Reviewer Report

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

Version: Original Submission **Date:** 10/11/2021

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Reviewer Comments to Author:

The authors have studied the cranial anatomy of the Proteus anguinus by x-ray tomography. In particular, they have imaged and segmented the sensory system of these animals across three different stages of development: larval, juvenile and adult. They find that although eye development starts in the larval stage, it gradually reduces its size to the point of blindness, probably due to adaptation to a cave environment. In contrast, axolotls fully develop their eyes.
Overall the scientific data provided by the authors will certainly lead to further studies on the evolution of the salamander brain in the contest of cave adaptation. However, I do not recommend the publication of this manuscript without a major revision.
The authors should segment not only the brain, olfactory epithelium, residual eye and skull but also ear labyrinth and muscles across the three different stages of development. They should provide a side-by-side comparison in Figure 5 with the axolotl giving inputs on the different predatory habits by comparing the muscles and the jaws of the two species.
The authors should also double check the scale bars of the top and middle image in Figure 1 as the dimensions of the animals are significantly different between the two stages of development.
It is not clear Figure 2 relates to the juvenile specimens from the text and this should be improved.
Furthermore the presentation of the synchrotron x-ray tomography of the axolotl brain in Figure 4 is redundant given the scope of the manuscript which is the study of Proteus anguinus sensory system. Either the authors present synchrotron images of the Proteus anguinus or they should omit the figure entirely as this is confusing to the readers.
Finally, it is not clear the reason why the authors have stained the specimens with PTA and Iodine for several weeks as the contrasting protocol initially developed by Brian Metscher involves only overnight or few days of staining. Perhaps the authors could share the reason for this very long method.

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