



401/D

Identification of an earthworm using molecular barcoding COI region from Polonnaruwa area

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Molecular techniques are helpful in the identification of earthworm species, especially when morphological characters are not taxonomically informative or difficult to discern. There are no reports on previous studies that have investigated molecular-based methods or combined morphological and molecular methods for earthworm taxonomy in Sri Lanka. The focus of the present study was to make the first approach using DNA barcoding to smoothen the way towards determining the earthworm richness in Sri Lanka. This study evaluated the use of both morphological and DNA barcodes to identify earthworm species inhabiting the Thamankaduwa in Polonnaruwa district of Sri Lanka. Adult clitellate earthworms were dissected and external and internal morphological identifications were conducted using 4 selected samples. According to morphological keys, resulting taxonomic features revealed that the earthworm belongs to the genus *Megascolex*. For molecular identification, genomic DNA extraction was carried out using 4 selected samples, and amplification of mitochondrial COI region was performed from extracted DNA. Sequences obtained were subjected to Basic Local Alignment Search Tool (BLAST) searches and compared to sequences available in GenBank. Results revealed 79.73% sequence similarity for the COI gene in Megascolecidae sp. Furthermore, it showed an A+T% bias, which is commonly observed in earthworms. Phylogenetic analysis performed using the Bayesian analysis showed that the obtained sequence was most closely related to *Megascolex laingii*. However, according to the morphological comparison, this finding was not fully supported, indicating the need for further taxonomic identification of this species. The average interspecific distance of sequences used for this study was 0.18%, giving a sequence threshold of 1.8%. About 95% of congeneric species pairs showed at least 15% sequence divergence, while levels of sequence divergence within a species were lower than 4%. This indicates a good separation of these species by genetic distance using the COI barcode. In conclusion, both morphological and molecular techniques confirmed that the studied earthworm belongs to the genus *Megascolex*. However, a further internal morphological examination is essential to identify the studied earthworm species to the species level.

Key words: *Megascolex*, earthworm, molecular barcoding

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