What's inside a fishy suction cup?

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Aims

The Northern Clingfish *Gobiesox maeandricus* is a small intertidal fish that is commonly found along the Pacific coast of the United States and Canada. A remarkable feature of the clingfish is its ability to adhere itself to rocks by using a ventral suction disk (Figure 1). Clingfishes that are attached to a surface can resist pulling forces that are beyond 200 times their own body weight even on rough substrates on which engineered rubber suction cups fail[1]. Although skeletal and muscular structures in the clingfish suction disk have been described previously[2,3,4], it remains cryptic, how the different elements function during attachment and detachment. Here we used desktop micro-CT imaging to study the functional morphology of the clingfish suction disk. The aims of this study were: (1) to provide a detailed description of the skeleton and musculature in the clingfish suction disk and (2) to reconstruct the movements of the suction disk during attachment and detachment to a surface.

Figure 1: The Northern Clingfish, *Gobiesox maeandricus*, adhering to a rock.

Method

We used an 82 mm specimen of *Gobiesox maeandricus*. This animal was collected in the intertidal zone of San Juan Island, WA, USA, euthanized and then stored in 70% ethanol. Prior to micro-CT imaging, we re-hydrated the specimen by stepwise (50%, 30%; each step was maintained for 24 h) decreasing the ethanol concentration until the specimen was transferred to distilled water. After re-hydration, we submerged the specimen for 24 h in 1% LUGOL's iodine-potassium iodide solution, which has been previously shown to significantly improve the contrast within soft-tissues for micro-CT imaging[5,6]. After the treatment with LUGOL's solution, we kept the specimen over night in distilled water.

To make sure that the specimen was not moving during the micro-CT scan, we gently squeezed the specimen into a cylindrical plastic tube that had approximately the same

dimensions as the clingfish. We then filled this specimen container with distilled water and mounted it into a Skyscan 1172 desktop micro-CT scanner (Figure 2A). The x-ray source voltage of the micro-CT scanner was set to 100 kV and the current was 100 µA. We operated the desktop micro-CT scanner with a 0.5 mm aluminum filter in front of the x-ray detector. The pixel size of the micro-CT scan was set to 26.66 µm. We recorded x-ray projections of the specimen with a step size of 0.4º over 360º. To reduce the noise in the captured x-ray projection images, we set the "*averaging*" option of the Skyscan micro-CT scanner to 10, i.e. ten images were captured for each rotational step and then an averaged image was kept for further processing. Because of its length, the clingfish extended well beyond the field of view of the x-ray detector and we had to scan the specimen in five subscans that were later automatically merged by the reconstruction software Nrecon 1.6.6 during the calculation of the volumetric dataset.

After analyzing the resulting micro-CT dataset, it became evident that the LUGOL's iodine-potassium iodide stain only penetrated the superficial tissue layers (Figure 2B). We then repeated the staining step with 1% LUGOL's but this time stained the specimen for seven days. After staining, we repeated the micro-CT imaging procedure with the exact same parameters as during the first micro-CT scan. This second micro-CT dataset showed much better soft-tissue staining, especially in the musculature (Figure 2C).

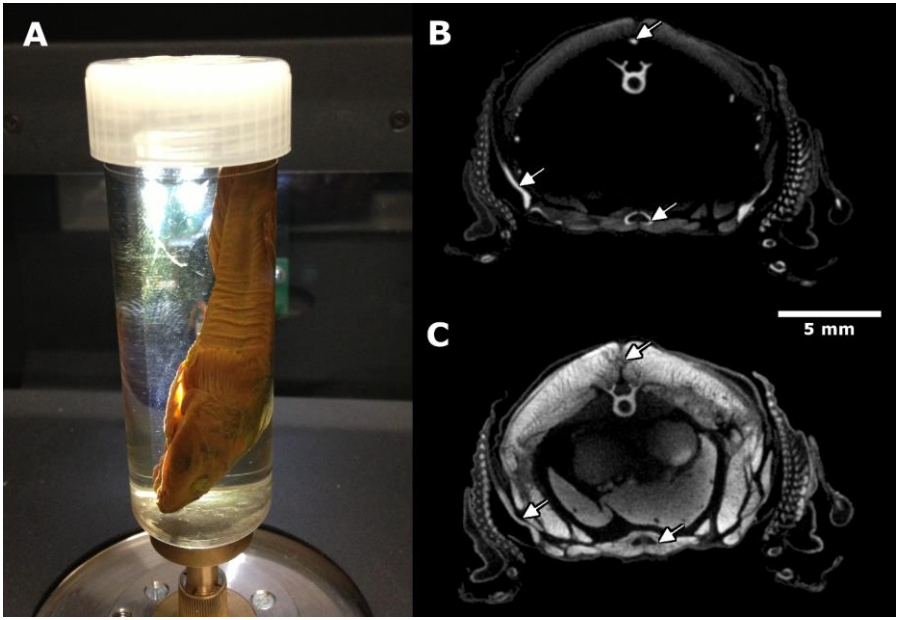

Figure 2: Micro-CT imaging of a clingfish. A: The specimen was stained in iodine-potassium iodide (LUGOL's) and then transferred to a plastic jar filled with distilled water for micro-CT scanning. B and C: Reconstructed transversal sections through the fish after CT imaging. In B, the fish was submerged in LUGOL's for only 24 hours; in C, the same specimen was stained for seven days. Longer staining times significantly improved the contrast within soft-tissues; however, especially smaller bones are much harder to identify in the strongly stained dataset (arrowheads).

Due to the stronger soft-tissue staining in our second trial, however, the differences in the x-ray attenuation coefficients (i.e. the exponential attenuation of x-rays in a material[7]) between soft and hard tissues were less pronounced than in the first scan, which made it harder to

automatically isolate skeletal elements from soft tissues in the second dataset. To account for this, we co-registered the two datasets by using the co-registration feature in the software tool DataViewer 1.5. For co-registration, we oriented the two datasets based on the skull and the vertebral column that were easily identifiable in both datasets. We then loaded both datasets simultaneously into the image analyzing software Amira 5.4 (Visualization Sciences Group) and displayed them by using Amira's built in Volume Rendering feature (*Volren*). The first (weakly stained) dataset was then used to segment bones, while the second (strongly stained) dataset was used to segment muscles.

Based on the segmented data, we converted each skeletal element that was part of the clingfish suction disk into a polygonal surface that were then used to create a physical model of the clingfish suction disk with a ZPrinter 310 rapid prototyping machine (3D printer). The 3D printed model was twenty times larger than the original structures in the clingfish. Further, we glued strings of yarn to the 3D printed model to highlight muscle attachments.

Results

By using micro-CT imaging in combination with LUGOL's solution as contrast agent, we were able to visualize bones and muscles in the clingfish. Co-registration of two datasets of the same individual allowed us to easily identify bones based on grey-value segmentation in the poorly stained dataset while we interactively segmented muscles in the stronger stained dataset. We segmented a total of 212 bones and muscles that were related to the jaw and the suction disk of the fish (Figure 3A). By generating an up-scaled physical model of the clingfish suction disk, we were able to interactively explore the degrees of freedom along which the individual elements can be transformed as well as the connectedness of the structures (Figure 4).

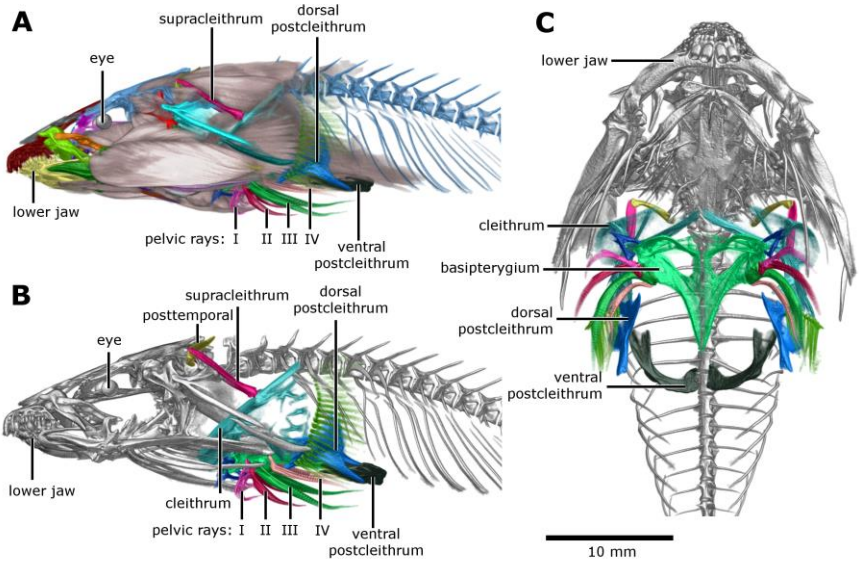

Figure 3: Volume renderings of the co-registered clingfish micro-CT datasets in lateral (A and B) and ventral (C) view. A: In total, 212 structures have been segmented, highlighted here by different colors. B and C: Only the skeletal elements that contribute to the suction disk are highlighted, muscles not shown.

Figure 4: Rapid prototyping. By using a rapid prototyping machine, we generated a twenty times up-scaled physical model of the clingfish suction disk. A: The bones of the suction disk were created as individual elements based on the segmented micro-CT data. B: The individual pieces were then reassembled. C: Yarn was used to highlight the position of muscles in the clingfish suction disk.

The suction disk skeleton is characterized by a strong integration of pectoral (i.e. corresponding to the shoulder) and pelvic (i.e. corresponding to the hip) elements as well as for fishes highly unusual flexible suspension of the pectoral girdle against the braincase. The center of the suction disk is build from the paired and widely enlarged bases of the pelvic fins (i.e. the basipterygia, Figure 3C), which can be folded against each other and thus provide a pumping mechanism to evacuate the suction disk's chamber. The anterior (i.e. towards the front) and posterior (i.e. towards the back) rims of the suction disk are supported by elements of the pectoral girdle – i.e. the cleithrum and the postcleithra (Figure 3). The entire suction disk is connected to the braincase by two rod-like bony elements (called the posttemporal and the supracleithrum; Figure 3A and B).

Conclusion

Micro-CT imaging allowed us to gain new insides on the anatomy and function of the ventral suction disk in the Northern Clingfish *Gobiesox maeandricus*. By post-processing of the micro-CT data to generate a physical 3D printed model of the suction disk, we were able for the first time to reconstruct the movements of the separate elements. Our results may potentially contribute to improve manufactured suction devices in a way to maintain high suction forces even on rough substrates.

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