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First evidence of epiphytic marine algae *Acrochaete leptochaete* identified from Beruwala reef, Sri Lanka by DNA barcoding

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Several green microalgal species that grow as epiphytes and/or endophytes associated with seaweeds have been identified from different locations worldwide. The composition of epiphytic marine algae in a marine ecosystem is an important indicator of environmental alterations. However, there are no previous records on epiphytic marine microalgae in the Sri Lankan coast. Therefore, this study aimed at identifying epiphytic marine microalgae inhabiting marine macroalgae in the Barbarian reef, Beruwala, and provides the first evidence of an epiphytic micro chlorophyte colonized within the seaweed genera from all the three marine macroalgal divisions. Morphologically different macroalgal specimens were collected in September 2018 from the Barbarian reef, Beruwala and they were identified using macroscopic and microscopic characters within two days of sample collection. DNA was extracted from the algal thalli and the nuclear ribosomal DNA Internal Transcribed Spacer regions (nrDNA ITS1-5.8S-ITS2, 650 bp) were amplified by polymerase chain reaction (PCR) from the extracted DNA using a primer pair (ITS1 and ITS4) specific for micro chlorophytes. PCR products were unidirectionally sequenced by the Sanger sequencing method using the forward primer. Sequences were checked and edited using BIOEDIT software version 7.2.6 and aligned with the nucleotide database (blastn) in National Centre for Biotechnology Information (NCBI). According to the morphological characterization of the macroalgae, nine, three and seven genera belonged to the Divisions Chlorophyta, Phaeophyta and Rhodophyta, respectively. The nrDNA ITS1-5.8S-ITS2 sequences showed a 95-96% similarity to *Acrochaete leptochaete* (JN104107.1) for the macroalgae *Chaetomorpha* (Division Chlorophyta), *Padina* (Division Phaeophyta) and *Gracillaria* (Division Rhodophyta). According to the fact that 93% sequence similarity should be achieved with that of the Genbank sequences for the species level determination of algae using the nrDNA ITS1-5.8S-ITS2 sequence, it could be confirmed that *A. leptochaete* is inhabiting *Chaetomorpha* (*A. leptochaete* - accession MK910762.1), *Padina* (*A. leptochaete* - accession MK910764.1) and *Gracillaria* (*A. leptochaete* - accession MK910760.1). This provides evidence for the broad host range owned by *A. leptochaete* among seaweed genera.

Keywords: *Acrochaete leptochaete*, DNA barcoding, Epiphytic algae, nrDNA-ITS