Investigation of the Function of the First Epicentral Intermuscular Bone and the First Anal-fin Radial Bone in *Polymixia lowei*

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Abstract

Beardfishes, members of the genus *Polymixia,* are seen as living fossils, as they are the sole survivors of a Late Cretaceous marine fish radiation. In this genus, the first intermuscular bone differs from the rest by lying in the horizontal septum and by being distinctively larger. In addition, the first anal-fin radial bone is distinctively enlarged. To investigate the function of these unique characteristics of *Polymixia*, we examined multiple specimens and perfected a method for staining nerves to reveal the innervation of the unique structures. In doing so, we hypothesize that the function of these structures is related to sound production involving the swim bladder, and that the nerves will reveal important clues to their functions.

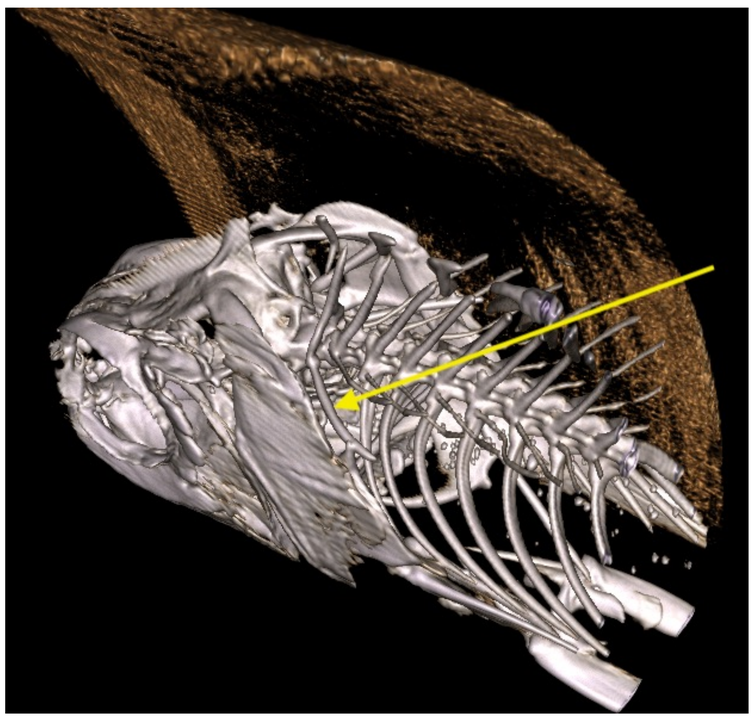

Fig. 1. µCT Scan of the anterior half of *Polymixia lowei*. Yellow arrow points out the first intermuscular (epicentral) bone on the centrum of the first vertebra (V1).

Introduction

Species of *Polymixia* are referred to as beardfishes for the pair of hyoid barbels underneath their chin. These species of fish have been found to reside over the outer areas of continental shelves and continental slopes in the Pacific, Indian, and Atlantic oceans. All species of *Polymixia* have the same unusual skeletal characteristics of a first intermuscular bone (Fig. 1) lying in the horizontal septum and a first anal radial that is distinctly enlarged. There has been much controversy regarding the classification of the first intermuscular bone of the vertebra. It was initially classified as an epineural due to its development in series with epineurals and the lack of epicentral characteristics in other teleost fish. However, more recent evidence has presented a compelling case for the reclassification of the bone. This reclassification has still not shed light on the function of the intermuscular bone. One possible function of this bone could be related to the uniquely enlarged first anal radial. It is known that the swim bladder rests in between the two skeletal features and might be involved in sound production. There is evidence that extrinsic muscles connect to the swim bladder or to other modified bones to produce sound in some fish species. To investigate a possible link between the unique skeletal structures of *Polymixia* and sound production, we examined specimens by µCT scanning (Fig. 1) and numerous specimens cleared and stained for bone and cartilage. Procedures for staining whole cleared specimens also for nerves were developed by Filipski & Wilson (1984) and have recently become more widely used. To identify the nerves associated with these unique bones, we worked with two specimens to modify and perfect procedures for staining whole cleared specimens for nerves.

Results

We were successful in staining for nerves, but were not able to find nerves that would have revealed a function for both the intermuscular bone and the first anal radial (Figs. 2–4).

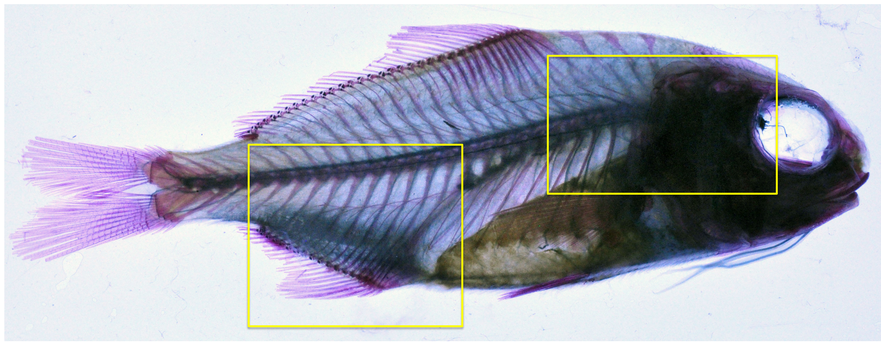

Fig. 2. Full body picture of Fish 2. This fish is double stained for nerves and bones. The elements stained in pink are bone. The elements stained in purple/black are nerves. The yellow rectangles show the regions seen in more detail in Figures 3 and 4.

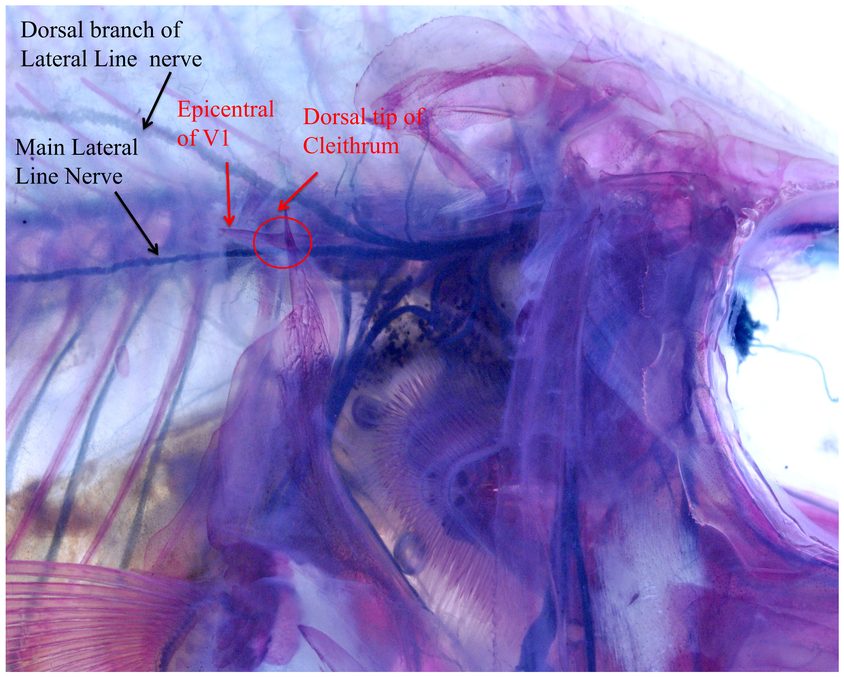

Fig. 3. Close-up of the area of the first intermuscular bone in Fish 2. The lateral-line nerve and its dorsal branch are indicated by black arrows. The intermuscular and the tip of the cleithrum are shown by red arrows. The red circle shows the close association between the cleithrum and the intermuscular bone.

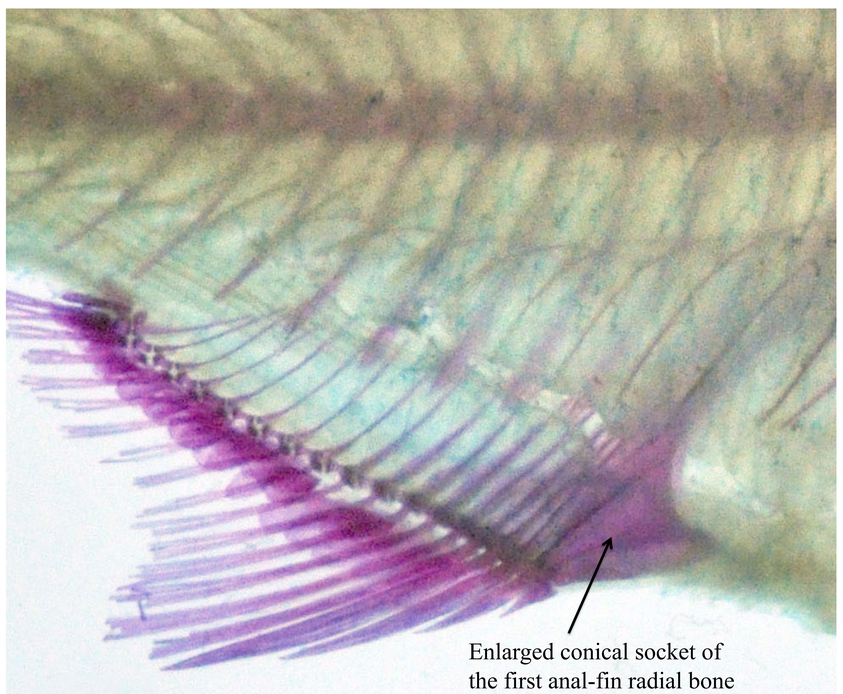

Fig. 4. Close-up of the enlarged and socketed first anal radial of Fish 1. This fish is double stained for Cartilage and Bone. The small blue elements are cartilage, and the pink elements are bone. The end of the airbladder fits into the socket of the radial.

Nerve-Staining Methods

To perfect the nerve-staining method (Fig. 5), two specimens of *Polymixia lowei* were used. In the fall semester, one fish was stained with Alcian Blue for cartilage and Alizarin Red S for bone. Staining for nerves with Sudan Black B was attempted but was not sufficiently successful. The second fish was stained only with Alizarin Red S for bone and Sudan Black B for nerves, and the timing for enzymatic maceration was shortened. The omitted step for the second fish was the initial transfer to Alcian Blue, which stains for cartilage but can obscure structures. After staining, both fishes were transferred to increasingly concentrated aqueous solutions of glycerin for clearing (making the flesh transparent) and study.

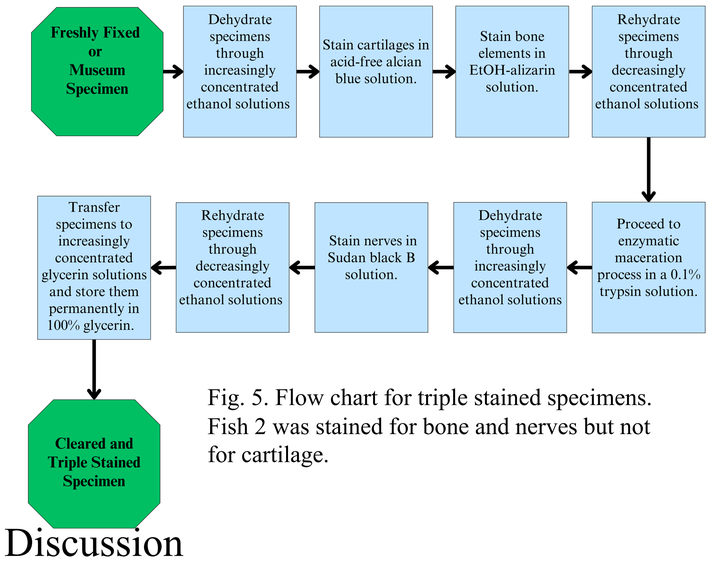

Fig. 5. Flow chart for triple stained specimens. Fish 2 was stained for bone and nerves but not for cartilage.

Discussion

In this investigatory study, we were unable to see proof of peripheral nerves closely associated with either the first intermuscular bone or the first anal radial. We also can see that the swim bladder is displaced some distance away from these bones by the staining procedure. However, one interesting revelation is the relationship between the first intermuscular bone and the cleithrum. It seems that the epicentral is in contact with the dorsal tip of the cleithrum. This is notable as it is well documented in catfish and other species that the cleithrum can be used in sonic production. Exploring this relationship can be the next step in future research. A possible experiment to investigate the nature of sound production would be to record audio of *Polymixia*. Another possible experiment would be to continue staining the fish with a special focus on the cleithrum and epicentral. Fish 1 was stained first in the fall semester and was triple stained to reveal bone, cartilage, and nerves. The bone and cartilage stains were successful, but unfortunately, the nerves were not stained to a satisfactory level. Esguícero and Bockmann (2022) outline many possible ways that nerve differentiation could have gone wrong. Sudan Black B stains the myelin sheaths that insulate the neurons from the outside. When the myelin sheaths become damaged, nerve differentiation is substandard or non-existent. Fortunately, the same mistake was not repeated on the second fish specimen as peripheral nerves are visible to the naked eye.

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