

## NEW DISTRIBUTIONAL RECORD OF THREE COCCAL GREEN ALGAE (CHLOROCOCCALES, CHLOROPHYCEAE) FROM THE ALPINE LAKES OF EASTERN HIMALAYAS, INDIA

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### INTRODUCTION

The Himalayas are the highest mountain ranges in the world. The Indian Eastern Himalayas located between 20°27' - 29°30' N latitudes and 87°59' - 97°30' E longitudes, include mainly the states of Arunachal Pradesh, Sikkim and Darjeeling of West Bengal, covering total area of 93,984 sq. km.. Vegetation of this region is mostly sub-alpine to alpine type with bountiful of lakes and other lotic freshwater bodies. Though being regarded as a biodiversity hot spot by Conservation International, the algal documentation of these area is scanty. Chlorococcalean algae from this region were only recorded by Bhakta & al. (2010), Das and Keshri (2012) and Das and Adhikary (2012, 2014).

In the present work three coccal green algal species, i.e. *Chlorococcum ellipsoideum*, *Neosporangiococcum gelatinosum* and *Dictyochloropsis splendida*, belonging to order Chlorococcales and class Chlorophyceae were documented from alpine lakes of eastern Himalayas, as first report in India.

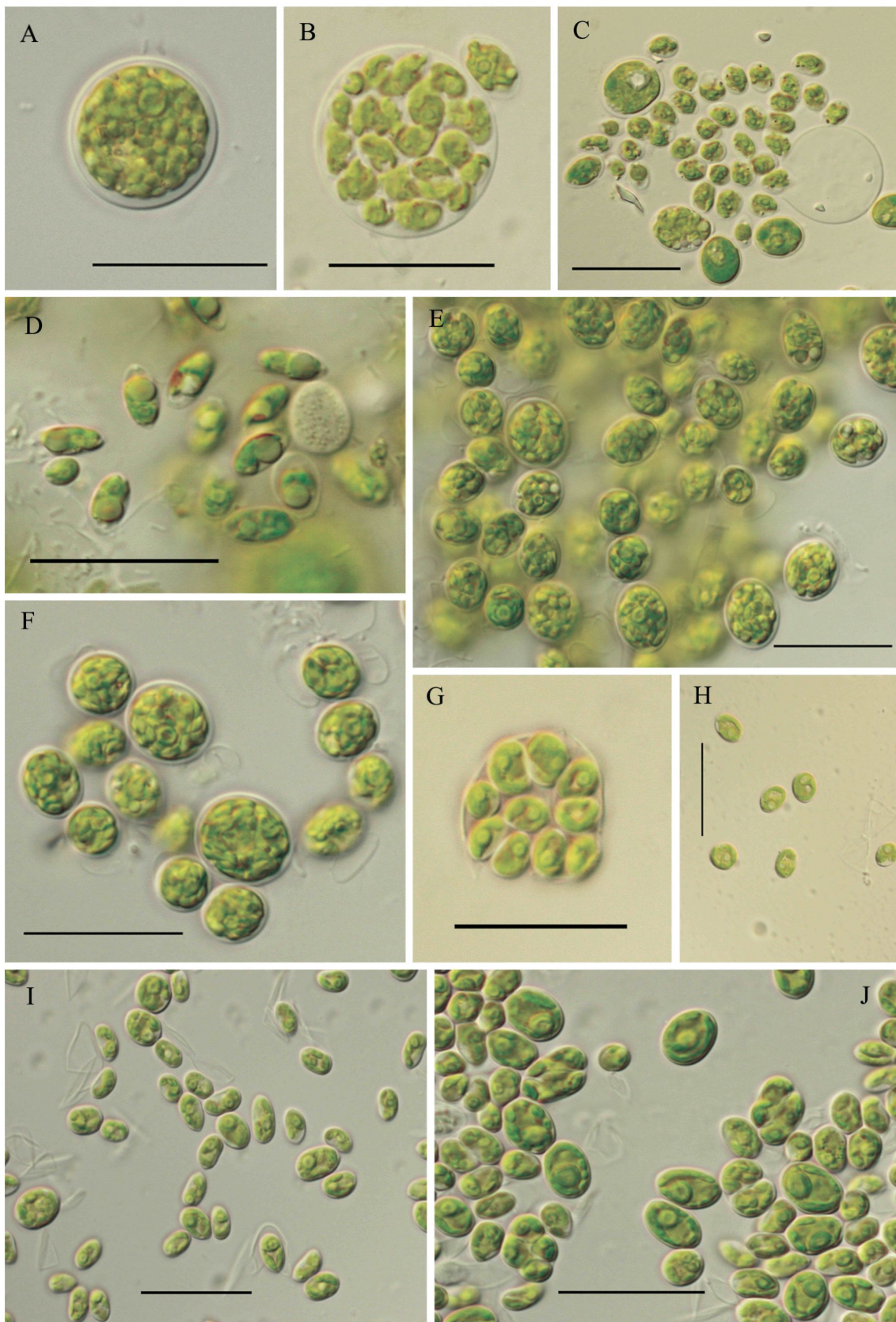
### MATERIALS AND METHODS

The coccal green algae were collected from the lakes of eastern Himalayas during several collection trips to Sikkim and Arunachal Pradesh, between October, 2010 and May, 2011. Samples were collected from the submerged rock surfaces using scalpel and tooth brush and from moist soils in the bank of the lake. A batch of the samples was assigned with a voucher number with date of collection, fixed on the spot by 4 % formaldehyde solution and deposited at Department of Biotechnology, Visva Bharati, Santiniketan. The GPS data of the collection sites as well as physico-chemical parameters of the water bodies like, pH, temperature, conductivity were noted on the spot. The coccal algae were identified and studied by isolating to pure cultures at Department of Botany, Comenius University in Bratislava, Slovakia. The cultures were grown and maintained in Petri dishes on agar plates as well as in test tubes in liquid Z medium according to Zehnder in Staub (1961) at 20°C temperature. Illumination up to 48.6  $\mu\text{mol. m}^{-2}.\text{s}^{-2}$  was provided by cool fluorescent lamps. The phenotypic characteristics were studied in regular intervals at different growth phases of the algae. Microphotography were made using the Leica DM 2500 optical microscope with Nomarski contrast, fitted with a Leica DFC 290 HD camera and operated by LAS 3.5.0. Identification of the taxa was followed by Ettl and Gärtner (1995) and Komárek and Fott (1983). All the strains were deposited at the Culture Collection of Algae at the Laboratory of Algology (CCALA), Trebon, Czech Republic as well as in the Department of Biotechnology, Visva Bharati, Santiniketan, West Bengal.

### RESULTS AND DISCUSSION

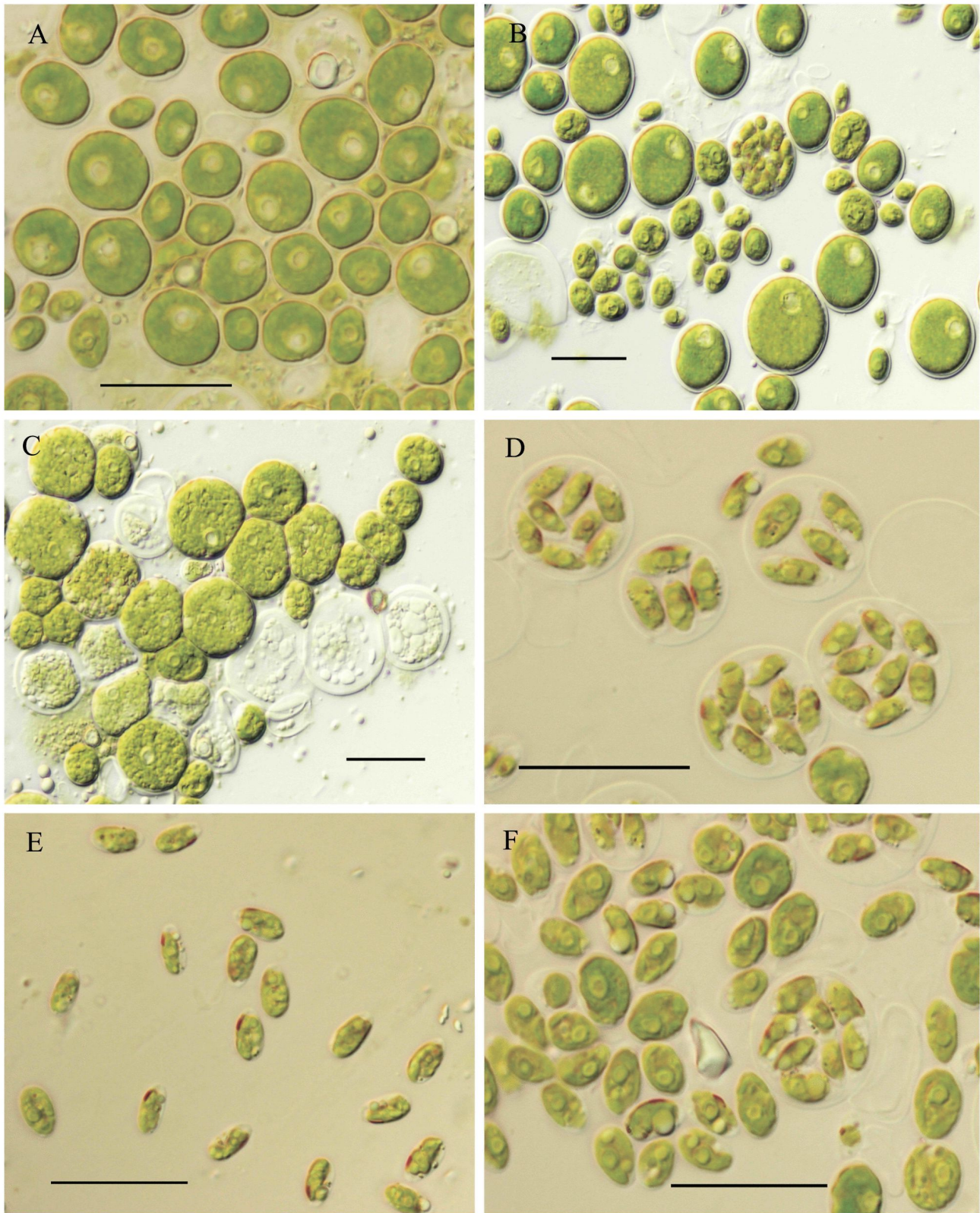
***Chlorococcum ellipsoideum* Deason & H.C. Bold in Univ. Texas Publ. 6022: 20, fig. 20-25, 90-92.1960  
(Fig. 1 A-F)**

Cells solitary, sometimes in group, spherical, 13 – 20  $\mu\text{m}$  in diameter (Fig. 1 A), younger cells irregular in shape (Fig. 1 E-F), 4.5 – 8  $\mu\text{m}$  in diameter; chloroplast entire, plate like, pyrenoid one, indistinct, sometimes



**Fig. 1:** Morphological features of *Chlorococcum ellipsoideum* (A-F) – A. single cell; B-C. formation and dispersion of zoospores; D. biflagellated zoospores; E-F. young cells; Morphological features of *Dictyochloropsis splendida* (G-J); G-I. Development, dispersal and morphology of zoospores; J. young cells. (Scale bar for Fig. 1 A-J = 20 μm)





**Fig. 2:** Morphological features of *Neospongiococcum gelatinosum*: **A-B.** broadly elliptical younger cells; **C.** spherical mature cells; **D-E.** formation of zoospores.; **F.** cellular development of zoospores. (Scale bar for Fig. 1 A-F = 20  $\mu$ m)

observed in mature cells; zoospores biflagellated, 8-16-32 zoospores formed in a single wall (Fig. B-D), with a central vacuole, 3–4 µm long and 1.5–2 µm broad.

*Habitat:* attached to submerged rocks in the periphery of the lake (pH – 6.4, conductivity – 77 µS and temperature – 7 °C).

*Specimen examined:* India, Sikkim, East Sikkim district, Manshu lake, 27°22.599' N, 88°48.344' E, c. 4477 m, S.K. Das, voucher no. 4117.

*Reference strains:* The cultured strain of the alga was deposited at CCALA and Visva Bharati as strain number Sudipta 2012/9.

**Neospongiococcum gelatinosum** (P.A. Archibald & H.C. Bold) H. Ettl & G. Gärtner in Nova Hedwigia 44: 513. 1987. *Chlorococcum gelatinosum* P.A. Archibald & H.C. Bold in Univ. Texas Publ. 7015: 29, fig. 19, 55. 1970. (Fig. 2 A-F)

Cells are oval to broadly elliptical when younger and almost spherical in mature stage (Fig. 2 A-C), younger cells 2.8 – 5.5 µm long and 2.5 – 5 µm broad, matures cells are up to 12 – 16 µm in diameter; cell wall is smooth, colourless and thickened, distinctly visible in older cells (Fig. 2 B-C); the chloroplast in mature cell is entire, compact, with one clearly visible pyrenoid, small perforations or incisions may appear to give a sponge like appearance (Fig. 2 C), in younger cells the chloroplast is more or less cup like (Fig. 2 B), parietal, with one pyrenoid; zoospores are elliptical, biflagellated (Fig. 2 E-F), 4-8-16 zoospores are formed in a single wall (Fig. 2 D), 3–4 µm long and 2.2–2.8 µm broad.

*Habitat:* attached to small rocks and pebbles in the periphery of the lake (pH – 6.5, conductivity – 38 µS and temperature – 5.5 °C).

*Specimen examined:* India, Arunachal Pradesh, Tawang district, Paradise lake, 27°38.278' N, 91°51.422' E, c. 3930 m, 29.10.2010, S.K. Das, voucher no. 2692.

*Reference strains:* The cultured strain of the alga was deposited at CCALA and Visva Bharati as strain number Sudipta 2012/14.

**Dictyochloropsis splendida** Geitler in Oesterr. Bot. Z. 113: 162, fig. 1-4, 1966. (Fig. 1 G-J)

Cells broadly elliptical to kidney shaped and irregularly spherical in mature form; 4.2 – 4.8 µm long and 2 – 2.5 µm broad, mature ones about 6 – 8 µm in diameter (Fig 1 G-I); cell wall is thin, smooth, with slight mucilage only distinct in mature cells; chloroplasts preperhal, irregular; pyrenoid one, always present towards one end (Fig. 1 J); biflagellated zoospores rare.

*Habitat:* on moist soils in the periphery of the lake (pH – 6.3, conductivity – 17 µS and temperature – 6 °C).

*Specimen examined:* India, Arunachal Pradesh, Tawang district, Nagula lake, 27°39.251' N, 91°51.779' E, c. 4147 m, 30.10.2010, S.K. Das, voucher no. 2731.

*Reference strains:* The cultured strain of the alga was deposited at CCALA and Visva Bharati as strain number Sudipta 2012/13.

*Notes :* The three newly reported coccal green algae were mostly confined to the semi-aquatic soil surface in the lake periphery (*Dictyochloropsis splendida*) or stone/rock surfaces fully or partially submerged in lake water (*Chlorococcum ellipsoideum*, *Neospongiococcum gelatinosum*) though their earlier distributional records from temperate countries were mostly from terrestrial habitats (Ettl & Gärtner, 1995). This documentation is a significant addition to the least explored phyco-flora of the eastern Himalayas.

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