 2012), Australia and New Zealand (Bostock & Holland, 2010: 257).

15. *C. prescottii* Drouet & Daily 1942, (Plate 1: Fig. B)

Source of identification: Komarek & Anagnostidis, 1999, P. 288, Plate 1: Fig. 379

Synonym: *Chroococcus gomontii* Nygaard sensu auctorum; *Eucapsis alpina* Sieminska 1967

General characters: Colony microscopic, free floating, irregular, oval to cylindrical or almost subspherical; cells enclosed in delimited envelope, often slightly lamellate; cell spherical or hemispherical with granular content, bright blue green, arranged in cubic or sarcinoid clusters; Cell diameter 3.8 µm.

Habitat/ Occurrence: This Cyanobacterium appeared in winter and summer as planktonic species.

Geographical distribution: Argentine (Mataloni & Tell, 1996: 103), Korea (Park, 2012: 10) and Pakistan (Leghari et al., 2009: 157).

16. *C. thermalis* (Meneghini) Nageli 1849, (Plate 1: Fig. H)

Source of identification: Komarek & Anagnostidis, 1999, P. 303, Plate 1: Fig. 401

Synonym: *Chroococcus turgidus* sensu auct.; *Pleurococcus thermalis* Meneghini 1837; *Protococcus thermalis* (Meneghini) Kutzing 1846; *Chroococcus turgidus* var. *thermalis* (Meneghini) Rabenhorst ex Hansgirg 1892; *Chroococcus yellowstonensis* Copeland 1936.

General characters: Cell diameter 9.4 µm; colony of 2, 3 or 4 cells with colorless indistinct mucilage; cells round, hemispherical, blue green with granular content, cell arrangement typical chroococcoid.

Habitat/ Occurrence: This species was collected from submerged rocks in July among other cyanobacteria.

Distribution: Australian thermal spring (McGregor & Rasmussen, 2007: 28), India (Patil & Patil, 2014: 511) and Pakistan (Leghari et al., 2005: 169).

17. *C. minutus* (Kutzing) Nageli 1849, (Plate 1: Fig. N, O)

Source of identification: Komarek & Anagnostidis, 1999, P. 297, Plate 1: Fig. 391

Synonym: *Protococcus minutus* Kutzing Kutzing 1843; *Chroococcus virescens* Hantzsch in Rabenhorst 1865; *Gleocapsa minuta* (Kutzing) Hollerbach in Elenkin 1938.

General characters: Cell diameter 9.6 µm; solitary or form microscopic colony; cell spherical, oval or hemispherical, yellow blue green with granular content, enclosed by a wide and distinct margin and granular content.

Habitat/ Occurrence: This species was collected throughout the year in 2011 growing as metaphyton.

Geographical distribution: Spain (Cuzman et al., 2010: 92), Portugal (Figueiredo et al., 2006: 149), Korea (Park, 2012: 10), Saudi Arabia (El-Naggar, 1994: 205), Egypt: (El-Sheekh et al., 2010: 2626), Turkey (Verol & Sen, 2014), China (Chu & Tiffany, 1951: 712), India (Choudhary, 2009: 59), Nepal (Rai et al., 2010: 337), Pakistan (Naz et al., 2004: 256; Leghari et al., 2005: 39; Husna et al., 2005: 144; Leghari et al., 2006: 34; Gul et al., 2007: 39; Hussain et al., 2008: 150; Munir et al., 2008: 217) and AJK (Khuhawar et al., 2009: 1910).

18. *C. turgidus* (Kutzing) Naegeli 1849 (Plate 1: Fig. G)

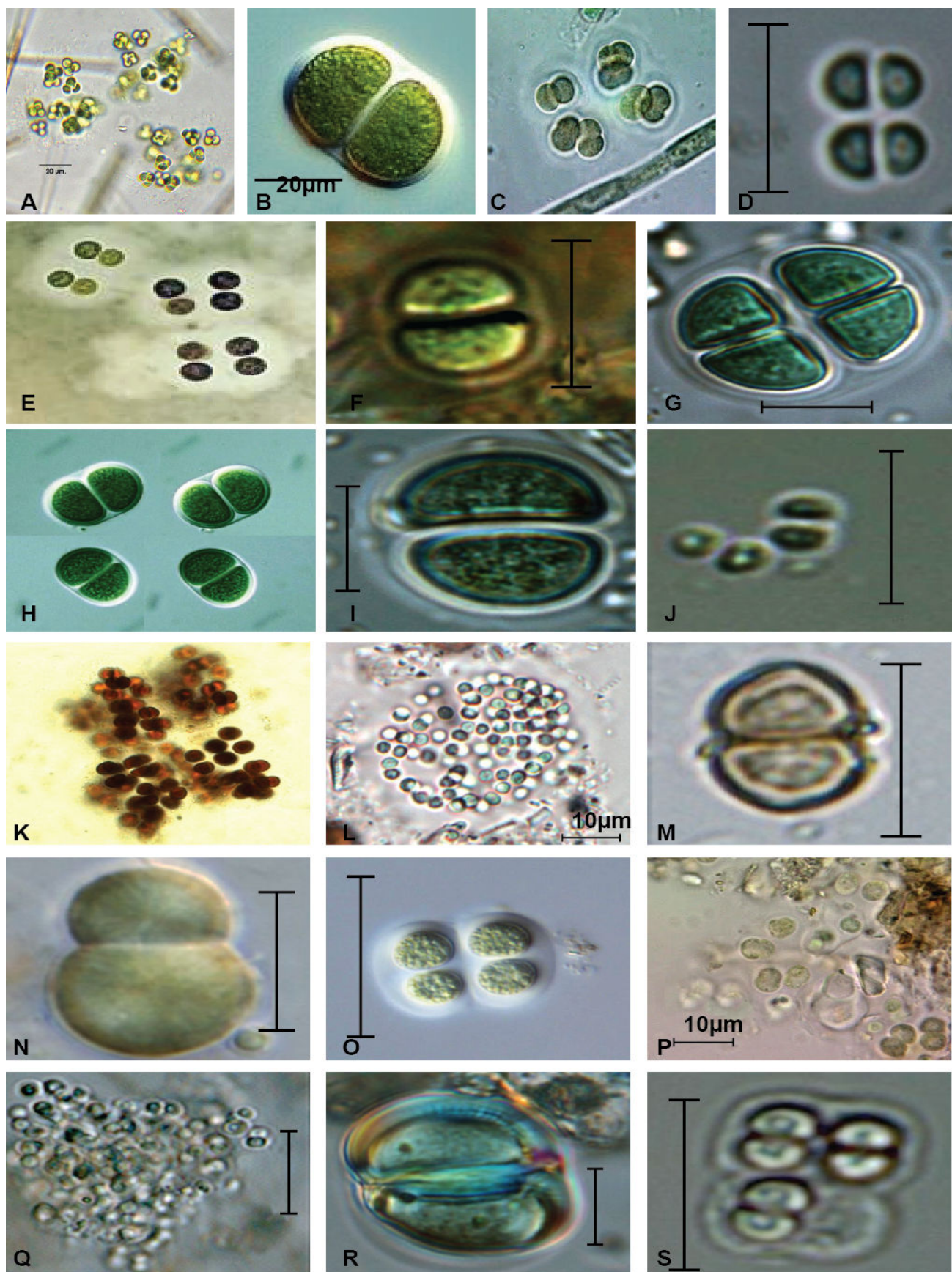
Source of identification: Komarek & Anagnostidis, 1999, P. 307, Plate 1: Fig. 407

Synonym: *Chroococcus dimidiatus* (Kutzing) Nageli 1849; *Anacystis dimidiatus* (Kutzing) Drouet & Daily 1952; *Gleocapsa turgida* (Kutzing) Hollerbach in Elenkin 1938; *Chroococcus turgidus* var. *maximus* Nygaard 1926.

General characters: Cell diameter 7.3 µm; colony of 2-8 cells, colorless, mucilaginous envelope around each cell or group of cells; individual cell lamellate; cell oval, hemispherical or segment of a sphere; dark blue green with granular content.

Habitat/ Occurrence: It was found as metaphyton with a lot of vegetation in stagnant lake water during summer and autumn months.

Geographical distribution: Europe: Spain (Cuzman et al., 2010: 92), Portugal (Figueiredo et al., 2006: 149); Korea (Park, 2012: 10), Turkey (Verol & Sen, 2014); China (Chu & Tiffany, 1951: 712), India (Mahajan & Mahajan, 1988: 50), Nepal (Rai et al., 2010: 337), Pakistan (Naz et al., 2004: 256; Husna et al., 2005: 146; Mehwish & Aliya, 2005: 118; Leghari et al., 2005a: 39; Leghari et al., 2005b: 155; Leghari et al., 2006: 34; Mehwish & Aliya, 2006: 150; Anjum et al., 1986: 120) and AJK (Khuhawar et al., 2009: 1910).



A. *Chroococcus planctonicus*, B. *Chroococcus prescottii*, C. *Chroococcus cohaerens*, D. *Chroococcus disperses*, E. *Chroococcus minor*, F. *Chroococcus lithophilus*, G. *Chroococcus turgidus*, H. *Chroococcus thermalis*, I. *Chroococcus westii*, J. *Chroococcus microscopicus*, K. *Chroococcus limneticus*, L. *Chroococcus minimus*, M. *Chroococcus polyedriformis*, N. *Chroococcus minutus*, O. *Chroococcus minutus*, P. *Chroococcus varius*, Q. *Chroococcus subtilissimus*, R. *Chroococcus schizodrmaticus*, S. *Chroococcus vacuolatus*, Magnification bar = 10µm

Plate 1. Microphotographs of *Chroococcus* species recorded from the Kallar Kahar Lake, Chakwal.

Conclusion

The *Chroococcus* species inhabit in fresh, brackish and marine water in tropical and temperate regions across the world due to their broad ecological amplitude and preference for a variety of habitats. This study recorded 18 *Chroococcus* species, including 5 new records viz., *C. microscopicus*, *C. planctonicus*, *C. polyedrififormis*, *C. subtilissimus* and *C. vacuolatus* for Pakistan and South Asian countries. Molecular studies are recommended to further ascertain its taxonomic complexities (Afzal *et al.*, 2015).

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