Ceylon Journal of Science 51(Special Issue) 2022: 577-588

RESEARCH ARTICLE

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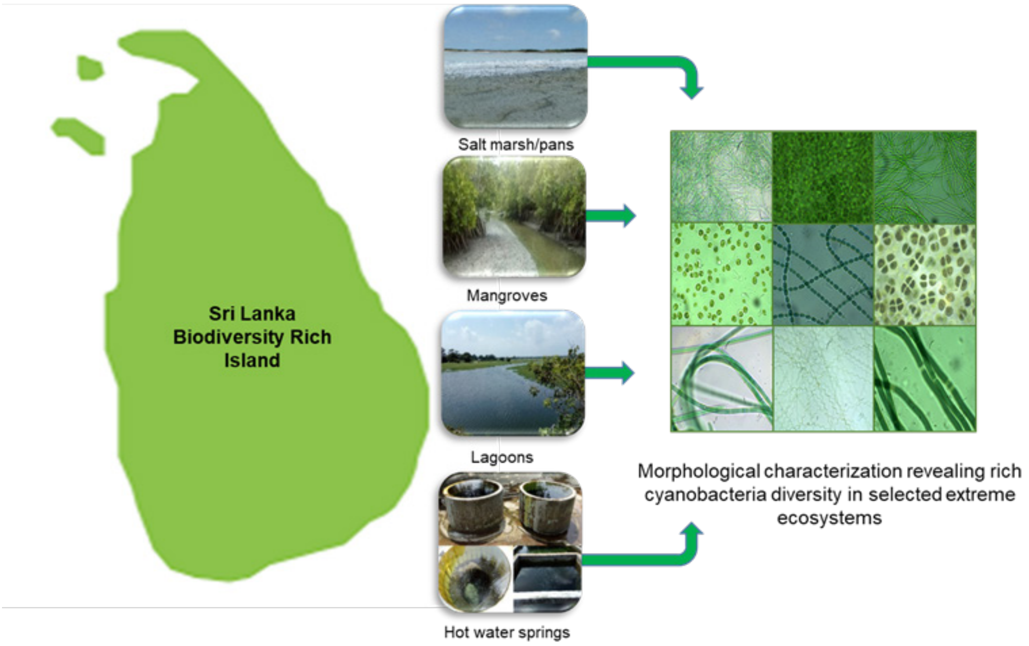

Highlights

- Morphological characterization revealed an impressive cyanobacterial diversity in selected extreme ecosystems.
- Eighteen culturable cyanobacterial genera were identified.
- *Leptolyngbya* was the most abundant in salt marshes and mangroves.
- *Pseudanabaena* was the most abundant in hot water springs.
- Their unusual adaptations provide interesting biotechnological perspectives for applied research.

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DOI: http://doi.org/10.4038/cjs.v51i5.8084

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Morphological characterization of culturable cyanobacteria isolated from selected extreme ecosystems of Sri Lanka

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Received: 30/06/2021; Accepted:04/02/2022

Abstract: Morphologically diverse cyanobacteria significantly contribute to higher microbial diversity in all Sri Lankan ecosystems. They promote promising, safe and low-cost natural alternative solutions for current global demand including food shortage, poverty, deteriorating health patterns, and environmental degradation. Only a few studies have recorded cyanobacterial diversity in some local extreme ecosystems such as hot water springs but most of them remain unexplored. Therefore, this study was carried out to investigate cyanobacterial abundance and diversity in selected extreme ecosystems of Sri Lanka based on the morphological characterization. Water sampling was carried out in selected salt marshes and salt pans, mangroves, hot water springs, and lagoons. Purified, monocultures were isolated by subsequent plate and liquid culturing in BG 11 and GO (N-free) media. Isolated monocultures were morphologically characterized using microscopic images photographed with IMAGE FOCUS 4.0 software. Among 143 isolates, 18 cyanobacterial genera namely: *Leptolyngbya*, *Oscillatoria*, *Nodosilinea*, *Anabaena*, *Geitlerinema*, *Gloeocapsa*, *Microcystis*, *Nostoc*, *Synechococcus*, *Lyngbya*, *Spirulina*, *Limnothrix*, *Pseudanabaena*, *Wilmottia/ Phormidium*, and *Fischerella* could be identified. Among them, one unidentified genera of Chroococcales, Oscillatoriales, and Nostocales were recorded. Salt marshes and salt pans showed the highest cyanobacterial diversity, *Leptolyngbya* being the most abundant genus. *Pseudanabaena* was the most abundant genus recorded in hot water springs. This study provides basic infomation of cyanobacteria diversity and abundance in extreme ecosystems in Sri Lanka for future related research and industrial applications, highlighting the necessity of their conservation.

Keywords**:** Characterization; cyanobacteria; diversity; extreme ecosystems; morphology.

INTRODUCTION

Cyanobacteria, also known as 'blue-green algae' is one of the earliest and largest groups of photosynthetic prokaryotes on the earth. Their successful ecological role as a primary producer provides the basis for ecosystem stability. Compared to higher plants, they are highly efficient CO_2 fixers having a special CO_2 concentrating mechanism (Lin *et al*., 2014) thus maintaining the CO_2-O_2 equilibrium in the environment. Nitrogen-fixing cyanobacteria fix nitrogen in soils, coral reefs, and various aquatic environments significantly contributing to the nitrogen cycle in a wide range of ecosystems; individually or sometimes in symbiosis with higher plants or fungi (Fay, 1992). Their ability to fix atmospheric nitrogen contributes to the utilization of atmospheric N_2 gas in many ecosystems. Cyanobacteria have the special ability in adapting themselves to rapidly changing environmental conditions. Thus, together with their ecological role, higher morphological, biochemical, and physiological properties enable them to colonize all possible ecosystems on Earth, including extreme environments such as hot water springs, volcanic regions, lagoons, hypersaline marshes, mangroves, and cold deserts (Soe *et al*., 2011). Diverse morphological, physiological, and biochemical properties of cyanobacteria provide a higher potential utilization in a variety of industrial applications. For example, some nitrogen-fixing cyanobacteria are promoted as an alternative for nitrogen fertilizers in rice cultivating countries (Roger and Kulasooriya, 1980) including India (Mishra and Pubbi, 2004), and Sri Lanka (Seneviratne *et al*., 2011). *Calothrix* sp. and *Anabaena* sp. have shown their effectiveness as both plant growth promoters and bio-control agents (Bidyarani *et al*., 2016). As bio-remediators, cyanobacterial species such as *Nostoc* sp., *Oscillatoria* sp., *Nodularia* sp., and *Cyanothece* sp. have shown their high potential individually and as mixed cultures in removing contaminants especially in industrial effluents (Dubay *et al*., 2011). As a source of bioenergy, cyanobacteria have been explored towards biodiesel production (Hossain *et al*., 2020b) and molecular H_2 gas production (Singh *et al*., 2016). Many studies have also explored their potential to be utilized in the pharmaceutical industry, cosmetic industry (Jain *et al*., 2005; Kim *et al*., 2008; Mourelle *et al*., 2017; Hossain *et al*., 2020a), and food industry (Desmorieux and Decaen, 2005; Hoseini *et al*., 2013; Singh *et al*., 2016; Hossain *et al*., 2020a).

Studies on identification, characterization and evaluation of cyanobacteria with industrial applicability have been mainly focused on cyanobacteria that are commonly found in normal environmental conditions (Chlipala *et al*., 2011; Sinang *et al*., 2015; Hossain *et al*., 2016; Hossain *et al*.,

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2020a; Hossain *et al*., 2020b). With the available records, show that there is a high potential within this group to be utilized in different applications for the well-being of both mankind and nature. With their ubiquitous distribution, morphology, and physiological and biochemical adaptations can vary and be diverse from ecosystem to ecosystem. Especially, cyanobacteria surviving in extreme environments can be differently adapted and, capable of producing completely different compounds that could be valuable in many aspects. Further, it can lead to the evolution of novel species and species with diverse properties, providing a broad area for research. Therefore, cyanobacteria from extreme ecosystems have currently become an interesting research topic for basic and applied research studies.

Studies on cyanobacteria from extreme ecosystems have revealed a higher diversity and novel records of taxa/species all around the world. The studies on geothermal springs in Eastern Algeria reported the occurrence of nineteen cyanobacterial morphotypes with the dominance of the genus *Leptolyngbya* (Amarouche-Yala and Benouadah, 2014). Prihantini *et al*. (2018) reported twelve culturable strains with three novel taxa of thermophilic cyanobacteria from geysers of Cisolok Hot spring in Indonesia. Cyanobacterial diversity in highly saline environments could also be higher, where hypersaline lagoons such as Araruama's lagoons in Brazil exhibited a high cyanobacterial diversity with 36 morphospecies belonging to 22 genera (Ramos *et al*., 2017). Marine ecosystems including mangroves have specific and fluctuating extreme environmental conditions. Most of these mangroves are in dry arid regions, with high salinity, and windy conditions, and undergo fluctuating temperatures with frequent tidal effects (Rigonato *et al*., 2012). Several studies on cyanobacterial communities restricted to mangrove ecosystems provide a clue on how diverse they are under these fluctuating environmental conditions. A study on cyanobacterial diversity in the phyllosphere of a mangrove forest in Brazil revealed the presence of 19 cyanobacterial genera only in the phyllosphere (Rigonato *et al*., 2012). Toledo *et al*. (1995) studied the diversity and seasonal nitrogen fixation of cyanobacteria associated with aerial roots of black mangroves in Northwestern Mexico. All these studies are evidences for a higher diversity and the possibility of finding novel species/taxa in these extreme ecosystems.

Sri Lanka is a tropical island enriched with remarkable floral, faunal, and microbial diversity. Diverse ecosystems representing a range of environmental conditions facilitate unlimited ecosystems for cyanobacterial distribution in Sri Lanka, contributing to a higher diversity (Kulasooriya and de Silva, 1981; Kulasooriya and Magana-Arachchi, 2016; Liyanage *et al*., 2016; Hossain *et al*., 2017; Hossain *et al*., 2020a). The occurence of cyanobacteria has been reported in reservoiurs of Sri Lanka (Silva and Wijeyaratne, 1999; Hossain *et al*., 2017; Senanayake and Yatigammana, 2017). Toxic cyanobacteria and their toxin production have also been extensively studied (Jayatissa *et al*., 2006; Kulasooriya, 2014; Liyanage *et al*., 2016; Kulasooriya, 2017). Only a few local research has been carried out to evaluate the potential of cyanobacteria in different industrial applications such as biofertilizer production (Kulasooriya, 1998; Seneviratne *et al*., 2011), biodiesel production (Hossain *et al*., 2020b), and food and pharmaceutical applications (Hossain *et al*., 2016; Hossain *et al*., 2020a). Specifically adapted cyanobacteria with undiscovered valuable properties, in extreme ecosystems could promote more promising safe and low-cost natural alternative solutions for current global issues such as food shortage, poverty, environmental degradation, and deteriorating health patterns. However, most of their existence and diversity in these extreme ecosystems is not discovered in Sri Lanka. Wanigatunge *et al*. (2014) have carried out a study on genetic diversity and molecular phylogeny of cyanobacteria from water and soil samples collected from freshwater reservoirs, lakes, rivers, brackish water, and tsunami-affected areas and paddy fields in Sri Lanka using light microscopy and 16S rRNA sequences. Based on the light microscopic observations, 24 different genera were identified from environmental samples and 33 genera were identified in cultures. They further reported the cyanobacterial diversity in salt pans in Hambanthota with the presence of seven cyanobacterial genera (Wanigatunge *et al*., 2014). Morphological characterization of uncultured and cultured cyanobacteria recorded eight different genera from Maha Oya hot springs (Medhavi *et al*., 2018) and three cyanobacteria genera from Mahapellessa hot springs (Samarasinghe *et al*., 2019) in Sri Lanka. Rajapaksha *et al*. (2014) also recorded the presence of some cyanobacteria genera such as *Nostoc*, *Oscillatoria*, *Lyngbya*, and *Gloeocapsa* in selected hot water wells in Mahapelessa. Later a few other studies identified and characterised industrially important bacteria from some hot water springs in Sri Lanka (Nandanee *et al*., 2015; Sadeepa *et al*., 2019). However, there are no comprehensive studies reported so far on cyanobacterial diversity in extreme ecosystems such as salt marshes/ salt pans and mangroves, and other lagoons and hot water springs in Sri Lanka. Therefore, this study was carried out to investigate the cyanobacterial diversity and abundance in some selected extreme ecosystems in Sri Lanka.

MATERIALS AND METHODS

Study sites and sampling

This study was carried out in four selected extreme ecosystems namely: salt marshes and salt pans, mangroves, hot water springs, and lagoons in Sri Lanka (Figure 1).

Nine major sampling locations of salt marshes and salt pans (in Mannar and Mannar South bar region), 19 in mangroves (Mannar region), 20 in five hot water springs (three from Kinniya, four from Rangiri Ulpotha, six from Mahaoya, six from Madunagala, and one from Jayanthi Wewa), and eight in lagoons (Nanthikadal, Bundala, Komari lagoon, Upper lagoon, Madakalapuwa, Periyakalappu, and two locations in Arugam bay) were selected for the sampling of water (Figure 2). Sampling locations representing salt marshes and mangroves were mainly chosen from the Mannar region as it is in the arid climatic zone representing extreme environmental conditions. Three samples were collected from each location into pre-sterilized 500 ml glass culture

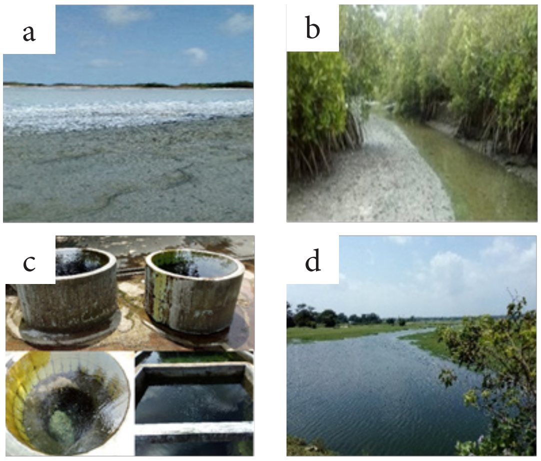


Figure 1: Sampling locations representing four selected extreme ecosystems: a. Salt pan (Mannar region) b. Mangroves (Mannar region) c. Hot water springs (tanks from Maha oya and Madunagala) d. Lagoons (Arugam bay).

bottles from the surface and the bottom of the water column. Water was directly collected into bottles if the sampling location had shallow water and the planktonic net was used to collect samples from deeper waters. Samples were collected from both free water and the walls of hot water wells as cyanobacterial growth were attached to the walls of the hot water wells. The coordinates of all the sampling locations were obtained using GPS (Geographical Positioning System) receiver (Garmin, eTrex 30).

Measurement of physico-chemical parameters of water

Physicochemical properties of water at sampling point were measured. Water temperature was recorded using a thermometer. The pH and the conductivity of the water were recorded using a digital portable pH meter equipped with both pH and conductivity probes (Eutech PCD 650, portable multi-parameter).

Sample preparation, culturing, and isolation

Microscopic analysis (Euromex BioBlue. Lab BB. 1153-PLi with fixed Euromex DC 5000 microscope camera) of representative natural water samples (collected from different sites/locations in selected extreme ecosystems) was performed prior to any treatment/culturing to observe the microbial diversity in the sample and natural morphology of the species present as it would provide an initial basis for morphological classification.

Both BG 11 and GO (N-free) media were used for cyanobacteria isolation. Sample preparation was carried out by mixing 10 mL of the water samples with 40 mL of BG 11 and GO media in sterile conical flasks in a laminar floor chamber for laboratory culture. They were incubated under the fluorescent light of 2,000 lux light intensity with constant illumination at 25 ℃ to 40 ℃ (Hossain *et al*., 2017) and maintained in 100 mL small conical flasks allowing sufficient aeration to the bottom of the flask. After cyanobacteria growth was observed, samples were sub-cultured into Petri-dishes by the spread plate method under a laminar airflow cabinet (Pulzand Gross, 2004; Yao *et al*., 2019) on Petri plates containing the same BG 11 and GO media solidified with 1.5% (w/v) bacteriological agar.

Culture plates were externally observed twice a week for their growth. Subculturing was frequently carried out with streaking techniques and growth patterns on plates were examined under the light microscope to isolate single colonies from each cyanobacteria streak plate (Hossain *et al*., 2017). Single colonies were selected using external morphological characteristics and uni-algal cultures were obtained by transferring isolated colonies repeatedly to newly prepared agar plates (Hossain *et al*., 2017). The purity of the culture was confirmed by repeated sub-culturing in plates and regular observations under the microscope.

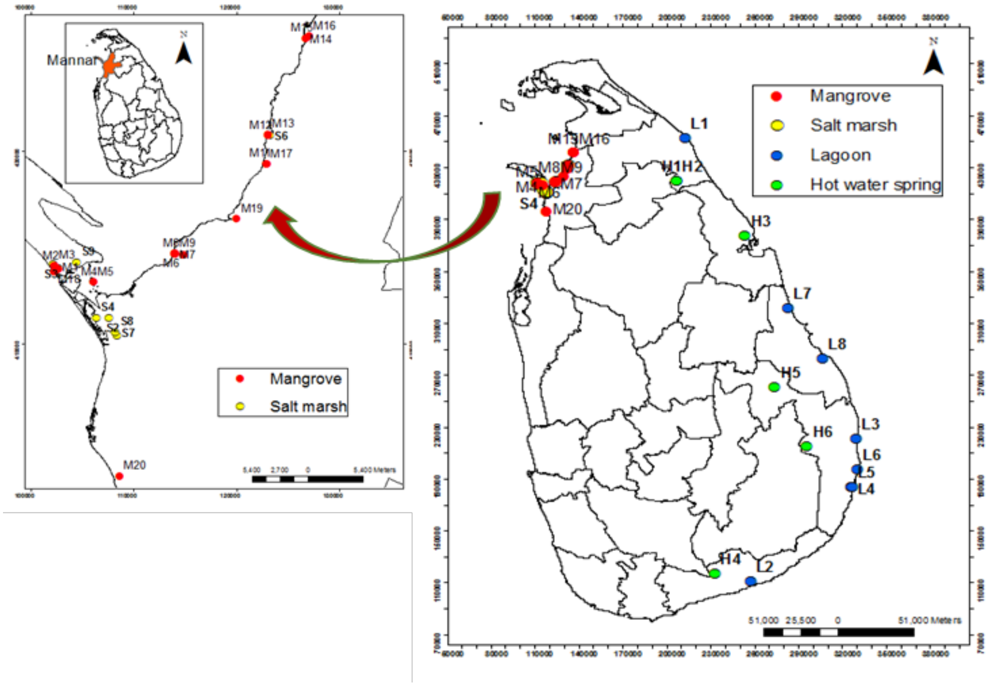


Figure 2: Map of Sri Lanka showing all major sampling locations representing four selected extreme ecosystems; Mangroves, Salt marshes, Lagoons, and Hot water springs.

Confirmed uni-algal cultures were then transferred to autoclaved 100 mL and 250 mL conical flasks containing sterile liquid BG 11 and GO media for storage purposes. Stored liquid monocultures were used for morphological characterization.

Morphological characterization of cyanobacteria strains through microscopic observation

Prepared slides of all purified liquid monocultures were observed under a compound light microscope (Euromex BioBlue. Lab BB. 1153-PLi). Microscopic images were photographed using IMAGE FOCUS 04 software. Morphological identification was carried out based on the morphological characteristics described by Desikachery (1959) and Mcgregor (2013, 2018). During identification, the following morphological characteristics were mainly considered; the shape of filaments, the shape of vegetative cells, the presence or absence of the mucilage sheath, their potential aggregation into colonies, the shape of colonies, and the presence of heterocysts and akinetes.

Statistical analysis

Numerical data were statistically analyzed using One-Way ANOVA and mean comparisons were carried out using Tukey pairwise comparisons in Minitab 17 software.

RESULTS AND DISCUSSION

Physico-chemical parameters of water collected from extreme ecosystems

Major physico-chemical parameters; pH, temperature, and conductivity measured for water samples collected from four extreme ecosystems are reported in Figures 3 and 4.

The mean temperature in hot water springs was significantly higher than in all other extreme ecosystems. Salt marshes and pans, mangroves, and lagoons showed similar mean temperatures in water. However, these conditions are highly variable with the factors such as the season and the time of sample collection. However, higher temperatures can create more stressful/extreme environments for the growth of many cyanobacteria. These stressful conditions can create space for specific cyanobacteria to adapt themselves accordingly and survive in these ecosystems. It could be the main reason for the occurrence of some cyanobacteria with specific adaptations, confined to specific ecosystems. Compared to other phytoplanktons, higher temperatures provide more favorable conditions for cyanobacteria growth in extreme environments. Higher temperatures will decrease the surface viscosity of water facilitating the buoyancy of many cyanobacteria while large nonmotile phytoplanktons are sedimented (O'neil *et al*., 2012). It also increases the nutrient diffusion through the cell surface of cyanobacteria. Frequent stratification of water due to higher temperatures enables buoyant cyanobacteria to uptake nutrients easily from deeper levels (O'neil *et al*., 2012). Therefore, cyanobacteria could compete with other phytoplanktons and survive successfully dominating extreme environments with higher temperatures.

The mean pH of the water of hot water springs was significantly lower than the water in the other three ecosystems which comparatively showed similar mean pH values. However, all four ecosystems showed pH values that were alkaline or closer to alkaline conditions. Usually, alkaline pH conditions are more favorable for many of the cyanobacteria growth while acidic conditions are creating stressful environments suppressing cyanobacteria growth (Vijayan and Ray, 2015). Therefore, the recorded pH of the water could have a positive impact on cyanobacteria growth and their diversity in all studied ecosystems.

The mean conductivity of water was significantly lower in hot water springs compared to the water in the other three ecosystems. There is no significant difference among the mean conductivity values of water in salt marshes, mangroves, and lagoons. However, the difference in mean conductivity in hot water and the other three ecosystems was significantly higher. Salt marshes and pans, mangroves, and lagoons are considered as natural coastal ecosystems, and they are usually affected by arid /semi-arid climate and coastal saline conditions (Weerakoon *et al*., 2020). Especially, coastal wetlands such as salt marshes are subjected to regular submerging conditions with seawater due to tidal effects. Lagoons are also lying along the boundary between the ocean and land, being frequently subjected to the effect of seawater (Weerakoon *et al*., 2020). Thus, the salinity and levels of dissolved ions of the water in these marine ecosystems are significantly higher (Weerakoon *et al*., 2020) compared to hot water springs. It could be a reason for higher conductivity in water and it also creates extreme conditions for cyanobacteria growth.

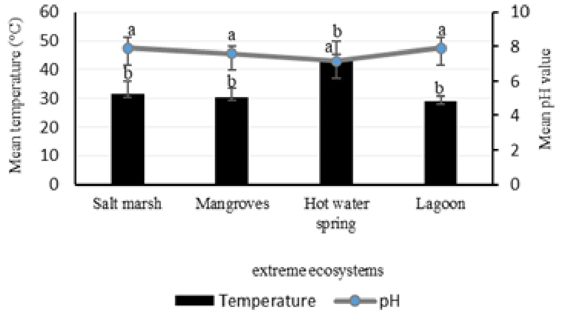


Figure 3: Mean temperature (°C) and pH of water in four ecosystems. Means with different letters are significantly different. (Tukey pairwise comparison, $p < 0.05$).

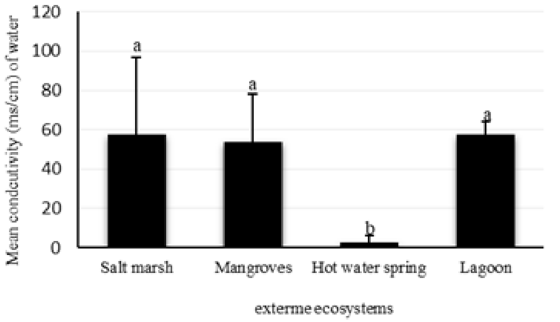


Figure 4: Mean conductivity (ms/cm) of water in four ecosystems. Means with different letters are significantly different. (Tukey pairwise comparison, $p < 0.05$).

Other than these physical parameters, fluctuations of wind currents and frequent submerged conditions due to tidal effects make these marine ecosystems more disturbed and stressful for cyanobacteria growth.

Isolation of cyanobacteria and morphological characterization of uni-algal cultures

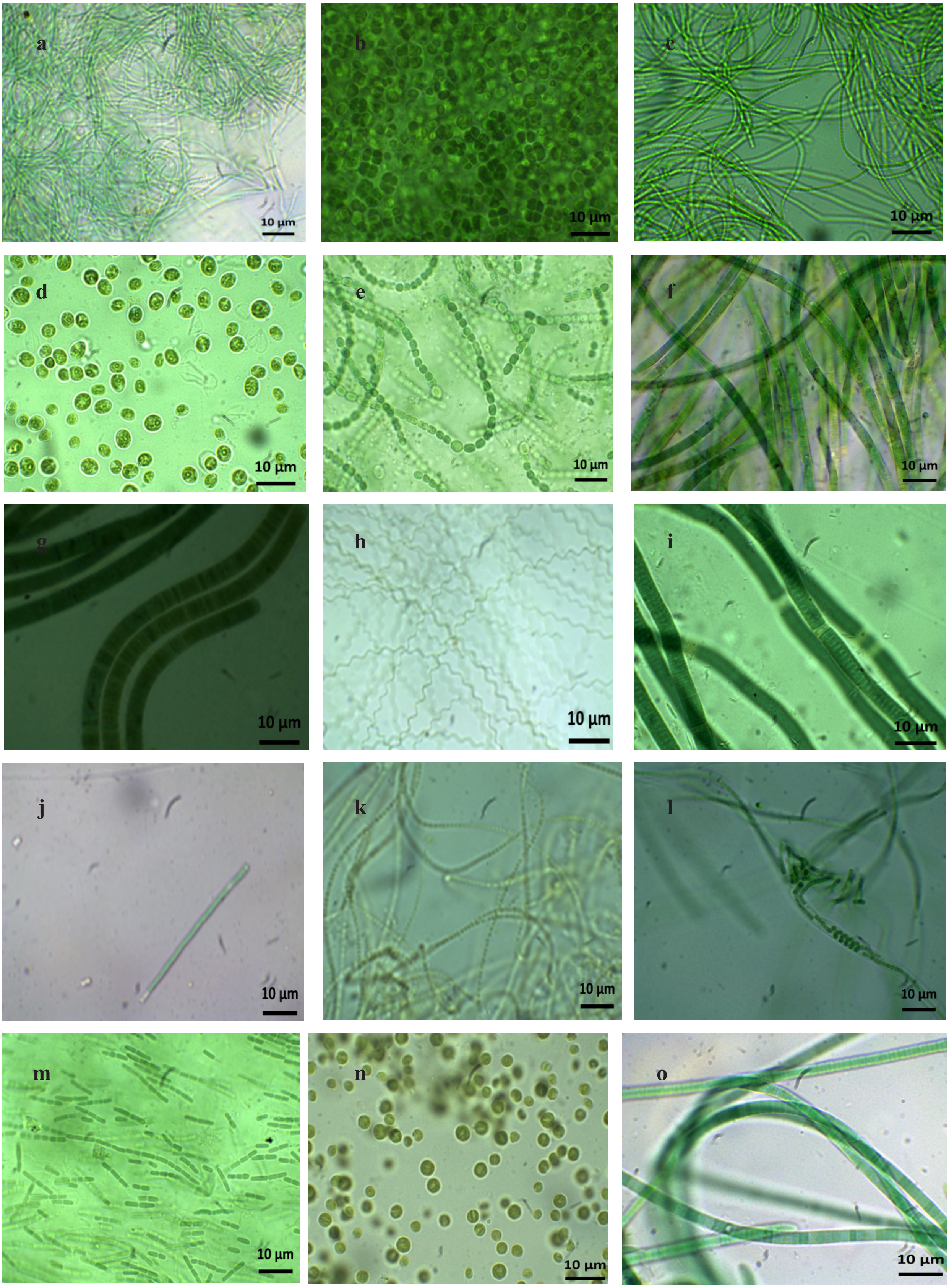
In the study, 143 purified uni-algal cultures were obtained and they were stored in liquid BG 11 and GO medium for further characterization. Among these monocultures, 56 cultures were from salt marshes and salt pans, 25 were from mangroves, 43 were from hot water springs and 19 were isolated from lagoons. All 143 monocultures were morphologically characterized. Based on the morphological characteristics, the 143 monocultures were placed in 18 different genera; *Leptolyngbya*, *Oscillatoria*, *Nodosilinea*, *Anabaena*, *Geitlerinema*, *Gloeocapsa*, *Microcystis*, *Nostoc*, *Synechococcus*, *Lyngbya*, *Spirulina*, *Limnothrix*, *Pseudanabaena*, *Wilmottia*/ *Phormidium*, *Fischerella*, an unidentified genus of Chroococcales, unidentified genus of Oscillatoriales, and unidentified genus of Nostocales. Microscopic images of some cyanobacteria cultures representing all 18 genera are shown in Figure 5. Compared to the morphology of uncultured cyanobacteria strains in natural water samples, few morphological features of some isolates were slightly affected. For instance, the colour and the width of the filaments were slightly changed in some strains. Also, some filaments were less twisted compared to their natural twisting pattern. However, the morphology of the isolates was not significantly affected by the isolation technique used and allowed morphological identification of the isolates without any confusion.

The abundance of each identified genus was studied with a view of gathering information on the cyanobacteria diversity in each ecosystem. The abundance of each identified genus in four studied extreme ecosystems; Mangroves, Salt marshes, Hot water springs, and Lagoons is illustrated in Figure 6.

The above genera were identified based on the major morphological characteristics described by Desikachery (1959), Mcgregor (2013, 2018). Out of 18 identified genera, only 15 could be identified up to the genus level and the other three could be identified only up to the order level. The major reason for this is that Order Chroococcales, Oscillatoriales, and Nostocales are larger and complex orders including many different genera. Many of them have very similar phenotypic characteristics by which two different genera sometimes cannot be differentiated. For example, Coccoid taxa in the order include solitary cells with different colonization patterns. They may have very small differences which differentiate one genus from another (Mcgregor, 2013). Due to their small size and simple and similar morphology, identification of many genera belonging to Order Chroococcales, Oscillatoriales, and Nostocales is therefore challenging. Some previous studies reported that even the use of 16S rRNA was limited in defining some genera in the Order Chroococcales (Wanigatunge *et al*., 2014).

Out of 18 genera identified, 16 genera (*Leptolyngbya*, *Oscillatoria*, *Nodosilinea*, *Anabaena*, *Geitlerinema*, *Gloeocapsa*, *Microcystis*, *Nostoc*, *Synechococcus*, *Lyngbya*, *Spirulina*, *Limnothrix*, *Pseudanabaena*, unidentified genus of Chroococcales, unidentified genus of Oscillatoriales, and unidentified genus of Nostocales) were recorded in salt marsh ecosystem showing the highest cyanobacteria diversity among all four ecosystems. Mean temperature, pH, and conductivity recorded for salt marsh ecosystems were 31.4 °C, 7.91, and 57.5 ms/cm, respectively. Usually, the temperature range of 25 – 35 °C provides favorable conditions for the growth of many cyanobacteria (Kim *et al*., 2020). Therefore, the studied locations in salt marshes and salt pans had favorable temperature conditions and alkaline pH conditions for cyanobacteria growth. These sampling locations selected in salt marshes and salt pans were also well exposed, not populated thus, undisturbed, and received an adequate amount of sunlight with sufficient light intensity (Figure 1a). These would facilitate more favorable conditions for the flourishing of a diverse collection of cyanobacteria in this ecosystem. Results obtained in the present study for salt marshes and salt pans were comparable with the study conducted by Wanigatunge *et al*. (2014) for salt pans in Hambanthota. Out of seven genera recorded in Hambanthota salt pans (Wanigatunge *et al*., 2014), five genera could be morphologically identified in this study. However, eight more genera namely, *Nodosilinea*, *Anabaena*, *Geitlerinema*, *Gloeocapsa*, *Microcystis, Spirulina*, *Limnothrix*, and *Pseudanabaena,* and 03 more unidentified genera from orders, Chroococcales, Oscillatoriales, and Nostocales could be identified from salt marshes and salt pans in this study other than the genera described in Hambanthota salt pans by Wanigatunge *et al*. (2014).

Six genera; *Leptolyngbya*, *Nodosilinea*, *Geitlerinema*, *Spirulina*, *Oscillatoria,* and an unidentified genus of Chroococcales order were recorded in mangroves while eight genera; *Leptolyngbya*, *Pseudanabaena*, *Geitlerinema*, *Oscillatoria*, *Wilmottia*/ *Phormidium*, *Gleocapsa*, *Fischerella* sp., and unidentified genus of Chroococcales order were recorded from hot water springs. *Oscillatoria* and *Gloeocapsa* were also recorded in a few studies previously done for Mahaoya and Mahapellessa hot water springs (Rajapaksha *et al*., 2014; Medhavi *et al*., 2018; Samarasinghe *et al*., 2019). *Leptolyngbya* was similarly recorded in Mahapellessa by Samarasinghe *et al*. (2019) however, it was not recorded in some other previous studies (Rajapaksha *et al*., 2014; Medhavi *et al*., 2018). Many members of Chroococcales were also recorded in Mahaoya (Medhavi *et al*., 2018). This study records the presence of *Geitlerinema*, *Wilmottia/Phormidium*, and *Fischerella* sp., which were not previously recorded in hot water springs of Sri Lanka. Only four genera, *Leptolyngbya*, *Geitlerinema*, *Oscillatoria*, and the unidentified genus of Chroococcales were recorded from lagoons recording the lowest cyanobacteria diversity. Compared to the number of sampling points, cyanobacteria diversity recorded in mangrove ecosystems was less. Light intensity is one of the major factors affecting photosynthetic cyanobacteria growth in natural environments (Vijayan and Ray, 2015). Light intensity could be a limiting factor in mangrove ecosystems as the selected water sampling sites were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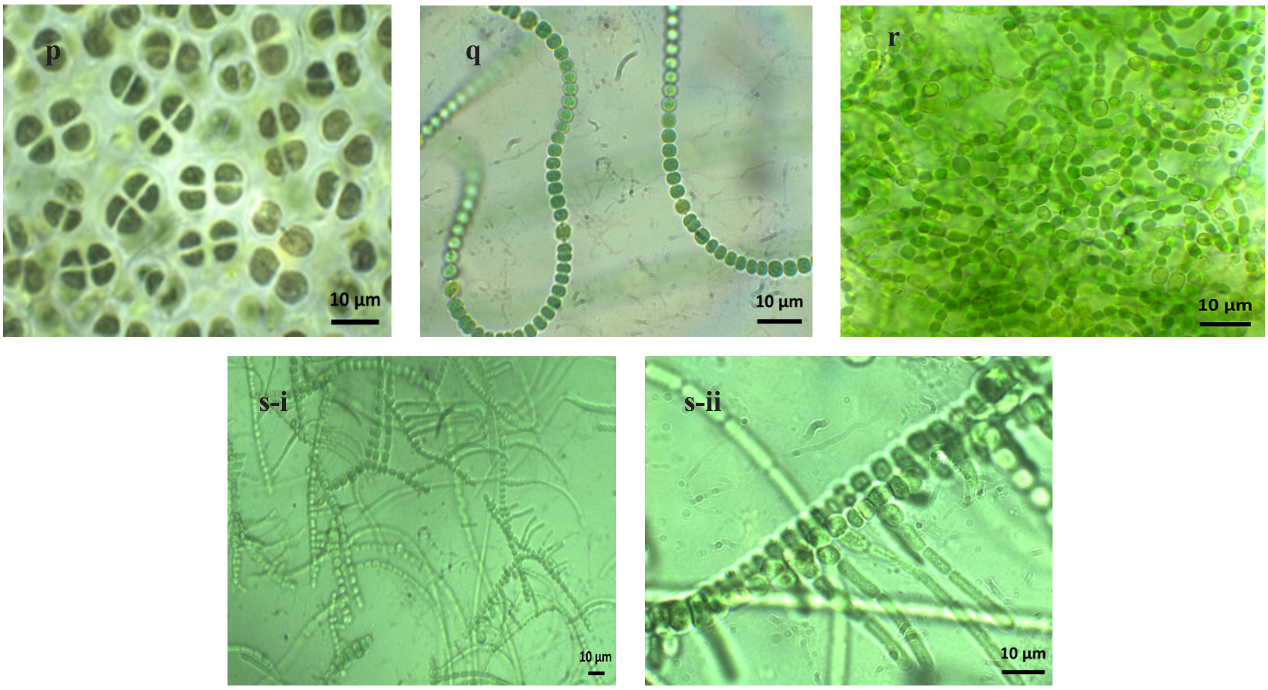

Figure 5: Microscopic images under oil immersion (Scale bar: 10 µm); a. *Geitlerinema* sp. b. *Gloeocapsa* sp. c. *Leptolyngbya* sp. d. *Microcystis* sp. e. *Nostoc* sp. f. *Wilmottia/Phormidium* sp. g. *Lyngbya* sp. h. *Spirulina* sp. i. *Oscillatoria* sp. j. *Limnothrix* sp. k. *Anabaena* sp.1 l. *Nodosilinea* sp. m. *Pseudoanabaena* sp. n. *Synechococcus* sp. o. An unidentified genus of Oscillatoriales p. Unidentified genus of Chroococcales q. *Anabaena* sp.2 r. An unidentified genus of Nostocales s-i. *Fischerella* sp. (x 400) s-ii. *Fischerella* sp. (x 1000).

covered with large mangrove vegetation (Figure 1b). Mangrove sites mainly located in the Mannar region were selected for the study as the Mannar region is in the Arid climatic zone usually representing extreme environmental conditions. However, due to human activities associated with both mangrove and lagoon ecosystems, such as shrimp cultivation and fishing, these sites were highly disturbed. This could be another reason for lower cyanobacteria diversity in these ecosystems.

Among all identified genera and orders*,* species belonging to *Leptolyngbya* were most frequently occurring in all four ecosystems (Figure 6). *Leptolyngbya* was the most abundant genus in both salt marsh and mangrove ecosystems, being present in 12 sampling sites in nine water sampling locations and 13 water sampling locations out of 19 in salt marsh and mangrove ecosystems respectively (Figure 6). Many previous studies on extreme environments also reported the frequent occurrence of *Leptolyngbya*. *Leptolyngbya* was recorded in all sampling points of geysers in Cisolok Hot spring in West Java, Indonesia, during the characterization of culturable cyanobacteria (Prihantini *et al*., 2018). The study on cyanobacterial composition in Eynal (Simav) hot spring in Kütahya, Turkey also recorded *Leptolyngbya* as the most abundant filamentous morphotype (YilmazCankilic, 2016). Not only in thermal springs but also in saline environments and mangroves, *Leptolyngbya* was one of the most abundant genera (Rigonato *et al*., 2012; Ramos *et al*., 2017). Easy morphological characterization and the ability to grow easily under laboratory conditions could be reasons for the higher detectability of this genus during diversity evaluations (Prihantini *et al*., 2018). Other than *Leptolyngbya*, *Geitelerinema*, *Oscillatoria*, and the unidentified genus of Chroococcales were recorded in all four ecosystems.

Pseudanabaena was recorded in 12 out of 20 sampling locations in highlighting their highest abundance and common occurrence in hot water springs. *Pseudanabaena* genus belonging to order Oscillatoriales can live in a wide range of habitats like freshwater, marine, and extreme environments including brackish water and thermal springs (Soe *et al*., 2011). The capability of developing adaptations according to changing environmental conditions ensures the successful survival of *Pseudanabaena* in extreme ecosystems. Bloom formation and toxin production ensure some of their distribution in extreme ecosystems suppressing the growth of other organisms (Oudra *et al*., 2002). Phycoerythrin possession is another major chromatic adaptation in some *Pseudanabaena* species to maximize the absorption of available light in extreme environments (Kehoe and Gutu, 2006).

Saltmarsh and salt pan ecosystems are highly exposed to frequent dehydration and rehydration, osmotic stress, and radiation-induced stress (Figure 1a). Genera such as *Nostoc*, *Synechococcus*, *Microcystis*, *Lyngbya*, *Limnothrix*, and *Anabaena* which were recorded only from salt marsh ecosystem (Figure 6) could therefore be well adapted with different adaptations such as bloom formation, toxin production, bearing gas vesicles for buoyancy (O'neil *et al*., 2012) and bearing heterocysts for efficient nitrogen fixation (Oudra *et al*., 2002) (Figure 7a) to tolerate these stressed conditions. Adaptations such as heterocyst formation in some cyanobacteria could be directly affected by the temperature of the water and available light conditions. *Fischerella* sp. isolated from hot water wells

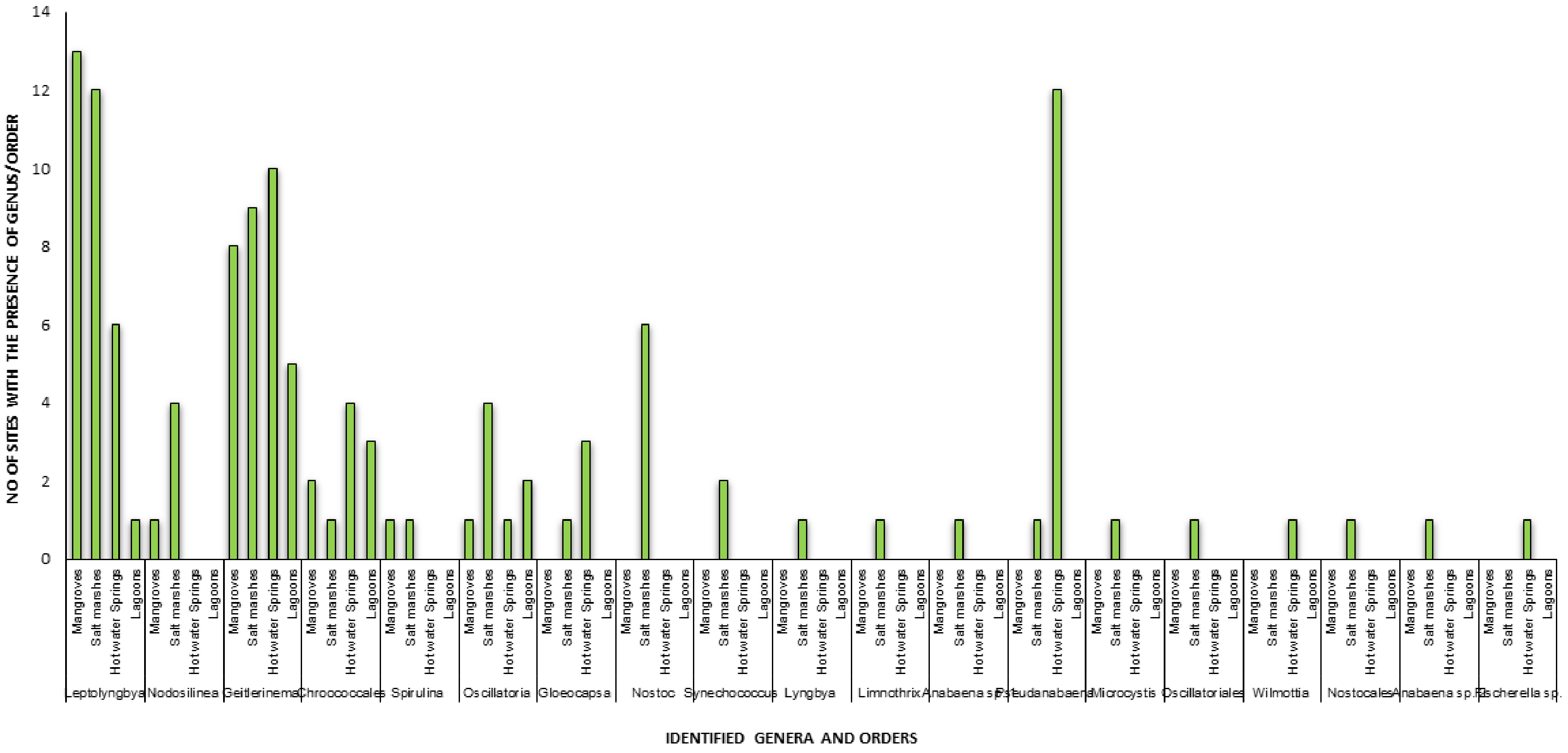

Figure 6: Abundance of each identified order with unidentified genus/genus in four studied extreme ecosystems; Mangroves, Salt marshes, Hot water springs, and Lagoons

are heterotrichous thalli with true branching filaments with round-shaped vegetative cells in the basal prostrate filaments and more cylindrical vegetative cells in the erect branches which are tapering at the ends (Figure 5s-i and 5s-ii). The solubility of oxygen and nitrogen in water can be reduced with the increase in water temperature resulting in the adaptation of heterocyst formation in heterocyst forming cyanobacteria (Staal *et al*., 2003). *Fischerella* sp. is known to be a major photosynthetic organism in many hot water springs (Alcorta *et al*., 2018) and forms heterocysts as an adaptation to survive in water with high temperatures (Figure 7b). They also have stable photosynthetic machinery to survive in higher temperatures (Alcorta *et al*., 2018).

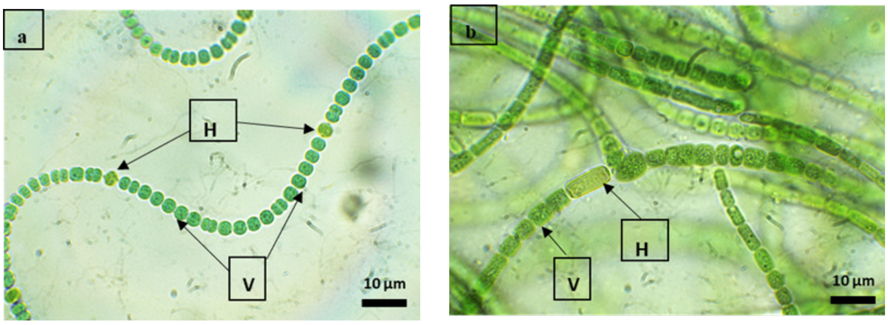

Figure 7: Heterocysts forming filamentous cyanobacteria; a. isolated from salt marsh ecosystem,b. isolated from hot water wells; **H**: Heterocyst and **V**: Vegetative cells of the filament (Scale bar: 10 µm).

Heterocyst formation enables them to compete with non-nitrogen fixing species and survive successfully in nitrogen limiting warm environments. It would be commercially beneficial to improve these nitrogen-fixing strains as biofertilizers. However, during isolation, these cyanobacteria would not form heterocysts in BG11 medium with combined nitrogen. Therefore, the use of only BG11 with combined nitrogen would limit the accurate morphological identification of certain heterocysts forming cyanobacteria. Nevertheless, these cyanobacteria are usually forming heterocysts in the GO medium where combined nitrogen is absent. Therefore, the morphology of heterocyst-forming cyanobacteria could be identified up to the genus level based on the morphology of heterocysts in the GO medium. Due to this, both BG11 and GO media were used in this study to recover most of the representative cyanobacterial diversity in each extreme ecosystem. However, the identity of these isolates will be further confirmed with molecular characterization.

Some cyanobacteria species could produce akinetes as an adaptation to survive under fluctuations of nutrients, temperature, and specially, desiccation (Carey *et al*., 2012) while many others can produce polyhydroxy saccharides to protect their cellular molecules from denaturation during fluctuating drought and wet conditions (Potts, 1994). As a response to extreme dehydrating conditions, some species can produce Water Stress Proteins (WSP) which are stable over a long period of time of desiccation (Billi and Potts, 2000). Therefore, further studies on the genetic makeup of these native strains would be useful to identify their economically beneficial genes and use them to develop genetically improved varieties, especially in agriculture. Species such as *Nostoc*, *Anabaena*, and many others in Nostocales, Chroococcales, and Oscillatoriales, surviving in extreme environments with high UV radiation could also produce ultraviolet radiation absorbing intracellular secondary metabolites such as Mycosporine-like amino acids (Jain *et al*., 2017). Thus, they could be promising to be used in the pharmaceutical and cosmetics industries. Cyanobacteria living in marine ecosystems are also capable of developing symbiotic relationships with other organisms like macroalgae, protozoans, and invertebrates. These symbiotic relationships have demonstrated remarkable changes in cyanobacteria physiology inducing the production of a variety of bioactive compounds which are not usually expressed and with interesting biotechnological aspects (Mutalipassi *et al*., 2021). Thus, cyanobacteria living in extreme ecosystems are differently adapted and these adaptations and their secondary metabolites could be beneficial in different industrial, agricultural, and pharmaceutical applications.

Morphological characterization would not be sufficient for the most accurate identification of the isolates up to the genus or species level. However, it will provide an overall evaluation of the diversity and abundance of microbial communities in different environments. This is evidenced by several previously published studies on the evaluation of microbial diversity only based on morphological and other qualitative methods (Toledo *et al*., 1995; Hanada *et al*., 2010; Chauhan *et al*., 2019). Similarly, though the identification may not be 100% accurate for some isolates, through the morphological characterization of all these isolates, morphologically distinct isolates which were different from each other could be identified in each ecosystem. Based on the phenotypic characterization, the number of morphologically different cyanobacteria isolated from each of these ecosystems could be further identified. Among them, some genera may have higher economic importance. Therefore, the results of this study would provide basic understanding of the cyanobacterial diversity and abundance in these extreme ecosystems for future studies

CONCLUSION

Morphological characterization has revealed a rich cyanobacterial diversity with 18 different genera, *Leptolyngbya*, *Oscillatoria*, *Nodosilinea*, *Anabaena*, *Geitlerinema*, *Gloeocapsa*, *Microcystis*, *Nostoc*, *Synechococcus*, *Lyngbya*, *Spirulina*, *Limnothrix*, *Pseudanabaena*, *Wilmottia*/*Phormidium*, *Fischerella*, an unidentified genus of Chroococcales, an unidentified genus of Oscillatoriales, and an unidentified genus of Nostocales in extreme ecosystems; salt marshes and salt pans, mangroves, hot water springs, and lagoons in Sri Lanka. However, cyanobacteria identification based on morphological characteristics solely would not support the most accurate and precise identification of some of the strains up to the genus or species level and it would be a major limitation of the study. Thus, molecular characterization of the strains would be the next step of this study to confirm the identity of the isolates. this study provides basic information about cyanobacterail diversity and their abundance in extreme ecosystems and highlights the necessity of conserving these natural resources while utilizing them sustainably.

ACKNOWLEDGEMENT

The authors acknowledge Mr.L. Mahadeva and Mrs. R.K.C.Karunaratne (Staff, NIFS, Sri Lanka) for their support during sample collection and analysis.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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