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RESEARCH NOTE

First report of genus *Chroococcidiopsis* (cyanobacteria) from Sri Lanka: a potential threat to human health

D.N. Magana-Arachchi* **and R.P. Wanigatunge**
Institute of Fundamental Studies, Hantana Road, Kandy.

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Cyanobacteria (Cyanophytes, Cyanoprokaryotes) are a large group of photosynthetic bacteria and one of the most fascinating groups of organisms on the earth. They show a considerable morphological diversity. Microscopy is the classical method of cyanobacterial identification and community assessment. It is difficult to classify cyanobacteria into accurate taxonomic groups since cyanobacterial morphology varies significantly in response to fluctuations of environmental conditions. Consequently, molecular biological techniques have become a popular tool for phylogenetic analysis of the cyanobacteria. Among the various gene sequences used to assess cyanobacterial biodiversity, 16S rRNA gene has been applied more frequently because it is ubiquitous, function is conserved, the gene is easy to sequence and a large database is available for sequence alignments and identification (Kumari *et al,* 2009).

A number of studies have been conducted in Sri Lanka for identifying fresh water cyanobacteria based on morphological features, and about 170 cyanobacterial species belonging to 45 genera have been described (Abeywickrama *et al*., 1986). *Microcystis aeruginosa*, *M. flos-aquaeM,. incerta*, *M. wesenbergi*, *Cylindrospermopsis*, *Aphanizomenon*, *Spirulina*, *Anabaena aphanizomenoids*, *Anabaena flos-aquae*, *Aphanocapsa*, *Chroococcus*, *Gloeocapsa*, *Nostoc* and *Coelosphaerium* have been frequently recorded from different parts of the country. However, there are no recorded reports on *Chroococcidiopsis* species from Sri Lanka.

Members of the genus *Chroococcidiopsis* are very primitive, photosynthetic, coccoid cyanobacteria and they have the capability to survive under high radiation, extreme temperatures, osmotic stress and extreme pH values. Genus *Chroococcidiopsis* have been found in freshwater, marine, hypersaline environments (Dor *et al*., 1991), hot springs, nitrate caves (Geitler, 1933; Friedmann, 1962), hot and cold deserts, airspaces of porous rocks from Antarctic valleys and in several lichens as cyanobionts (Büdel *et al.,* 2000).

While investigating the biodiversity of cyanobacteria in different parts of Sri Lanka, *Chroococcidiopsis* species were identified from the Yala National Park, Hambantota, Sammanthurai, the Kondawatuwana Tank and the Mahaweli River using molecular techniques. It is interesting to note that until now there have been no reports indicating the occurrence of *Chroococcidiopsis* species in Sri Lanka.

Water and soil samples were collected into sterilized brown glass containers from Yala, Hambantota, Sammanthurai, the Kondawatuwana Tank and the Mahaweli River (Table 1). Collected water samples were concentrated by centrifugation (3500 rpm, 10 min). An aliquot of 500 µL from the centrifuged pellet and 500 µL from the supernatant and soil samples were inoculated into cyano specific media. Morphological observations were made from each sample using compound light microscope (Olympus BH2) (400 – 1,000×) and standard identification keys for cyanobacteria. Cultures were incubated at 28 ± 2 °C and under fluorescent light with a 12:12-h D/L cycle. An aliquot of 500 µL of the cultured sample from each site along with 500 µL of standard

* Corresponding author (nayomam@yahoo.com)

M. aeruginosa culture [obtained from the Pasteur Culture Collection (PCC 7941), France] were subjected to DNA extraction according to the Boom's method (Boom *et al*., 1990) using silica particles and guanidium isothiocyanate. DNA amplification was performed for the 16S rRNA gene to identify the presence of cyanobacteria with the modified protocol of Nübel*et al*. (1997) using the cyanobacteria-specific primers, forward primer CYA359F (5'-GGGGAATYTTCCGCAATGGG-3') and the reverse primer CYA781Rb (5'-GACTA CAGGGGTATCTAATCCCTTT-3'). All PCR reactions were performed in a 50 µL reaction mixture containing 0.6 µM of each primer, 100 µM of each deoxynucleoside triphosphate, 10 µL of 5x PCR buffer, 2 mM $MgCl_2$, 2.5 U of *Taq* DNA polymerase (Promega, Madison, Wisconsin, USA) and template DNA. Thermal cycling was performed in a Techine TC 3000 DNA Thermal Cycler. The initial denaturation step at 94 °C for 5 min was followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min and a fiinal extension step at 72 °C for 15 min. Aliquots of the resulted amplified products were electrophoresed in 1.5 % agarose gels containing 10 μgmL^{-1} ethidium bromide and documented through a Gel Documentation System (Syngene, UK). PCR products were carefully excised and purified with the GenElute™ Gel Extraction Kit (SIGMA) according to the manufacturer's instructions and DNA sequencing was carried out by Macrogen Inc., South Korea using ABI 3730XL sequencers with the corresponding reverse primer. DNA sequences obtained in this study were deposited in GenBank under accession numbers EU276382, EU276383, EU310420, EU310430, EU310432, GU300772 and GU594024.

Microscopic observation of wet mounts of cultures identified the unicellular nature of the cyanobacteria. The mature undivided cells were spherical, dark blue green in colour. They formed round or quadratic shaped colonies. There were no considerable morphological variations among the colonies (Figure 1). According to the gel profiles obtained, all DNA samples submitted to PCR reactions with cyanobacterial specific oligonucleotide primers yielded the unique fragment of ~ 450 bp. 16S rRNA sequences of the tested isolates showed a DNA sequence similarity of 96 to 99 % with previously reported *Chroococcidiopsis* isolates (Table 1).

The occurrence of *Chroococcidiopsis* species in Hambantota, Ampara as well as in the Mahaweli River (Kandy) shows the diverse geographical distribution of the species in Sri Lanka. This new occurrence may indicate the selection of clones adapted to present environmental conditions or a large physiological tolerance to environmental parameters in this species. Further, ecophysiological experiments are needed to unravel this problem. According to a study by Bahl *et al.* (2011), massively parallel pyrosequencing of environmental samples collected from all continents

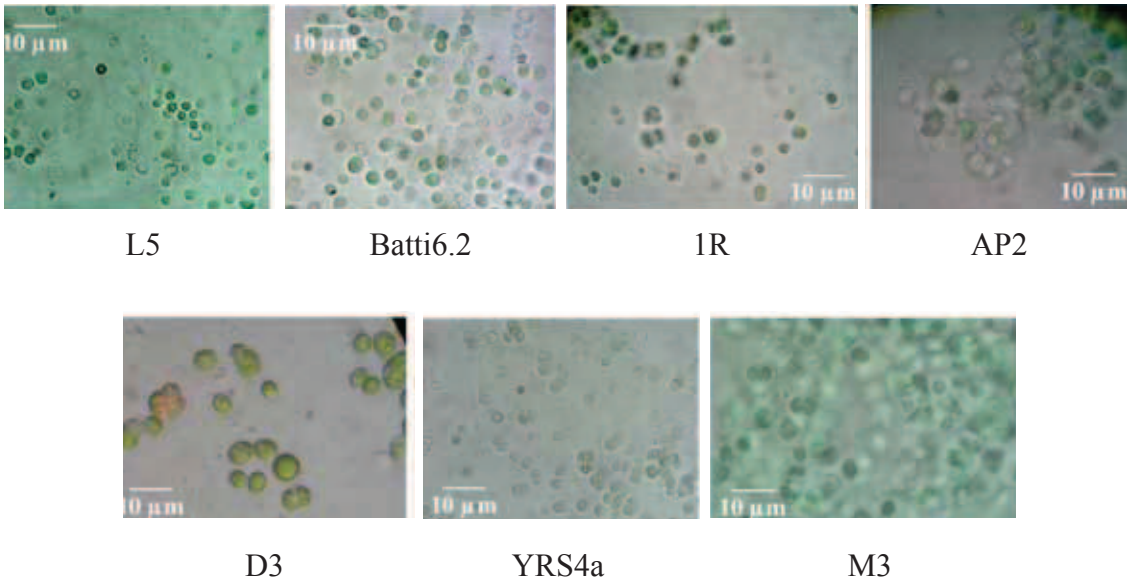

Figure 1: Light microscopic view of *Chroococcidiopsis* isolates identified from five locations of Sri Lanka (magnification x1000)

Table1: Details of the sample collection of local isolates of Chroococcidiopsis sp. and comparison of 16S rRNA sequences

Sample Code	Location	Location co-ordinates	Source of isolation	Accession number	Highest match with NCBI database	% similarity
YRS4a	Yala National Park	6.16N, 81.15E	Crust from beach rock	EU310420	*Chroococcidiopsis* sp. SAG 2025 (AM709635)	97
D3	Hambanthota	6.11N,81.11E	Soil (Nonagama)	EU310430	*Chroococcidiopsis thermalis* CCALA 048 (HM630155)	97
L5	Sammanthurai area	7.36N, 81.80E	House hold well water mixed with tsunami waves	EU276383	Uncultured Chroococcidiopsis sp. clone 1P-2-N12 (EU705152)	99
1R	Sammanthurai area	7.36N, 81.80E	House hold well water mixed with tsunami waves	EU276382	Uncultured *Chroococcidiopsis* sp. clone 1P-2-N12 (EU705152)	99
Batti6.2	Sammanthurai area	7.36N, 81.80E	House hold well water mixed with tsunami waves	EU310432	*Chroococcidiopsis thermalis* PCC 7203 (FJ805841)	98
AP2	Kondawatuwana tank	7.28N, 81.64E	Water	GU300772	Uncultured *Chroococcidiopsis* sp. clone 1P-2-N12 (EU705152)	99
M3	Mahaweli River	8.46N, 81.23E	Water	GU594024	Uncultured *Chroococcidiopsis* sp. clone 1P-2-N12 (EU705152)	96

confirmed that *Chroococcidiopsis* variants were specific to either hot or cold deserts and the distribution was dependent on contemporary climate. The global distribution for *Chroococcidiopsis* has been limited by barriers to long distance dispersal and/or invasive colonization, with regional gene pools maintained over geological timescales. Environmental selection may also have exerted a major role in colony establishment in different geographical regions (Bahl *et al*., 2011).

Finally, as *Chroococcidiopsis* species are known to produce β-N-methylamino-L-alanine, a neurotoxic amino acid (Cox *et al*., 2004) and hepatotoxic microcystin, the toxicity of *Chroococcidiopsis* species should be determined. Furthermore, the risk of contamination of water used for drinking purposes by *Chroococcidiopsis* species should be assessed in order to prevent the risk to human health.

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