New records of green-algae (chlorophyceae) from the Punjab state of India

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Abstract
This study is the first report on Chlorophyceae members at species level from Ropar wetland (Ramsar site), Punjab (India). During this study, the algal samples were collected from different locations of the wetland, and were identified on the basis of their morphological characteristics. A total of 16 species of 14 genera (Ankistrodesmus, Cladophora, Coelastrum, Cylindrocapsa, Golenkinia, Gonium, Hydrodictyon, Micractinium, Monoraphidium, Oedogonium, Pediastrum, Scenedesmus, Spirogyra and Tetraedron) belonging to 6 orders of 10 families of the class Chlorophyceae were recorded from the study area. All the members of Chlorophyceae documented during this study at species level, except Cylindrocapsa geminella are new records for Punjab state of India.

Keywords: Algae, Chlorophyceae, new records, Punjab, Ropar wetland.

Introduction
Algae are oxygenic-photosynthetic organisms, including both prokaryotic as well as eukaryotic forms, and are considered as first autotrophic plants on earth (Lone et al. 2017, Khalil et al. 2021). Algae are diverse and highly specialized group of varied micro- & macro-organisms in terms of size, shape, color, structure and habitat, and are adapted to wide range of ecological conditions. In any aquatic ecosystem, algae are primary producers and play an important role in aquatic food chain, on which fishes and zooplanktons depend for their food (Severes et al. 2018). Algae are one of the significant organisms which help CO₂ sequestration and water purification, due to their ability to absorb various organic and inorganic pollutants along with heavy metals (Khalil et al. 2021). On the basis of their habitat, the freshwater algae can be categorized into two groups: lotic (growing in flowing water) and lentic (growing in stagnant water). The lotic system includes streams, rivulets, rivers, waterfalls whereas, lentic system comprises small ponds, ditches, lakes, puddles etc. All the members of algae are not fresh water algae, but members of Cyanophyta, Chlorophyta, Charophyta and Bacillariophyta display their high diversity and abundance in fresh water (Leliaert et al. 2012, Whitton 2012, Sharma et al. 2018).

Chlorophyceae is one of the ecologically diverse and largest assemblages of algae found to grow abundantly in lakes, ponds, rivers and streams etc. (Rai et al. 2020). This group comprises eukaryotic, motile or non-motile organisms occurring in different forms as solitary unicells, colonies or in filamentous forms. Chlorophycean members are found in all types of habitats including aquatic, terrestrial, subaerial, endophytic and symbiotic, but some algae may have different ecological preferences (Leliaert et al. 2012). The distribution of Chlorophyceae in India is very wide, and thousands of species are recorded from different water bodies across different regions of the country (Anand 1998, Kant and Gupta 1998, Sau and Gupta 2005, Jena and Adhikary 2007, Mallick and Keshri 2008). Therefore, very comprehensive work has been done on occurrence and distribution Chlorophyceae around the country.

From time to time members of algae have also been reported from different habitats of Punjab including, lakes, rivers, wetlands, ponds, ditches, puddles and paddy fields (Rattan 1963a-c, 1964a, b, 1967, 1968a,b, 1971,1989;Sharma et al. 2013, Prabha and Dua 2018, Komal et al. 2021) and new algal species are reported every time. It indicates that knowledge about the diversity and distribution of...
Chlorophyceae in Punjab is not fully known and remains unexplored. In the present study, an attempt has been made to study the diversity and distribution of Chlorophyceae for the addition of more knowledge to algal flora of Punjab region of India.

Materials and Methods

Collection and Identification of algal samples
Algal samples were collected from the Ropar wetland (Ramsar site) located at 30° 57’- 31° 06’ North latitude and 76° 25’- 76° 36’ East longitude in Roopnagar district of Punjab state of India during 2018 and 2020 (Figure 1). The photographs of some habitats of algae of the collection sites are shown in Figure 2. All collected samples were preserved in 4% formalin solution on spot during collection and were brought to the laboratory for further analysis. Each sample was assigned with a particular herbarium number (WUA) of Sri Guru Granth Sahib World University. The temporary slides were prepared from collected algal samples and observed under Olympus CH20i microscope at 40X and 100X using immersion oil. The algal representatives were identified on the basis of morphological features viz. shape of cell, size, number of chloroplasts, number and arrangement of pyrenoids, spines etc. by following standard literatures (West 1892, Prescott 1951, Philipose 1967, Prasad and Misra, 1992, Yamagishi 2010).

Results

During the present study a total of 16 members of chlorophyceae belonging to 14 genera (Micractinium, Gonium, Ankistrodesmus, Monoraphidium, Tetraedron, Hydrodictyon, Pediastrum, Scenedesmus, Coelastrum, Golenkinia, Cylindrocapsa, Spirogyra, Cladophora and Oedogonium) of 6 orders (Chlamydomonadales, Chlorellales, Cladophorales, Oedogoniales, Sphaeropleales and Zygamenales) of class Chlorophyceae were recorded. Out of 16 chlorophycean taxa, fifteen are new reports for Ropar wetland as well for the Punjab state of India. The microphotographs of recorded species are shown in Figures 3 and 4.

Order: Chlorellales
Family: Chlorellaceae
Micractinium pusillum Fresenius 1858: 236, pl. XI [11]: figs 46-49
Plate 1 (a)

Description
Colonies 4-8 celled with triangular shape, easily collapsed into uni-cells; cell diameter 4-8µm, setae 30-50µm in length; cells spherical; spines attenuated towards tip, 2-7 in number; cup-shaped chloroplast having one pyrenoid. Collection No.: WUA 12, WUA 19, WUA 27, WUA 52 and WUA 55.

Order: Chlamydomonadales
Family: Goniaceae
Gonium pectorale O.F. Müller 1773: 60
Plate 1 (b)

Description
Cells in colonies; embedded with gelatinous sheath; 16-32-celled colonies with octagonal shape, with 4 central, 10 intermediate and 18 peripheral cells, cells 15-19µm in length, 10-12 µm in breadth; almost globose; embedded in gelatinous sheath;

Distribution

Order: Sphaeropleales
Family: Selenastraceae
(i) Ankistrodesmus falcatus (Corda) Ralfs 1848: 180, pl. XXXIV [34]: figs a-c
Plate 1 (c)

Description
Cells 10-20µm in length, 1.5-2.0 µm in breadth; acute to hardly lanceolar; tapering to acuteapices; typically in 4-8
(ii) Monoraphidium contortum (Thuret) Komárková-Legnerová in Fott 1969: 104, pl. 18: figs 1-5
Plate 1 (d)

Description
Cells 25-40µm in length, 3-6µm in breadth; extended, solitary and fusiform; almost straight to somewhat slender; sigmoid; thin cell wall; cell ends pointed; single chloroplast having no distinct pyrenoids.

Collection No.: WUA 12, WUA 19, WUA 27, WUA 52 and WUA 55.

Distribution
Eastern and North-eastern States of India (Jena and Adhikary 2007), Odisha (Behera et al. 2020).

Order: Sphaeropleales
Family: Hydrodictyaceae

(i) Tetraedron caudatum (Corda) Hansgirg 1888: 131
Plate 1 (e)

Description
Cell 12-14µm in diameter; small, cells having five sides, four sides concave whereas fifth forms the notch of varied depth; having rounded angles that produces short and fine spines.

Collection No.: WUA 07, WUA 15, WUA 24, WUA 27 and WUA 32.

Distribution
Bhopal (Bhat et al. 2015), Karnataka (Gupta 2012), Andhra Pradesh (Mallikarjuna et al. 2019).

(ii) Hydrodictyon reticulatum (Linnaeus) Bory 1824: 506
Plate 1 (f)

Description
Cells 51.6-54.4µm in length, 9.3-10µm in breadth; extended; tubular; coenocytic; 6 cells together interconnected at their ending walls to form polygonal network; cell wall smooth; reticulate chloroplast with numerous pyrenoids.

Collection No.: WUA 06, WUA 17, WUA 25 and WUA 42.

Distribution
Eastern and North-eastern States of India (Jena and Adhikary 2007), Maharashtra (Patil et al. 2012), Meghalaya (Kalita et al. 2015), Rajasthan (Meena 2017).

(iii) Pediastrum tetras (Ehrenberg) Ralfs 1845: 469
Plate 1 (g)

Description
Cells in colonies; colonies without perforations; marginal cells 6-14µm in length, 6-15µm in breadth; marginal cells of basal parts triangular to trapezoidal; 2 triangular processes

Figure 3: Micro-photographs of green algal species identified from study area. (a): Micractinium pusillum; (b): Gonium pectorale; (c): Ankistrodesmus falcatus; (d): Monoraphidium contortum; (e): Tetraedron caudatum; (f): Hydrodictyon reticulum; (g): Pediastrum tetras; (h): Pediastrum araneosum; (i): Scenedesmus acuminatus (Scale= 10 µm)
produce deep incision; cell walls smooth; reticulate chloroplast with single pyrenoid.

**Collection No.** WUA 10, WUA 18, WUA 24, WUA 33 and WUA 52.

**Distribution**
Bhopal (Bhat et al. 2015), Eastern and North-eastern States of India (Jena and Adhikary 2007), Rajasthan (Meena 2017).

(iv) **Pediastrum araneosum** (Raciborski) G. M. Smith 1916: 476
Plate 1 (h)

**Description**
Cells in colonies: colonies with slight perforations; cells 17-23µm in length, marginal cells flat at the basal portion, ranged from quadrate to trapezoid in shape; development of hollow incision due to two opposite short lobes; inner cells hexagonal to trapezoid in shape; minute undulation in the cell walls due to rough reticulated edges; net like chloroplast with one large pyrenoid.

**Collection No.** WUA 09, WUA 16, WUA 25 and WUA 43.

**Distribution**
Jammu and Kashmir (Kant and Gupta, 1998), Karnataka (Suxena 1984), Tamil Nadu (Philipose 1967), West Bengal (Mallick and Keshri 2008).

**Order:** Sphaeropleales

**Family:** Scenedesmaceae

(i) **Scenedesmus acuminatus** (Lagerheim) Chodat 1902: 211
Plate 1 (i)

**Description**
Cells 10-15µm in length, 2-7µm in breadth; bowed and fusiform; arranged linearly in a series of 4-celled or in 8-celled colony by contacting with the side; smooth cell wall; plate like chloroplast with parietal position having single pyrenoid.

**Collection No.** WUA 12, WUA 19, WUA 27, WUA 52, WUA 97 and WUA 107.

**Distribution**

(ii) **Coelastrum proboscideum** Bohlin in Wittrock, Nordstedt and Lagerheim 1896: no. 1240
Plate 2 (a)

**Description**
Cells in colonies; colony consists of 8-16 cells; 44-98µm in diameter; spherical or hollow in shape; cells spherical to polygonal shaped; attached by small interconnecting protuberances made of mucilaginous sheaths, with sharp spines; parietal chloroplast with single pyrenoid.

**Collection No.** WUA 15, WUA 21, WUA 28, WUA 44 and WUA 54.

**Distribution**
Eastern and North-eastern States of India (Jena and Adhikary 2007), Odisha (Behera et al. 2020).

**Order:** Sphaeropleales

**Family:** Cylindrocapsaceae

**Golenkinia radiata** Chodat 1894: 305, pl. III [3]
Plate 2 (b)

**Description**
cells circular; Cell diameter 8-13µm, setae 20-26µm long; possess spines 2-3 times longer than the diameter of cell; chloroplast cup shaped.

**Collection No.** WUA 05, WUA 14, WUA 26, WUA 32 and WUA 55.

**Distribution**
Meghalaya (Hajong and Ramajunam 2021), Tamil Nadu (Philipose 1967), West Bengal (Smith 1920).

**Order:** Sphaeropleales

**Family:** Zygnemataceae

(i) **Spirogyra crassa** (Kützing) Kützing 1843: 280, pl. 14: fig. 4
Plate 2 (d)

**Description**
Filaments green, long and unbranched; cells 24-45µm long, 14-17µm wide; cells oblong to ellipsoid, arranged in uniseriate manner, surrounded by tough gelatinous cell wall as a whole; massive chloroplast with single pyrenoid.

**Collection No.** WUA 03, WUA 14, WUA 28, WUA 38 and WUA 57.

**Distribution**

**Order:** Zygnematales

**Family:** Zygnemataceae

(ii) **Coelastrum proboscideum** Bohlin in Wittrock, Nordstedt and Lagerheim 1896: no. 1240
Plate 2 (b)

**Description**
Cells in colonies; colony consists of 8-16 cells; 44-98µm in diameter; spherical or hollow in shape; cells spherical to polygonal shaped; attached by small interconnecting protuberances made of mucilaginous sheaths, with sharp spines; parietal chloroplast with single pyrenoid.

**Collection No.** WUA 15, WUA 21, WUA 28, WUA 44 and WUA 54.

**Distribution**
Eastern and North-eastern States of India (Jena and Adhikary 2007), Odisha (Behera et al. 2020).

**Order:** Sphaeropleales

**Family:** Neochloridaceae

**Golenkinia radiata** Chodat 1894: 305, pl. III [3]
Plate 2 (b)

**Description**
cells circular; Cell diameter 8-13µm, setae 20-26µm long; possess spines 2-3 times longer than the diameter of cell; chloroplast cup shaped.

**Collection No.** WUA 05, WUA 14, WUA 26, WUA 32 and WUA 55.

**Distribution**
Meghalaya (Hajong and Ramajunam 2021), Tamil Nadu (Philipose 1967), West Bengal (Smith 1920).

**Order:** Sphaeropleales

**Family:** Cylindrocapsaceae

**Cylindrocapsa geminella** Wolle 1887: 104
Plate 2 (c)

**Description**
Filaments green, long and unbranched; cells 24-45µm long, 14-17µm wide; cells oblong to ellipsoid, arranged in uniseriate manner, surrounded by tough gelatinous cell wall as a whole; massive chloroplast with single pyrenoid.

**Collection No.** WUA 03, WUA 14, WUA 28, WUA 38 and WUA 57.

**Distribution**

**Order:** Zygnematales

**Family:** Zygnemataceae

(i) **Spirogyra crassa** (Kützing) Kützing 1843: 280, pl. 14: fig. 4
Plate 2 (d)

**Description**
Filaments green, long and unbranched, slimy to touch; vegetative cells 150-220µm long, 35-40µm wide; plane end walls; Single chloroplast making 4-5 spiral turns.

**Collection No.** WUA 06, WUA 17, WUA 29, WUA 40 and WUA 53.
Distribution
Kerala (Ushadevi and Panikkar 1994), Meghalaya (Hajong and Ramajunam 2021).

(ii) Spirogyra hyalina Cleve 1868: 17, pl. 2; pl. 3: figs 1-6
Plate 2 (e)

Description
Filaments green, long, unbranched; vegetative cells 80-240µm long, and 47-56 µm wide; end walls plane; range of chloroplasts 2-4 making 1-3 spiral turns.

Collection No.: WUA 08, WUA 16, WUA 29, WUA 41 and WUA 43.

Distribution
Arunachal Pradesh (Gupta et al. 2002), Himachal Pradesh (Seth 2005), Jammu and Kashmir (Kant and Gupta 1998), Maharashtra (Barhate and Tarar 1985; Patil et al. 2012), West Bengal (Sikdar et al. 2012).

Order: Cladophorales
Family: Cladophoraceae
Cladophora glomerata (Linnaeus) Kützing 1843: 266
Plate 2 (f)

Description
Macroscopic; filaments uni-seriate; slight to profusely branched; entangled filaments; slender; green; upper parts crowded; long terminal branches; cells 30-60µm long, 10-15µm wide; cells cylindrical to barrel shaped; each cell with parietal net like chloroplast with numerous pyrenoids.

Collection No.: WUA 04, WUA 13, WUA 27, WUA 45 and WUA 55.

Distribution

Order: Oedogoniales
Family: Oedogoniaceae
Oedogonium globosum Nordstedt ex Hirn 1900: 94, pl. V [5]: fig. 30
Plate 2 (h)

Description
Filaments long uni-seriate and unbranched; cells long, cylindrical, 25-45 µm long; 8-13.2 µm wide; large single swollen oogonium; oogonium globose to sub globose; 12-15 µm in diameter; thick spore wall; smooth; chloroplast reticulate with numerous pyrenoids.

Collection No.: WUA 06, WUA 13, WUA 25, WUA 44 and WUA 55.

Distribution
Meghalaya (Hajong and Ramajunam 2021), Odisha (Behera et al. 2020).

Discussion
In the present study, a total of 16 species of Chlorophyceae has been identified on the basis of morpho-taxonomic characteristics, which included unicellular, colonial and filamentous unbranched and filamentous branched forms. These species belonged to 6 orders of 10 families, out of which majority of the species were of order Sphaeropleales. The diversity as well as the distribution of presently reported Chlorophycean genera has earlier been studied throughout the India. Out of these 16 species, Ankistrodesmus falcatus, Pediastrum sp., Cladophora sp., and Spirogyra sp. are reported to be well distributed and as dominant genera in Dodi Tal Wetland and some Northern parts of India (Jindal and Thakur 2013, Sharma and Singh 2018). Whereas, Coelastrum proboscideum, Oedogonium globosum, Cladophora glomerata are well distributed in Eastern zone of Odisha (Behera et al. 2020). The present study advocates the cosmopolitan nature of above-mentioned genera. The comparison of present study with other studies conducted in India (Kant and Gupta 1998, Paul and Anand 2009, Harsha et al. 2017, Behera et al. 2020), revealed comparatively low diversity of Chlorophycean members from the study area. Though, some of the Chlorophycean members have earlier been reported from different water bodies of Punjab but these reports are only upto genus level viz. Spirogyra, Scenedesmus, Hydrodictyon, Pediastrum, Coelastrum, Cladophora, Oedogonium (Sharma et al. 2013, Braich and Saini 2015, Kaur and Singh 2017, Prabha and Dua 2018, Akhter and Braich 2020b). Thus, out of 16 chlorophycean taxa reported during this study are fifteen are first report from Ropar wetland as well as from Punjab. Therefore, the present study added more species to the diversity of the earlier reported algal flora of Punjab.

Conclusions
In present study, the diversity of Chlorophyceae members (up to species level) from the Ropar wetland, Punjab (India) has been investigated for the first time. This wetland does not support rich diversity of green algae may be due to different climatic conditions in comparison to other regions of the country. This is purely speculative and needs further investigation. The present study revealed the cosmopolitan natures of Coelastrum, Cladophora, Oedogonium, Spirogyra, Scenedesmus, Hydrodictyon and Pediastrum as these genera are well distributed throughout India. The present study added 15 more members at the species level to the list of known members of Chlorophyceae from Punjab State of India.
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