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# Establishing DNA-barcode libraries of Scandinavian clitellates

Christer Erséus<sup>\*†1</sup> and Mårten Klinth<sup>2</sup>

<sup>1</sup>University of Gothenburg, Biological and Environmental Sciences – Box 463, SE-40530 Göteborg, Sweden

<sup>2</sup>Station Linné – Färjestaden, Sweden

## Abstract

Species of Clitellata (oligochaetes and leeches) are common in aquatic and terrestrial ecosystems, and they will be of great importance when large-scale barcoding becomes an everyday approach in biodiversity assessment. During 2019-2022 and with funding from the Swedish Environment Protection Agency, we have compiled a large dataset of DNA-barcode sequences obtained in our lab over the last two decades. The objective is to establish taxonomically verified barcode reference data of as many as possible of species primarily occurring in Norway and Sweden. We have material also from Denmark and other countries in North Europe. Important is to account for haplotype variation of the genes within the species, and the latter are in many cases species hypothesis rather than formally described, binomial taxa. Four libraries, one for each of Cytochrome C Oxidase subunit I (COI), 16S rDNA (both mitochondrial), and two loci in the nuclear genome, the Internal Transcribed Spacer Region 2 (ITS2), and Histone 3 (H3) are assembled. We successfully PCR-amplified and sequenced DNA (of at least one gene marker) for totally ca. 21,000 worms (9,800 from Sweden; 9,800 Norway; 500 Denmark; 900 other countries), and we thus obtained at least Cytochrome C Oxidase subunit I (COI) mtDNA, or (if COI is missing) 16S mtDNA, sequences for all individuals. The intra-specific variation is generally lower in ITS2 and H3, and they were more selectively sampled for sequencing. We are in the process of uploading our libraries on Genbank, initially using tools in the Barcode of Life Data (BOLD) Systems. BOLD normally publishes only COI data for animals, but ultimately, all four markers will be publicly available on GenBank. At the end of 2022, we will have uploaded 12,700 sequences (ca. 4,000 COI, 3,550 16S, 2,700 ITS2, 2450 H3) from 4,000 specimens, representing ca. 530 species.

**Keywords:** DNA libraries, Scandinavia, Clitellata

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<sup>\*</sup>Speaker

<sup>†</sup>Corresponding author: [christer.erseus@bioenv.gu.se](mailto:christer.erseus@bioenv.gu.se)