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Distribution of Oscillatoriales from Kalimpong District of Darjeeling Himalaya, India

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ABSTRACT

Kalimpong district belongs to the Darjeeling Himalayan mountain range, covering Neora Valley National Park present in the northern part of West Bengal, India. The elevation and tranquil environment favor photosynthetic microorganisms to flourish here naturally in different capacities. It includes cyanobacteria, which are Gram-negative prokaryotes and primary producers exhibiting epipelic, epiphytic, epilithic and other modes of occurrence in lentic and lotic ecosystems. The order Oscillatoriales consists of simple unbranched nonheterocytous forms of cyanobacterial thallus and it serves as a bridge between unicellular coccoid forms and comparatively more complex heterocytous filamentous forms. Some symbolic works had been earlier conducted to record cyanobacterial flora in this region. Therefore, considering the significance and need for updated validation regarding recordkeeping of different cyanobacterials strains under the order Oscillatoriales in this site, this investigation was thoroughly performed. The morphometric taxonomic enumeration of the collected species was achieved using selected monographs and databases. In addition, from the collection sites, their physicochemical parameters, their respective elevation from sea surface and frequency of occurrence of species were also recorded. This helped to compute their correlation matrices required to point out the ecosystem as stable against anthropogenic activities such as tourism, farming and urbanization. Altogether eighteen nonheterocystous filamentous forms of cyanobacteria belonging to nine genera were documented, among which *Phormidium* outwitted as the most common and dominant genera of Kalimpong with nine different species followed by *Oscillatoria* and other members belonging to the order of discussion.

Keywords Kalimpong, Cyanobacteria, Oscillatoriales, Diversity, Physicochemistry.

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INTRODUCTION

Kalimpong is situated at the northern region of West Bengal, India, popularly known as North Bengal. This phytoecological region belongs to the range of Eastern Himalaya and is popular for travelling as well as tea and terrace farming. The vast biodiversity present in the district is still considered to be unexplored and somewhat untouched by mankind. Still the area to some extent is disturbed by anthropogenic activities such as tourism, home staycations, and farming. Kalimpong was previously considered as Dalingkot Sub-Division of Western Duars during British Indian colonization. It is situated at the north of Jalpaiguri District, south of Sikkim and Bhutan, west of Jaldhaka River, and east of the Tista River. Being in the Darjeeling Himalaya it roughly belongs to the region between latitudes 26°52' and 27°11'N and longitudes 88°22' and 88°52'E. The elevations of this hilly district ranges from 64 to 3187 meters with slopes approximately from 0° to 78°. The only areas with flat topography are the southern part of Chel and Jaldhaka (Ghosh and Lepcha 2024). The Kalimpong Sadar is the only subdivision in the Kalimpong district that presently comprises four community development blockes namely Kalimpong I, Lava, Pedong and Gorubathan.

The environment and physicochemical parameters of the habitats suit several orophilic and photosynthetic cyanobacteria to grow naturally in this region with the ability of enhancing surface fertility as well as phycoremediating pollutants. Alongside the ability to produce invaluable secondary metabolites, cyanobacteria hold immense potential for application as primary microbial colonizers in ecosystems, heavy metals and ions omitting agents and soil conditioners (Dey *et al.* 2021, Joseph and Ray 2025).

The cyanobacteria are divided into three major groups based on their physical traits known as coccoid, filamentous non-heterocytous and filamentous heterocytous forms (Desikachary 1959). Oscillatoriales are one of the most ecologically as well as evolutionarily significant groups among other orders of cyanoprokaryotes because of their rapid growth, mat forming abilities, and composite unbranched structure more complex than unicellular coccoids but simpler than hetercystous forms. Members of this order are distinguished by their filamentous thallus lacking akinetes and heterocysts (Komárek and Anagnostidis 2005). These non-heterocystous filaments are very common forms and are reported from different habitats of India (Anand 1989, Dey and Bastia 2009, Dey *et al.* 2011, Debnath and Dey 2022, Mallick *et al.* 2025), but most of them could not reach Kalimpong. Therefore very minimum information is available on non-heterocystous forms particularly on the members of the order Oscillatoriales from this diversity rich district. Around forty year ago a survey was performed that obtained twenty freshwater blue green algae from this site that included the genera Oscillatoria and Lyngbya from the order Oscillatoriales (Mahajan and Mahajan 1990). Another morphotaxonomic account documented one species of the genera Oscillatoria from one site of this district (Satpati *et al.* 2012). The works on cyanobacteria in Kalimpong is thus very limited, older and so on the order Oscillatoriales. The objective of this study was to investigate the diversity of the order Oscillatoriales in Kalimpong, West Bengal. Documentation and study of diversity was carried out in order to generate comprehensive information concerning the cyanobacterial flora of North Bengal.

MATERIALS AND METHODS

The study area is Kalimpong district of West Bengal, India, present in the Darjeeling Himalayan range, about 68 km. towards north from the town of Siliguri, the 'Gateway of North-East India'. The district covers 1,053.60 sq. km area. The climate of Kalimpong is himalayan, temperate, Summer during May to June, Monsoon during June to September and Winter during December to February. The highest temperature hits 27°C during summer and average temperature marks around 5°C during the winter season. The relative humidity remains between 87% to 91% while average rainfall is measured as 2200 mm (IMD, n.d.). The Neora Valley National Park represents around 88 sq. km. area that belong to the Kalimpong district. It is considered as a virgin forest patch and one of the oldest Reserve Forests in India (Fig. 1). The samples were collected randomly in vials from different habitats, e.g. drains, moist soils, agricultural field's soils, tree barks and stone surfaces

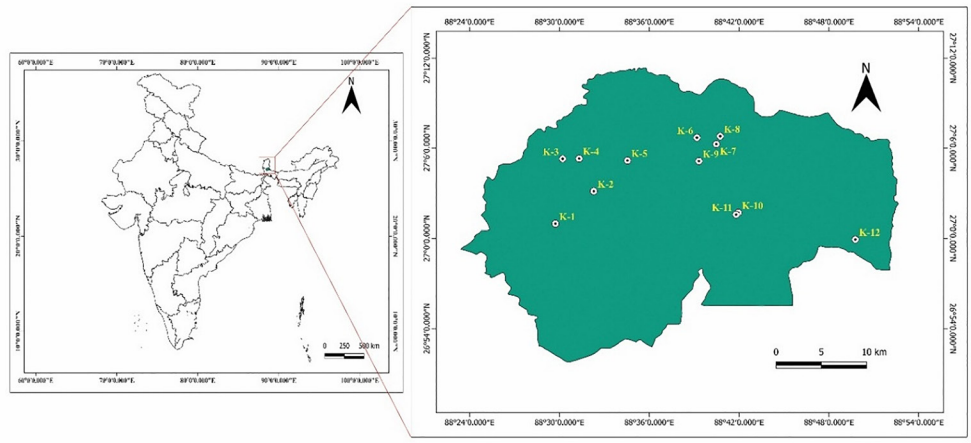


Fig. 1. A. Map of India makred with Kalimpong subdivision; **B.** Map of Kalimpong sub division marked with collection sites.

from various places in Kalimpong District of West Bengal, India during different seasons in the year 2024 and in 2025, which includes the common accessible area that belongs to the Neora Valley National Park. At the collection sites pH, temperature, electrical conductivity and total dissolved solids were measured using universal pH paper, thermometer, EC and TDS meter (Aquafresh) respectively. The samples were collected from 12 different spots. The slides were duly prepared and observed under a compound microscope (Carl Zeiss Primo Star Microscope 4150500-0054-000) and photographs for identification and recorded measurement. Identification were done up to the level of species using monograph and literature (Komárek and Anagnostidis 2005). The frequency of occurrence of individual species were determined using the following formula where n refers to the number of sampling sites from where the species were obtained and N denotes the total number of sampling sites (Simpson 1949).

Frequency of Occurrence (FO) of one species

$$(\%) = \left(\frac{n}{N}\right) \times 100$$

RESULTS AND DISCUSSION

The collection sites included different types of habitats belonged to the elevation from 677.27 to 2182.66 meter above the sea level. The sampling sites were cited as K-1, K-2, K-3, K-4, K-5, K-6, K-7, K-8, K-9, K-10, K-11 and K-12 respectively. Cyanobacterial samples were found attached to the muddy trunk of tea plants, moist soils, cemented surfaces, drains, bricks, wet surfaces near small and large mountain streams, waterfalls. The fluctuation of temperature with seasonal changes, dynamic microclimates were observed including cloudy, rainy, bright sunny weather, shadows and sunlight invading dense and light forests of large trees, and anthropogenic interventions due to farming and development of townships in some areas. The temperature during the entire collection tenure ranged from 16.7 to 23.7 °C during the time of sample collection, particularly daytime. All the cyanobacterial samples were found between 6.2-8.2 pH (Table 1).

Multivariate analysis showed Dim1 and Dim2 accounted for 46.6% and 33.7% respectively and together they accounted for 80.3% of the overall variances (Fig. 2). Dim1 showed notable positive loading for the TDS and EC. It was found that ionic gradient influenced the variations related to the different study sites. In Dim2, temperature and elevation i.e. altitude showed negative correlation whereas pH and TDS showed weak and positive correlation. Site 6 (K-6) and 7 (K-7) are highly similar in the context of environmental variables as also observed for the

Table 1. Details of sampling sites and Oscillatoriales members recorded from Kalimpong.

Sites	Lattitude (N) Longitude (E) Altitude (m)	Temp. (°C) pH EC (µs/cm) TDS (mg/L)	Name of the species
K-1	27.016117 88.495537 1079.50	19.87±1.76 7.06±0.13 207±11.86 125.67±18.8	*Planktothrix suspensa* (Pringsheim) Anagnostidis et Komárek; *Phormidium macedonicum* Čado; *Symploca thermalis* Gomont ex Gomont; *Phormidium laetevirens* (Crouan ex Gomont) Anagnostidis et Komárek
K-2	27.0522232 88.5382240 935.26	21.1±1.07 7.77±0.33 273.33±42.21 156.67±31.9	*Phormidium laetevirens* (Crouan ex Gomont) Anagnostidis et Komárek
K-3	27.088122 88.503580 1683.10	20.53±2.16 7.13±0.21 194.33±49.71 108.33±34.26	*Pseudanabaena spelaea* Anagnostidis; *Phormidium formosum* (Bory ex Gomont) Anagnostidis et Komárek
K-4	27.088409 88.521933 1507.41	20.63±0.57 7.77±0.26 263.67±96.92 139.33±51.34	*Phormidium macedonicum* Čado; *Jaaginema pseudogeminatum* (Schmid) Anagnostidis et Komárek
K-5	27.086189 88.575838 1302.15	19.93±0.63 6.87±0.13 232.33±85.89 123.67±44.92	*Phormidium macedonicum* Čado; *Phormidium formosum* (Bory ex Gomont) Anagnostidis et Komárek
K-6	27.1115990 88.6530013 2062.39	18.13±0.82 7.43±0.13 274±27.07 157.33±30.94	*Phormidium californium* Drouet; *Phormidium chlorinum* (Kützing ex Gomont) Anagnostidis
K-7	27.104436 88.674528 1563.77	17.8±0.9 7.83±0.33 346.67±36.34 174.33±19.14	*Leptolyngya subtruncata* (Voronichin) Anagnostidis; *Phormidium corium* Gomont
K-8	27.113182 88.678935 1320.82	18.6±1.1 6.73±0.1 59.33±21.45 26±11.05	*Phormidium corium* Gomont; *Phormidium laetevirens* (Crouan ex Gomont) Anagnostidis et Komárek
K-9	27.085556 88.655278 2182.66	19.83±1.28 7.73±0.17 337.33±45.55 174±34.71	*Phormidium autumnale* [Agardh] Trevisan ex Gomont; *Oscillatoria lutea* Agardh ex Gomont; *Phormidium irriguum* (Kützing ex Gomont) Anagnostidis et Komárek; Oscillatoria sp. 1
K-10	27.028374 88.699007 814.87	20.9±1.02 7.17±0.17 139.33±23.11 70.67±7.36	*Schizothrix californica* Drouet; *Phormidium boryanum* (Bory Anagnostidis et Komárek)
K-11	27.026229 88.696608 845.83	21.8±1.8 7±0.16 81±21.65 39.67±12.55	*Phormidium laetevirens* (Crouan ex Gomont) Anagnostidis et Komárek; *Phormidium macedonicum* Čado
K-12	26.998889 88.829167 677.27	19.8±1.02 6.57±0.29 333±32.96 163.33±9.67	*Phormidium autumnale* [Agardh] Trevisan ex Gomont; *Romeria chlorina* Böcher; *Phormidium corium* Gomont; *Oscillatoria* sp. 1

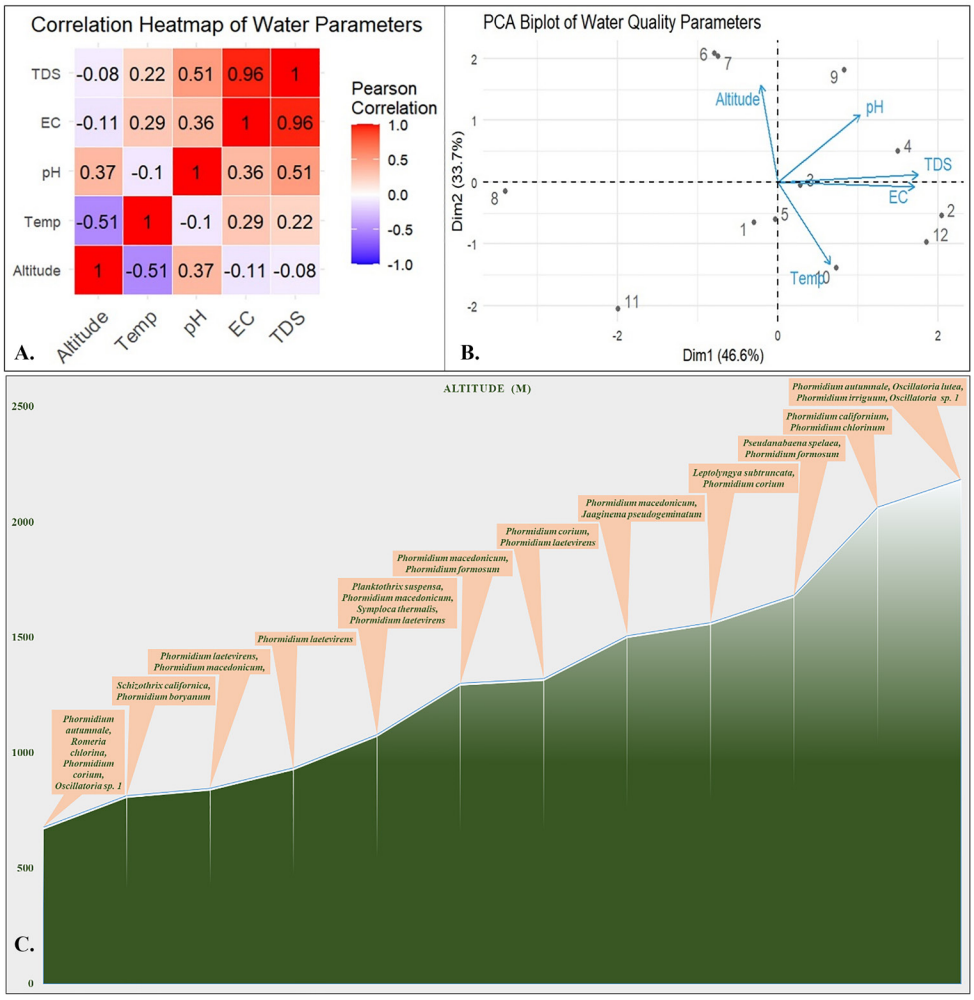
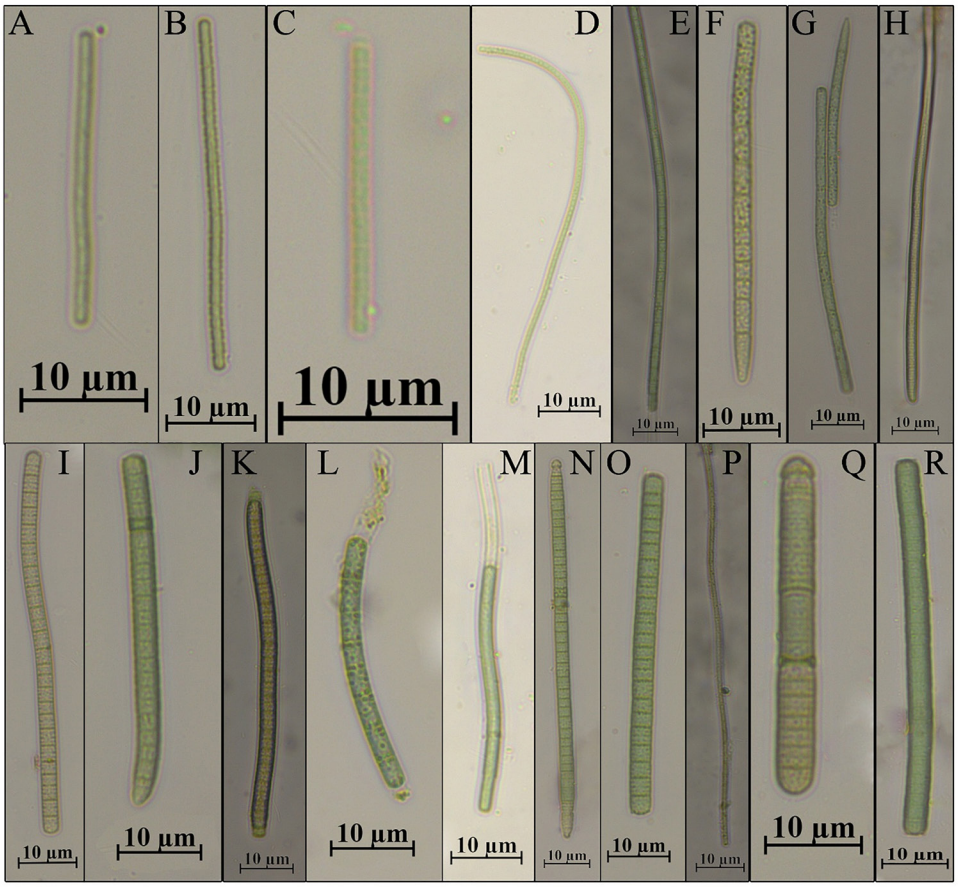


Fig. 2. A. Heatmap of water parameters markes with Pearson correlation; **B.** PCA biplot of water quality parameters. **C.** Occurrence of Oscillatoriales across different altitudes of Kalimpong.

Site 2 (K-2) and 12 (K-12). Site 8 (K-8) and site 11 (K-11) showed distinct differences in respect of the physicochemical and environmental properties. This principle component analysis was aided by the Pearson correlation matrix. EC and TDS with very strong positive correlation (r=0.96) as determined by the analysis indicated mutual dependence on dissolved mineral concentration. On the other hand strong negative correlation was obtained in between altitude and temperature (r=-0.51) which pointed out that the relative decrease of temperature was observed with increasing elevation. Temperature vs EC and TDS vs altitude showed weak correlation respectively. A likely correlation have been observed in freshwater environment with response to the seasonal water cycles and mineral inputs (Chamizo *et al.* 2024).

During the entire study period thirty two samples were collected from twelve different collection sites and a total of eighteen different species of Oscillatoriales were recorded. Among the obtained species nine belonged to the genus *Phormidium*, two belonged to the genus *Oscillatoria,* and one each to the genera *Romeria, Pseudanabaena, Jaaginema, Leptolyngya, Planktothrix, Schizothrix* and *Symploca* (Photoplate no. 1).



Photoplate no. 1. A. *Romeria chlorina* Böcher; **B.** *Pseudanabaena spelaea* Anagnostidis; **C.** *Jaaginema pseudogeminatum* (Schmid) Anagnostidis et Komárek; **D.** *Leptolyngya subtruncata* (Voronichin) Anagnostidis; **E.** *Planktothrix suspensa* (Pringsheim) Anagnostidis et Komárek; **F.** *Schizothrix californica* Drouet; **G.** *Phormidium californicum* Drouet; **H.** *Phormidium laetevirens* (Crouan ex Gomont) Anagnostidis et Komárek; **Fig. I.** *Phormidium boryanum* (Bory Anagnostidis et Komárek); **J.** *Phormidium formosum* (Bory ex Gomont) Anagnostidis et Komárek; **K.** *Phormidium macedonicum* Čado; **L.** *Phormidium chlorinum* (Kützing ex Gomont) Anagnostidis; **M.** *Phormidium corium* Gomont; **N.** *Phormidium autumnale* [Agardh] Trevisan ex Gomont; **O.** *Phormidium irriguum* (Kützing ex Gomont) Anagnostidis et Komárek; **P.** *Symploca thermalis* Gomont ex Gomont; **Q.** *Oscillatoria lutea* Agardh ex Gomont; **R.** *Oscillatoria* sp. 1

The highest diversity was found at sites referred to as K-1, K-9 and K-12 having four different members each while lowest diversity was recorded at K-2 with one genera. *Phormidium* were observed as most common genera distributed in diverse nature of habitat in this region of which *Phormidium laetevirens* was the most frequent species followed by *Phormidium macedonicum* with 33.33 and 25 % frequency of occurrence respectively (Table 2).

Based on the morphometric features and overall colony characteristics the taxonomic enumeration of the obtained species was achieved and noted down hierarchically. The recording of their occurrence at ranges of phytoecological habitats has emerged as an enriched information. It comprised of one of the most earliest described species Romeria chlorina Böcher as well as later described species Oscillatoria lutea Agardh ex Gomont in the monograph (Komárek and Anagnostidis 2005). The outcome showed the presence of diverse, primitive and comparatively preceding members of the order Oscillatoriales in the

Table 2. Description, reference and frequency of occurrence (FO) of the recorded species.

Name of the species	Description & Occurrence	Reference*	FO (%)
Romeria chlorina Böcher	Solitary trichomes, pale-green, 1 µm wide, motile, cylindrical, blunt sterminal cell. Occurrence: Tea plantation mud.	Page 61 Fig. 25	8.33
Pseudanabaena spelaea Anagnostidis	Thallus yellow green, trichome pale green, sheath present, I µm wide, constricted cross walls, cylindrical, apical cell rounded. Occurrence: cluster in moist rock pavement	Page 85 Fig. 64	8.33
Jaaginema pseudogeminatum (Schmid) Anagnostidis et Komárek	Thallus pale blue green, trichome straight, more or less isodiametric cells, 0.8-1.3 µm wide. Occurrence: messy moist green wall adjacent to moist soil base.	Page 113 Fig. 113	8.33
Leptolyngya subtruncata (Voronichin) Anagnostidis	Thallus mat, blue green, trichomes curved, 1-1.5 µm wide, thin sheath, more or less isodiametric cell. Occurrence: moist inclined garden soil.	Page 186 Fig. 224	8.33
Planktothrix suspensa (Pringsheim) Anagnostidis et Komárek	Solitary trichomes, free-floating, olive green, cylindrical, slighlty curved, 2 µm wide, longer rounded apical cell. Occurrence: waterlogged holes withing cemented steps.	Page 358 Fig. 498	8.33
Schizothrix californica Drouet	Pale green, clear sheath, 2-4 µm wide, cells isodiammetric. Occurrence: Roadside moist mud crusts.	Page 518 Fig. 766	8.33
Phormidium californicum Drouet	Thallus wide, trichomes blue green, around 3 µm wide, cells longer than wide, slightly constricted cross-walls, narrow towards ends. Occurrence: roadside drains.	Page 412 Fig. 587	8.33
Phormidium laetevirens (Crouan ex Gomont) Anagnostidis et Komárek	Thallus dark green, trichome straight, 1.9-3 µm wide, slight bending at terminals, more or less isodiametric. Occurrence: most rocky surface beside small stream, stagnant water.	Page 415 Fig. 588	33.33
Phormidium boryanum (Bory Anagnostidis et Komárek)	Trichome solitary, olive green, slightly wavy, 4.2-6.1 µm wide, isodiametric to half as long as wide, granulated, rounded apical cell. Occurrence: bank of dirty flowing roadside drain.	Page 418 Fig. 595	8.33
Phormidium formosum (Bory ex Gomont) Anagnostidis et Komárek	Thallus dull blue green, trichome straight, long, pale blue green, colorless sheath present, cells nearly isodiametric 1.6-2.8 µm wide, obtuse apical cell. Occurrence: attached to the mud.	Page 421 Fig. 602	16.66
Phormidium macedonicum Čado	Mucilaginous thallus, dark brown, colorless thin sheath, Trichomes rod-shaped, bluish green, apical cell rounded, 2-4 µm wide. Occurrence: attached to mud bricks.	Page 436 Fig. 633	25
Phormidium chlorinum (Kützing ex Gomont) Anagnostidis	Thallus fine, sheath present, trichomes straight, slightly flexible, yellow green, cells 2.4-4 µm wide, 2.6-4 µm long, not granulated at cross walls, apical cell slightly flattened or rounded or acute. Occurrence: bank of dirty flowing roadside drain.	Page 436 Fig. 635	8.33
Phormidium corium Gomont	Thallus thin, meshy, brownish green, trichomes blue green, long, curved, thin sheath present, cells longer than wide, 3-4.8 µm wide. Occurrence: moist garden soil consists of decaying leaves.	Page 450 Fig. 660	25
Phormidium autumnale [Agardh] Trevisan ex Gomont	Thallus leathery, greenish brown, filaments straight, sheatch absent, motile, Cells 2-4.3 µm wide, isodiametric to 2X longer than wide. Occurrence: inclined moist soil of tea plantation.	Page 473 Fig. 707	16.66
Phormidium irriguum (Kützing ex Gomont) Anagnostidis et Komárek	Thallus blackish, trichome blue green, straight, 6-8.2 µm wide, granulated cross-walls, cells shorter than width. Occurrence: moist rocky surface.	Page 481 Fig. 720	8.33
Symploca thermalis Gomont ex Gomont	Thallus bright blue green, distinct colorless sheath, trichomes pale blue green, 1-2 µm wide, granulated, round apical cell. Occurrence: Wet rock near small stream.	Page 487 Fig. 727	8.33
Oscillatoria lutea Agardh ex Gomont	Trichomes solitary, pale blue-green, 5-6 µm wide, flattened-rounded apical cell, thick outer wall. Occurrence: cluster at the bottom of waterlogged reservoir	Page 593 Fig. 884	8.33
Oscillatoria sp. 1	Isopolar filament, 6-8 µm wide, solitary trichome, cylindrical, straight, sheath absent. Occurrence: Moist soil.	Page 576	16.66

*Reference Source: Komárek, J. & Anagnostidis, K. (2005) .

Table 3. Taxonomic enumeration of the obtained species from Kalimpong.

Family: Pseudanabaenaceae Anagnostidis & Komárek
Subfamily: Pseudanabaenoideae Anagnostidis et Komárek
Genus: *Romeria* Koczwara in Geitler
Species Name: *Romeria chlorina* Böcher
Genus: *Pseudanabaena* Lauterborn
Species Name: *Pseudanabaena spelaea* Anagnostidis
Genus: Jaaginema Anagnostidis et Komárek
Species Name: *Jaaginema pseudogeminatum* (Schmid) Anagnostidis et Komárek
Subfamily: Leptolyngyoideae Anagnostidis et Komárek
Genus: Leptolyngya Anagnostidis et Komárek
Species Name: *Leptolyngbya subtruncata* (Voronichin) Anagnostidis
Family: Schizotrichaceae Elenkin
Genus: *Schizothrix* Kützing ex Gomont
Species name: *Schizothrix californica* Drouet
Family: Phormidiaceae Anagnostidis et Komárek
Subfamily: Phormidioideae Anagnostidis et Komárek
Genus: *Planktothrix* Anagnostidis et Komárek
Species Name: *Planktothrix suspensa* (Pringsheim) Anagnostidis et Komárek
Genus: *Phormidium* Kützing ex Gomont
Species Name:*Phormidium californicum* Drouet
Phormidium laetevirens (Crouan ex Gomont) Anagnostidis et Komárek
Phormidium boryanum (Bory Anagnostidis et Komárek)
Phormidium formosum (Bory ex Gomont) Anagnostidis et Komárek
Phormidium macedonicum Čado
Phormidium chlorinum (Kützing ex Gomont) Anagnostidis
Phormidium corium Gomont
Phormidium autumnale [Agardh] Trevisan ex Gomont
Phormidium irriguum (Kützing ex Gomont) Anagnostidis et Komárek
Genus: *Symploca* Kützing ex Gomont
Species Name: *Symploca thermalis* Gomont ex Gomont
Family: Oscillatoriaceae [S.F. Gray] Harvey ex Kirchner
Subfamily: Oscillatorioideae
Genus: *Oscillatoria* Vaucher ex Gomont
Species name: *Oscillatoria lutea* Agardh ex Gomont

Kalimpong district (Table 3).

CONCLUSION

Kalimpong is a major part belonging to the Eastern Himalayan biodiversity hotspot. This district provides wide range of heterogeneous landscape that includes rivers, forests and different microhabitats with immense potential for high microbial diversity and ecosystems. On the basis of present investigation, it is concluded that Kalimpong district has diverse members of non-heterocystous cyanobacteria and there are good chances of new strains being present, which may be new to the science as well as addition to the microbial flora of Darjeeling Himalaya. Members of *Phormidium* genus are abundantly present in this study area. It needs more extensive study to develop comprehensive information on such important group of microorganisms with tremendous potential. Furthermore, the need for long-term monitoring and conservation strategies can be concluded to preserve the microbial biodiversity in the Darjeeling Himalaya affected by anthropogenic stresses such as agriculture, urbanization and tourism. In order to enhance our understanding regarding cyanobacterial diversity and their significant roles in the Himalayan ecosystems, morphological, ecological and physicochemical assessment can further be integrated with adequate molecular approaches.

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