

Reviewer Report

Title: Living in darkness: Exploring adaptation of *Proteus anguinus* in 3D by X-ray imaging

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Reviewer name: Jia Jia

Reviewer Comments to Author:

The blind white olm (*Proteus anguinus anguinus*) are unique in many aspects of its biology, including geographic distribution, development and particularly its salient troglomorphy, which resulted from its morphological adaptation to underwater cave-dwelling lifestyle. As mentioned in this technical study, our knowledge on this particular salamander species was accumulated bit by bit over the past few hundred years. Now we are standing at a point where details of morphological and developmental changes of this taxon can be better revealed by non-destructive methods based on more readily available specimens raised in labs due to the advancement of high-resolution microCT scan and contrast enhancing techniques. Sharing of high-resolution CT images of endangered species that are significant to advance our understandings of certain scientific questions should always be encouraged.

This manuscript authored by Tesař et al. provided a dataset of contrast-enhanced microCT images for the head region of three specimens (a larva, a juvenile and an adult) of *Proteus anguinus anguinus* and also images for two specimens (a larva and an adult) of *Ambystoma mexicanum* for the purpose of comparative studies. Besides many annotations I made in the word file as attached, here are the rest of my other concerns:

- 1) The comparison between the olm and the axolotl is reasonable considering both species are neotenic and one is cave dwelling and the other is surface dwelling, and therefore avoid introducing noises from changes created by metamorphosis; however, why not choose any neotenic species in the sister group genus of *Proteus*, *Necturus*, for comparison, considering their phylogenetic closeness and disparate lifestyles (*Necturus* is surface dwelling in slow-moving streams)?
- 2) After checking the dataset by loading dicom files into software VG Studio, it seems that the larval specimen of the olm has some shrink in the head region, which may be unavoidable, but I would appreciate if you can enrich the descriptions for the contrast-enhancing experimental procedures by providing the length of the rehydration process for each specimen.
- 3) Most of the datasets contain a complete head region except the one for the adult specimen of the axolotl, which has the posterior part of the hyobranchial apparatus missing. It is highly recommended to provide a more complete dataset for the axolotl adult specimen.
- 4) The dataset for the adult specimen of the olm can not be properly loaded in visualization softwares like VG Studio and Photoshop, because it has three damaged dicom files, i.e., "Proteus_anguinus_adult_0661.dcm", "Proteus_anguinus_adult_2023.dcm" and "Proteus_anguinus_adult_2349". The first image is 590 kb, and the latter two are 0 kb in size, in contrast to most other images in the same dataset which are 637 kb. It's also noticeable that 233 images ranging from "Proteus_anguinus_adult_2116.dcm" to "Proteus_anguinus_adult_2348.dcm" are 2317 kb, and can not be properly loaded into software with files of 637 kb.

Generally speaking, the manuscript is clearly written, and the dataset is easily accessible and well controlled. I look forward to its formal publication. Feel free to contact me (jia.jia@ucalgary.ca) directly if any of my comments are unclear.

Best wishes,

Jia

October 8, 2021

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