3D imaging of mysterious cave-dwelling salamander reveals adaptations for life in the dark

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Image of Proteus anguinus with inset showing a 3D rendered image of a proteus head. Credit: Gregor Aljančič

A showcase of evolutionarily designed sensory organs is revealed

through high-resolution images of the head of the exclusively cave-dwelling blind salamander, Proteus anguinus. The proteus is an evolutionary masterpiece of an unusual set of adaptions for surviving in lightless caves. Even its physical appearance is unique, to the extent that locals in the 1600s believed it was a baby dragon. The research here, carried out by an international collaboration led by Czech scientists Jozef Kaiser and Markéta Tesařová, was aimed at gaining detailed information about these unusual evolutionary changes. The scientists used X-ray computed microtomography (microCT) scans to produce stunning 3D reconstructions of the soft tissue in the head of the proteus, allowing them to directly view the extensive changes that have occurred in the salamander over time. The research has been published in the journal *GigaScience*.

Proteus anguinus lives in pitch-dark karst caves in Central and Southeastern Europe. It is also known as the olm or "human fish." The work in this study brings to light extensive details of the proteus' unique evolutionary mechanisms. Additionally, since these images and 3D models are digital data, scientists from all over the world can also access and use these in their own research, which is particularly valuable, given the proteus is endangered and making physical samples nearly impossible to share. The published article includes colorized 3D reconstructions and an easy-to-explore online model (which is also viewable using a VR headset). Additionally included is a video abstract showcasing live and animated images, helping highlight the main findings.

The proteus is the largest cave tetrapod, and the only European amphibian that lives exclusively underground. It is a carnivorous creature, lives off small crustaceans, and swallows its prey whole. Often highlighted as one of the animals with the strangest adaptations to survive, it is snake-like in appearance, colorless, and can live up to 100 years. Due to living in an underground environment, one of its

adaptations is starvation resistance— allowing it to survive up to 10 years without eating. Proteus also has both gills and lungs, unlike most amphibians that as adults lose their gills and move out of the water.

Early naturalists were fascinated by this creature of darkness, including Charles Darwin, who described the species as a "wreck of ancient life." The work in this study using advanced modern-day imaging technology, finally allows centuries-old questions about this strange creature to be answered.

For their research, the scientists used X-ray microCT scanning technology and 3D modeling from multiple samples, including getting images from a closely related surface-dwelling salamander, the axolotl. This provided the researchers with a range of data to assess the changes in the form, shape, and structure of organs of proteus.

The researchers make note of the breadth of analyses these data provided them, saying:

"We accessed several collections to cover developmental stages from larval to adult specimens. Thus, the data can be used to study developmental and evolutionary differences between the stages. Also, making data of proteus and axolotl accessible allow to make an exemplary comparison between cave- and surface-dwelling paedomorphic salamanders."

The ancestors of the proteus lived above ground and had functional eyes, but once they began living in lightless caves, selective pressure for retaining vision was gone. The result has been that the proteus' visual organs are small and incompletely formed, leaving them blind. As a holdover from their ancestors, however, the rudiments of eyes are present in the early stages of life, and the loss of eyes happens during development from juvenile to adult. Because the data generated by the

researchers includes images from both juvenile and adult proteus' heads, researchers can get far better information about changes of visual development and loss, including changes in position, shape and size as the animal grows into adulthood.

Loss of unnecessary elements can happen during evolution, but if survival requires new mechanisms, physical or behavioral changes appear. For the proteus, without vision, other sensory organs needed to change to allow them to obtain food and other resources. To investigate such changes, the Czech research group did microCT scans at different stages of the axolotl to look at different parts of the skull and sensory organs for comparison. The new microCT data and 3D modeling available provide such high resolution, researchers can now get extremely fine details of these changes, rather than more basic changes, such as the proteus odor sensing organ is much larger in the proteus than in axolotl.

Although research using these new data have focused on investigating physical changes, these could also aid in another area of evolution research: investigating behavioral changes important for survival.

Author Lucia Mancini, from the Elettra Sincrotrone synchrotron facility in Trieste (Italy), highlights the significance of imaging methods that use electromagnetic radiation (a synchrotron):

"The application of advanced 3D imaging technologies can give insights into the living strategies of the proteus. In fact, thanks to the combined use of synchrotron and laboratory-based analyses, future studies could help to model the mechanisms of behavioral adaptation, to better understand the habitat use."

Her colleague Edgardo Mauri from the Speleovivarium Erwin Pichl adds, "The data will allow us to study perceptive abilities through three-

dimensional models and to research behavioral responses in relation to chemical cues, auditory frequencies or emission of signals."

Given the rarity of the proteus, it is extremely difficult for researchers to obtain physical samples. Now that these imaging resources are available in a shareable digital format, scientists throughout the world can take advantage of this resource to gain new insight into evolutionary mechanisms. Such studies, as well as providing new basic understanding of how species change over time, with climate change and its impact on survival of species, can improve understanding of evolutionary needs for survival.

More information: Markéta Tesařová et al, Living in darkness: Exploring adaptation of Proteus anguinus in 3 dimensions by X-ray imaging, *GigaScience* (2022). DOI: 10.1093/gigascience/giac030

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