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Molecular Systematics of *Limnodrilus* (Annelida: Clitellata)

Yingkui Liu

Institutionen för biologi och miljövetenskap
Naturvetenskapliga fakulteten

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Abstract

The freshwater *Limnodrilus* worms (Clitellata: Naididae: Tubificinae) are segmented hermaphroditic annelids, bearing a unique clitellum ("girdle") during sexual maturity. Some abundant and common taxa of *Limnodrilus* are ecologically and economically important in many respects, but taxonomic controversy, especially regarding the diagnosis of the cosmopolitan *Limnodrilus hoffmeisteri* Claparède, 1862 (the type species of the genus), has lasted for more than a century. In addition, the phylogenetic position of *Limnodrilus* within the subfamily Tubificinae has been uncertain.

Taxonomic studies based on molecular data, e.g., using DNA-barcoding (for animals, the mitochondrial marker COI), have revealed several examples of cryptic speciation among widely distributed clitellate morphospecies. In this thesis, I used both mitochondrial COI barcodes and nuclear ITS data to explore primary species hypotheses from a sample of the morphologically defined *L. hoffmeisteri* collected in the northern hemisphere, and a final conclusion about species boundaries was based on the congruence of the mitochondrial and nuclear phylogenies. Furthermore, the phylogeny of *Limnodrilus* was estimated based on multiple-loci data of several *Limnodrilus* species, including a new one described as *L. sulphurensis* Fend, Liu & Erséus, 2016 from a sulphur cave in North America, and other naidid taxa. Finally, as the commonly used ITS primers are neither efficient nor specific for clitellates, two new pairs of clitellate-specific ITS primers were proposed.

The molecular study showed that the well-known taxon "*L. hoffmeisteri"* actually represents a species complex (with at least ten species) rather than a single, cosmopolitan, species with great morphological variation. This work also showed that DNA barcoding, without using additional nuclear data, is likely to overestimate the number of species. Therefore, the ITS primers specific for clitellates will facilitate future research on species delimitation and the evaluation of mitochondrial DNA barcoding in Clitellata as a whole. In addition, by combining morphological and genetic information, a neotype of *L. hoffmeisteri sensu stricto* was designated, and the new species *L. sulphurensis* was discriminated from the other members of this genus. The neotype of *L. hoffmeisteri* is a baseline for future taxonomic work on the many cryptic species.

The phylogenetic hypothesis presented in this thesis contributes to our understanding of *Limnodrilus sensu stricto,* which is a well-demarcated, monophyletic genus of the naidid subfamily Tubificinae, containing at least three main evolutionary lineages (i.e., three species groups). The sister lineage of *Limnodrilus* in our taxon sample is a group of three genera, *Baltidrilus*, *Lophochaeta* and *Varichaetadrilus*. However, *Limnodrilus rubripenis* Loden, 1977 is phylogenetically closer to *Varichaetadrilus* than to other *Limnodrilus* species.

Keywords: Cryptic diversity, Species delimitation, Oligochaeta, Clitellata, Sludge worms, *Limnodrilus*, Integrative taxonomy, Neotype, DNA-barcoding, Phylogeny, Primers.