## A comparison of macrobdellid leech morphology using microCT

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## Abstract

Micro-computed tomography (MicroCT) is a powerful tool for studying the internal morphology of soft-bodied invertebrates, such as leeches, and has advantages over destructive techniques like dissection and serial sectioning. We used microCT to compare the morphology of the internal reproductive organs of two morphologically similar, yet molecularly distinct, species of North American medicinal leeches, Macrobdella mimicus and Macrobdella decora. Macrobdella mimicus is morphologically similar, yet molecularly distinct, from the type species of the genus, Macrobdella decora. Our goals were to compare the internal reproductive morphology of these two species, to assess the influence of breeding condition (i.e., seasonality) on the reproductive organs, as well as to assess scan quality of specimens recently collected (< five years) versus historical specimens. Six specimens each of M. mimicus and M. decora were stained with 0.3% phosphotungstic acid and 3% DMSO in 70%ethanol for 2 weeks and scanned using a GE Phoenix v—tome—x micro-CT scanner with the 180 kV Nanofocus tube configuration. The best scan of each species was segmented for 3D reconstruction using the software package Amira v. 2019.1. We found subtle morphological differences between the two species, although size differences of the male reproductive systems and the accessory organs was likely influenced by the breeding condition of the leech. The oviduct of *M. decora* was more convoluted and longer than that of *M. mimicus*, and the oviducts join inside the vagina of *M. mimicus* rather than outside as in *M. decora*. The epididymes of *M. decora* had more coils and the internal vas deferens were much longer than observed in *M. mimicus*. Historical specimens seemed to absorb stain better, but all specimens resulted in high quality scans. MicroCT is a promising technology for documenting fine scale internal morphology of soft-bodied invertebrates, but is not yet scalable for collection-wide digitization efforts.

Keywords: imaging, Hirudinea, 3D reconstruction

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