A guide for optimal iodine staining and high-throughput diceCT scanning in snakes

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Abstract

Diffusible iodine-based contrast-enhanced Computed-Tomography (diceCT) visualizes soft-tissue from microCT (μCT) scans of specimens to uncover internal features and natural history information without incurring physical damage via dissection. Unlike hard-tissue imaging, diceCT datasets are currently limited to a few individual specimens and taxonomically underrepresented. To initiate best practices for diceCT in a non-model group, we outline a guide for staining and high-throughput μCT scanning in snakes. We scanned the entire body and one region of interest (i.e., head) for 23 specimens representing 23 species from the clades Aniliidae, Dipsadinae, Colubrinae, Elapidae, Lamprophiidae and Viperidae. We generated 82 scans that include 1.25% Lugols iodine stained (soft tissue) and unstained (skeletal) data for each specimen. We found that duration of optimal staining time increased linearly with body size; head radius was the best indicator. Post-reconstruction of scans, optimal staining was evident by evenly distributed grayscale values and clear differentiation among soft-tissue anatomy. Under and over stained specimens produced poor contrast among soft-tissues, which was often exacerbated by user bias during "digital dissections" (i.e., segmentation). Regardless, all scans produced usable data from which we assessed a range of downstream analytical applications within ecology and evolution (e.g., predator-prey interactions, life history, and morphological evolution). Ethanol de-staining reversed the known effects of iodine on the exterior appearance of physical specimens, but required substantially more time than reported for other de-staining methods. We discuss the feasibility of implementing diceCT techniques for a new user, including approximate financial and temporal commitments, required facilities, and potential effects of staining on specimens. We present the first high-throughput workflow for full-body skeletal and diceCT scanning in snakes, which can be generalized to any elongate vertebrates, and increases publicly available diceCT scans for reptiles by an order of magnitude.

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