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DNA barcoding as a tool for conserving endemic orchids in Sri Lanka

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Orchidaceae is a well-known family of flowers, consisting of more than 30,000 species worldwide. In Sri Lanka, there are over 188 species, including one endemic genus and 55 endemic species. According to the National Red List of Sri Lanka, 71% of the orchid species are categorized as threatened, of which 2%, 9% and 29% are categorized under the critically endangered possibly extinct CR(PE), critically endangered (CR) and endangered (EN), respectively. Additionally, three endemic orchid species have been grouped under the data deficient category (DD) due to lack of knowledge on current basic information. Abundance of endemic orchids in their natural habitats is declining at an alarming rate. Hence, it is required to take immediate action for their conservation. Although legal restrictions have been enacted, one of the main threats that affects the survival of endemic orchids is trafficking. Exporting orchid plants collected from the wild under false names in export permit documents is a common practice. Unavailability of a precise, scientific species identification techniques has severely affected the efficiency of the prevention of wildlife trafficking in Sri Lanka. DNA barcoding provides a platform for accurate species identification without using morphological traits. It uses relatively a small, standardized DNA fragment as a tag to define a species. Eleven endemic orchid species with comprehensive morphological description were selected for this study to develop DNA barcodes. DNA was extracted from young leaves by using the cetyltrimethylammonium bromide (CTAB) method and the DNA was amplified using polymerase chain reaction (PCR) using the primers for the two plant barcoding regions, chloroplast *matK* and nuclear ribosomal ITS. DNA sequences were obtained by performing bi-directional sequencing using the Sanger sequencing method. The resultant consensus sequences obtained were compared against preexisting sequences of similar orchid species in the NCBI database. DNA barcodes were developed for the ITS sequences of 9 species and the matK sequences of 8 species. The developed DNA barcodes were submitted to the Barcode of life database (BOLD). This method does not require flowers to be inspected or expert taxonomic knowledge to identify suspected plant samples. Further, identification can be done with small tissue samples obtained from any part of the plant, and the process is fast and reproducible.

Keywords: Conservation, DNA barcoding, Endemic orchids