STRUCTURAL DIFFERENCES BETWEEN ALLIGATOR PIPEHORSE AND BAY PIPEFISH TAILS

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ABSTRACT

This study compares the structure and mechanical properties of the bony plates from the tails of the Alligator pipehorse and the Bay pipefish. These bony plates provide the fish support and protection. The tail structures of both species were investigated by optical and scanning electron microscopy. Partial deproteinization of the samples revealed differences between the connections of the bony plates. The plates of the pipehorse overlap, allowing for flexibility of its tail. In contrast, the plates in the pipefish interlock, making the tail more difficult to bend. These mechanisms contribute to the prehensility and non-prehensility of the tails of the pipehorse and pipefish, respectively.

1. INTRODUCTION

Advancements in engineering mechanics and sciences often evolve from studying the natural world. Many field of engineering draw inspiration from the observation of biological materials at a high magnification. Several examples of successful engineering innovations inspired by nature are Velcro® inspired by plant burrs, adhesive tape inspired by the gecko's feet, and the design of wind turbines based on humpback whale tubercles.

We previously investigated the structure and unusual deformation mechanisms of the seahorse (Hippocampus kuda) tail, and now extend the analysis to other members of the family Syngnathidae. Two species of pipefish, one with a prehensile tail (Alligator pipehorse, Syngnathoides biaculeatus), and one without a prehensile tail (Bay pipefish, Syngnathus leptorhynchus), were studied to observe structural differences between the tails. Both species belong to the family Syngnathidae along with seahorses and seadragons. Ahnesjö and Craig expressed the significance of the Syngnathidae ability to make unusual biological adaptations; recommending this family as a model for studies in evolution.

The Alligator pipehorse has a body that is tapered at both ends; therefore, sometimes it called a "double-ended pipefish." For security and safety, the pipehorse dives vertically down and grasps onto sea grasses for camouflage. The Bay pipefish is also characterized by its slender body shape and camouflages in sea grasses for protection. However, the pipefish does not grasp onto sea grass or swim with a vertical posture. Figure 1 shows photographs of both species of pipefish.

Both the pipehorse and pipefish are weak swimmers, using their dorsal and pectoral fins as their principal organs of locomotion. Regardless of their similarities, the pipehorse has a prehensile tail that allows it to grasp onto sea grass, while the pipefish lacks prehensility.
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Figure 1. Photographs of (a) Alligator pipehorse and (b) Bay pipefish used in this study.

Similar to the seahorse, the pipehorse and pipefish have external bony plates that are organized along the length of their body. The seahorse has a body covered by ring-like segments made of bony plates connected by rotating and sliding joints that allow it to bend its tail into a logarithmic spiral. Additionally, these bony plates provide body structure and support. The distinct S-shape of the seahorse and its horse-like head distinguishes it from pipefish. Porter et al. found the ring-like segments could withstand large deformations without fracture through local buckling and joint sliding, thereby providing the animal with crushing protection from predators.

There are many articles that discuss seahorses and pipefish from a biological point of view, but little research has been done on the structure of the pipefish armor. Due to the similar habitat and external appearance of both the pipehorse and pipefish, the bony plated structure may be one of the reasons (along with other musculoskeletal components, such as the vertebrae and muscles) of their dissimilar mechanical functionality (prehensility in the former and non-prehensility in the latter). Furthermore, this study may provide a better understanding of the mechanisms that provide prehension in Syngnathidaes, which could inspire advancements in mechanical design technologies.

2. MATERIALS AND METHODS
2.1 Sample preparation
A fully grown Alligator pipehorse and a Bay pipefish (see Figure 1) were donated by the Birch Aquarium at Scripps Institute of Oceanography, University of California, San Diego, in June 2012. They died naturally in the aquarium and were kept frozen for several weeks until use. Prior to analysis, the specimens were thawed by immersing them in water at room temperature. Once defrosted, the pipefish were preserved in 70% isopropanol. Several small sections (~ 5 mm length) from the midsection of the tails were cut for analysis.

2.2 Partial deproteinization
The tails were immersed for ~2-3 days in 10-25 ml of an aqueous 0.5 N NaOH solution until the skin was removed and the samples fell apart into individual bony plates. The NaOH solution was replaced daily and sample observations were recorded using an optical microscope.

2.3 Optical microscopy
Partially deproteinized (to better visualize the structure of bony plates and connections) and untreated samples were analyzed by optical microscopy using a Zeiss Axio imager equipped with a CCD camera (Zeiss Microimaging Inc., Thornwood, NY) and a VHX-1000 digital microscope system equipped with a CCD camera (KEYENCE Corporation, Osaka, Japan).
2.4 Micro-computed tomography (μCT) analysis

Whole body samples were investigated by a micro-computed tomography (μCT) scanner, Skyscan 1076 (Kontich, Belgium). The samples were wrapped in a tissue paper that was first moistened with a phosphate buffer saline (PBS) solution and placed in a sealed tube to prevent the specimens from drying out during the scanning process. The samples were scanned using a 0.5 mm aluminum filter. Imaging was performed at 9 μm isotropic voxel size with an electric potential of 48 kV and a current of 200 μA. A beam hardening correction algorithm was used for image reconstruction. Skyscan's Dataviewer and CTVox software were used to analyze and developed images and 3-dimensional (3D) models.

2.5 Scanning electron microscopy

Samples for scanning electron microscopy (SEM) were dried using a critical point dryer (Tousimis Autosamdry-815, Rockville, MD). The dried samples were sputter-coated with iridium using an Emitech K575X sputter coater (Quorum Technologies Ltd., West Sussex, UK) prior to imaging. The samples were observed at 10 kV with a Philips XL30 field emission environmental scanning electron microscope (ESEM) (FEI-XL30, FEI Company, Hillsboro, OR).

2.6 Microhardness

The microhardness of the untreated bony plates were measured with a micro-hardness testing machine (LECO M-400-H1) equipped with a Vickers indenter. Individual bony plates of each tail segment were embedded in epoxy resin and polished until the surfaces of the samples were exposed. A load of 10 gr was utilized to indent the exposed surfaces. The Vickers hardness of the bony plates was evaluated by:

\[ HV = \left( \frac{1.854F}{d^2} \right) \times 9.81 \]

where \( HV \) is the Vickers hardness number in MPa, \( F \) is the applied load in kgf, and \( d \) is the arithmetic mean of the two measured diagonals in mm.

3. RESULTS AND DISCUSSION

The structural framework of the pipehorse is composed of a vertebral column and bony plates that form ring-like segments. Each segment consists of four bony plates that overlap at the dorsal (back), ventral (front), and lateral (side) mid-lines. The plates decrease in size from the proximal to distal end. At the most distal segments, there are no plates and only the vertebrae remain (Figure 2a). The lack of plates at the distal end is believed to play an important role in the prehensility of Alligator pipehorse tail. μCT imaging in Figure 2a shows the lower part of the pipehorse body, including its prehensile tail. A cross-sectional view (Figure 2b) shows the four bony plates that form the four corners of each segment that surround the central vertebrae.

The pipefish is also composed of a vertebral column and bony plates forming ring-like segments that decrease in size towards the distal end (Figure 2c). Compared to the pipehorse, the main difference is that the plates cover the whole length of the body, and therefore, do not allow prehensility. Figure 2d illustrates that the cross-section of the pipefish tail is also square-shaped, consisting of four bony plates that form each of the four corners. Within each segment, the bony plates overlap at the ventral, dorsal, and lateral mid-lines.

Similar to the pipehorse and pipefish, the bony plates of the seahorse overlap at the mid-section of the dorsal, ventral, and lateral sides of each tail segment. Hale describes the different joints that connect the bony plates and vertebrae in the seahorse. The transversal and haemal spines (Figures 2b and 2d) connect to the bony plates as pivoting joints, governed by collagenous connective tissues. This is similar to a ball-and-socket joint that provides three degrees of rotational freedom.
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Within each segment of the seahorse, the bony plates are connected to each other with gliding joints, allowing for translational sliding motion. Gliding joints also connect the segments to one another.

Figure 2. Micro-computed tomography images of the tails and corresponded cross-sections of (a,b) the Alligator pipehorse and (c,d) the Bay pipefish. Colors were added to enhance clarity.

Figure 3 shows SEM images of individual bony plates of the pipehorse and pipefish. The plates of the pipehorse overlap, while the plates of the pipefish interlock (tab and slot). The overlapping area of the pipehorse plates is apparent in the optical micrograph of the midsection of the tail shown in Figure 4. The interlocking joints of the pipefish are shown in the SEM images in Figure 5, showing a tab and slot configuration. This interlocking connection between the bony plates of the pipefish restricts movement between each segment, making it difficult for the tail to bend. In contrast, the overlap of the plates in the pipehorse provides a higher range of mobility between adjacent segments. This helps give the tail the ability to bend ventrally.

The dorsal views of both tails show connections of the bony plates between each adjacent segment (Figure 6). From these μCT images, it is clear that the bony plates of the pipehorse overlap, allowing for more freedom of movement between each segment, and an overall more flexible tail structure. In contrast, the bony plates between each segment of the pipefish are more restricted, giving the tail a more rigid structure.
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Figure 3. SEM images of individual bony plates of (a) Alligator pipehorse, and (b) Bay pipefish (due to extreme thinness, the plate was curled on either side from dehydration). Areas that connect the adjacent segments of the plates are circled in red.

Figure 4. Overlapping bony plates of the Alligator pipehorse under optical microscopy. The section where the two plates overlap is circled.

Microhardness data for both pipehorse and pipefish are summarized in Figure 7. Microhardness testing of the bony plates reveals a similar distribution of hardness across the cross-sections for both species of pipefish that does not seem to vary with location. The average hardness of the pipehorse plates is $400 \pm 40$ MPa, while that of the pipefish plates is $410 \pm 60$ MPa. Microhardness data for both species correlate well with microhardness measurements for seahorse bony plates taken with the same load. The average hardness of the seahorse plates was found to be $420 \pm 50$ MPa\textsuperscript{6}. These data indicate that the prehensile ability if the tail does not arise from differences in the mechanical properties of the bone tissue.
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Figure 5. SEM imaging of Bay pipefish bony plates. (a) Top view of a single plate with a magnified view of its ends. (b) Interlocking connection between two plates.

Figure 6. Micro-computed tomography images of the dorsal view of the bony plates of the (a) Alligator pipehorse and (b) Bay pipefish. A segment connection in each tail is circled.
4. CONCLUSIONS

The structure and mechanical properties of the bony plates from the Alligator pipehorse tail and the Bay pipefish tail were analyzed by microscopic methods and hardness testing. It can be concluded that:

- Both species have structural anatomies that are similar, including segments that consist of four bony plates overlapping in the midsection of all four sides;
- The bony plates in adjacent segments overlap in the pipehorse, providing a more flexible structure. In agreement with other researchers, the lack of plates at the distal end of its tail provides the pipehorse prehensility;
- The bony plates in adjacent segments interlock in the pipefish, giving it a more rigid structure;
- There is no significant difference between microhardness values of the plates of the prehensile (pipehorse) and non-prehensile (pipefish) tails.

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