2008

Morphology of the inner and peripheral ear of the loricariid catfish Pterygoplichthys gibbiceps K.

Brian David Rodgers
Western Kentucky University

Morphology of the inner and peripheral ear of the loricariid catfish *Pterygoplichthys gibbiceps* K.

by

Brian David Rogers

A Capstone Experience/Thesis

submitted in partial fulfillment of the requirements of

University Honors College at

Western Kentucky University

2008

_____

Michael E. Smith, CE/T Advisor

_____

John Andersland

_____

Craig T. Cobane

TABLE OF CONTENTS

ABSTRACT

The morphology of the peripheral and inner ear structures was studied in the loricariid catfish *Pterygoplichthys gibbiceps*. Specimens (n=6) were preserved in fixative (4% paraformaldehyde, 2% glutaraldehyde in 0.1M phosphate buffer) and dissected for examination of the gross morphology (using light microscopy) and ultrastructure of the auditory sensory epithelia (using scanning electron microscopy). One additional specimen was cleaned in a dermestid beetle colony in order to examine the osteology of the skull. The swim bladder of *P. gibbiceps* is divided along the midline of the fish into two reduced but equal lobes residing in two laterally oriented bony encapsulations. Immediately lateral to the swim bladders, fenestrations were observed in the pterotic + supracleithrum. A single Weberian ossicle was attached to the medial apex of the bladder, which translates external sound pressure energy into interaural hydrodynamic motion of the fluid within the pars inferior. The single ossicle bends 90^{o} through a bone which acts as a pivot point allowing linear motion at the extreme ends of the ossicle. Otoliths (solid calcareous bones in the inner ear) were similar in shape to those of other loricariids. The asteriscus was disk-like and had a large crescent shaped sulcus that covered the macular striola. Sagittae were slender at their caudal apex and exhibited two wing-like projections about the rostral region of the otolith. Utricular otoliths were thick, having a bulbous rostral region and a laterally flattened triangular caudal region. On its ventral surface there was a deep sigma-shaped sulcus which was not in contact with the utricular maculae. Auditory endorgan-specific patterns of the orientation of sensory hair cell kinocilia were observed on each macular surface. Maculae exhibited areas of reversed hair cell orientation called the striola. Sacculi possessed a vertical striolar pattern. The lagenar patterns were crescent shaped in similar fashion as the sulcus of the otolith, and the pattern of the utricle was unlike the shape its otolith and curved sigmoidally to terminate at the lateral extremities of the otolith. In general, while there are unique peripheral auditory structures in *P. gibbiceps* (bi-lobed and encapsulated swim bladders and a single Weberian ossicle), the inner ear maculae and striolar patterns found in *P. gibbiceps* are similar to those found in other catfishes.

CHAPTER ONE: A General Introduction

Thesis Organization

This thesis is organized into three chapters. The first and present chapter provides an introduction to the auditory structures of fishes and defines the objective of this thesis. The thrust of the thesis is to provide preliminary data, thus the greatest emphasis was placed on chapter two the experimental study chapter. While the aim of the study was to examine the anatomy of a morphologically interesting species of catfish, functional speculations could be made based on some of the more obvious adaptations of this highly specialized species. Therefore, the final chapter discusses briefly the implications of this morphological study and proposes hypotheses for future investigation.

Background

Unlike the mammalian ear, fishes' auditory structures are entirely inside their bodies. This holds true with teleost (ray-finned) and non-teleost (lobe-finned) fishes, as well as Chondricthyes (cartilaginous) fishes. The structures homologous to the mammalian ear are located in their cranium. Within teleost fishes, there are groups of fishes with known specializations that increase the sensitivity and range of bandwidths at which they hear. Fishes with such specializations are known commonly as hearing 'specialists'. Those without such auditory specializations are considered 'generalists'.

The Superorder Ostariophysi consists of approximately 65% of all freshwater species of fishes (Nelson, 1994). The subgroup Otophysi (catfish, goldfish, carp, minnows, loaches; Rosen & Greenwood, 1970) possesses unique auditory modifications that make them hearing

specialists. A growing body of evidence demonstrates that this subgroup of fishes detects sounds at a higher sensitivity and broader bandwidth of frequencies than other teleost fishes (Stetter, 1929; von Frisch, 1938; Jenkins, 1977; Ladich, 1999; Ladich & Wysocki, 2003; Lechner & Ladich 2008). This acoustic sensitivity is achieved with the aid of what might be thought of as an internal amplifier.. Modified vertebral elements physically connect the swim bladder to the inner ear. As the gas filled swim bladder oscillates when sound passes through it, the chain of vertebral elements is oscillated and vibrations are transmitted to the fluid filled spaces of the inner ear. Thus the sound pressure energy in the water is transferred into hydrodynamic energy within the inner ear. This interesting modification of the vertebral elements, termed the Weberian apparatus, is one of the definitive features of Otophysan fishes (Chardon & Vandewalle, 1997; Fink & Fink, 1981)

Generally, the swim bladder serves a hydrostatic function, i.e. regulation of the buoyancy of a fish. However, when Weber discovered the apparatus that connected the swim bladder to the inner ear structures, it seemed that the apparatus and the swim bladder also served an auditory function (Weber, 1820). This was contested with regularity during the late 19th and early 20th centuries (Bridge & Haddon, 1889, 1892; Hartridge, 1920), but the controversy was resolved when von Frisch (1923) conclusively demonstrated the auditory role of the Weberian apparatus via behavioral studies following extirpation of a member of the Weberian ossicle chain. It appears that when one member of the chain is removed a significant loss of hearing occurs.

There have been numerous scientific advances in the study of fish hearing since the time of Weber and multiple theories have arisen concerning the process of fish audition. The prevailing theory is that the ear is separated into two distinct functional regions: the pars superior and the pars inferior. The pars superior is believed to serve a primarily vestibular function, while

the pars inferior is thought to be the locus of sound detection and some degree of sound processing (Popper et al, 2003; Popper & Schilt, 2008). The pars inferior is composed of two otolithic endorgans, the saccule and the lagena. Each is composed of a sensory epithelium and a solid calcareous mass termed the otolith. While hypotheses vary, the saccule is generally accepted to be the primary hearing organ, though the lagena seems to play a role as well (Popper & Platt 1983; for a complete overview see Ladich and Bass 2003).

Justification

Among otophysans, peripheral auditory structures, such as the Weberian ossicles, and swim bladder modifications, exhibit an interesting array of variability. Although it has been nearly 200 years since the discovery of the Weberian apparatus, much of the morphology, physiology, and embryology of the auditory structures remain unexplored within a relatively large number of Otophysan species. Within the Otophysan order Siluriformes, i.e. catfish, an especially peculiar family appears to possess not only acoustically advantageous internal modifications, but external modifications as well. In many teleost fishes, the swim bladder is singular and located within the center of the body, and the skull is solidly fused together. However, members of the family Loricariidae possess a highly adapted skull structure with holes, or fenestrae, adjacent to a pair of modified swim bladders. How these structures relate to their hearing ability remains unknown. Further, there have been only a handful of studies to date that have endeavored to describe catfish inner ear morphology and even fewer have been conducted on loricariids (Jenkins, 1977, 1979; Bleckmann et al., 1991; Lechner & Ladich 2008).

Objective

Often descriptive studies of the ear are undertaken to gain a better understanding of the biology of a taxonomic group of interest. Electrophysiology can then be performed to determine hearing capabilities by detecting brainwave patterns in response to tone stimuli. After control specimens are tested, a wide array of variables may be changed in order to precisely examine auditory characteristics (e.g., bandwidth range and hearing threshold limits) of a species of interest. In this way, morphological data and electrophysiological evidence together provide a complete picture of how interesting structures, like those in loricariids, potentially aid in their ability to hear. Thus, the purpose of this study was to describe the morphology of the inner ear, the peripheral auditory structures, and the morphology of the sensory epithelia of the inner ear of a loricariid catfish, *Pterygoplichthys gibbiceps*. Future experiments will directly examine the hearing capabilities of this species.

CHAPTER TWO: Morphology of *Pterygoplichthys gibbiceps*

Introduction to Research

One of the largest groups of fishes in the Superorder Ostariophysi is the catfishes (Order Siluriformes). Catfish families comprise one of the most speciose categories of fishes in the world, approaching 2400 species (Arratia et al., 2003). As with other members of the subgroup Otophysi, catfish possess a Weberian apparatus and are able to hear a broad range of frequencies. Certain families even possess unique external characteristics that are potentially advantageous to hearing. One such family is the family Loricariidae. However, at present, little work has been done concerning the auditory structures of the family Loricariidae. Descriptions concerning gross morphology of their skeleton and overall shape of the inner ear have taken place over the past century (Retzius, 1881; Sagemehl, 1885; Bridge & Haddon, 1892; Bridge & Haddon,1893; Chardon, 1968; Chardon et al., 2003; Lechner & Ladich, 2008), but a thorough description of the peripheral and sensory structures of the inner ear is still lacking.

The family Loricariidae is the largest catfish family, designating over one-fourth of all catfish species with approximately 646 accepted species (Armbruster, 2003). Loricariidae, the family of armored sucker-mouth catfish, possess interesting morphological features distinct from other catfishes. Instead of possessing one large swim bladder, loricariids possess two relatively small swim bladders separated into laterally oriented lobes. Additionally, this family demonstrates dozens of fenestrations (lateral openings) in their cranium behind their opercula. These fenestrations are filled with fat and contact both the outside environment and the swim bladders. The functional significance, if any, of these openings is unknown.

Loricariids also have a Weberian apparatus that is adapted to match the reduced, bi-lobed swim bladders. Rather than having a chain of several ossicles attached to a single bladder located medially in the peritoneal cavity as with channel catfish, they possess two (or possibly one) ossicle attached to each swim bladder. These ossicles are currently believed to be the fused modification of the ancestral tripus and scaphium (Coburn & Grubach, 1998). Recent data has demonstrated a correlation between swim bladder size, number of Weberian ossicles, and hearing sensitivity in the families Ariidae, Auchenipteridae, Heptapteridae, Malapteruridae, Mochokidae, Pseudopimelodidae, Challichthyidae, and Loricarridae (Lechner & Ladich, 2008). An investigation into the morphology of the ossicles is thus necessary to gain a more complete understanding of audition among otophysans.

While there are limited data concerning the peripheral auditory structures in loricariids, there has been an examination of the sensory epithelium of the pars inferior in other catfish families (Jenkins, 1977). The saccule and lagena of the pars inferior are believed to be the locus of hearing in fishes (von Frisch, 1936; Dijkgraaf, 1960; Popper & Fay, 1973; Fay & Popper, 1980; Saidel & Popper, 1983; Popper et al, 2003). In each auditory structure (termed an endorgan), the sensory epithelium (or macula) contains several thousand sensory cells called hair cells (Lombarte & Popper, 2004). Their name is derived from the hair-like appearance of specialized microvilli (stereocilia) organized in a staircase of rows behind a true cilium (called a kinocilium) at the apical surface of the cell. Further, kinocilia tend to face one particular direction in a given region on the macular surface, with minimal variance. These regions of kinocilia orientation form distinctive patterns on each endorgan, which are shared by other members of their taxon (Popper and Coombs, 1982).

I hypothesize that the unique peripheral structures surrounding the loricariid ear may indicate adaptations of inner ear maculae to improve hearing. Thus providing a detailed description of these structures is needed before experiments can be designed to test for specific acoustical functionalities. Although there have been some anatomical descriptions of the skull and swim bladder that surround the loricariid ear (Shaefer, 1987; Bleckmann ,1991; Arratia et al., 2003; Weitzmann, 2005), a complete description of the inner ear, ultrastructure, and its connection to the swim bladder has not been made. The purpose of this study was to describe a) the gross morphology of the inner ear, b) the surrounding bony structures and swim bladder and, c) the ultrastructural morphology of the sensory epithelia of the inner ear of the loricariid catfish *Pterygoplichthys gibbiceps*.

Methods

Animals

We chose to examine *Pterygoplichthys gibbiceps* (commonly known as the sailfin or leopard plecostomus) as a representative loricariid catfish due to their hardiness and availability in the aquarium trade. They possess an anatomical structure similar to that of other loricariid species (i.e. fenestrated pterotic plus posttemporosupracleithrum; paired, encapsulated swim bladders; and modified Weberian ossicles).

The specimens were purchased from local commercial suppliers and immediately housed in the Western Kentucky University Biology Department animal care facility in a self-contained flow-through aquarium system. Six *P. gibbiceps* ranged in total length from 10.0-14.3 cm with masses ranging from 8.9-21.5 g. Prior to dissection, fish were killed via an overdose of tricaine methane sulfonate (MS-222), a commonly used fish anesthetic.

Light microscopy

After recording the total length and mass of each fish, the brain casing was exposed and a fixative solution of 4% paraformaldehyde, 2% glutaraldehyde, and 1 mM Ca^{2+} in 0.1M PBS buffer at pH 7.4 (using sodium phosphate stock solutions) was injected into the brain casing adjacent to the otolithic organs. Each animal was decapitated and the head placed into the same fixative solution for at least 12 hours. The Weberian ossicles, swim bladders, and ears were then dissected out of the cranium in 0.1M PBS buffer. Whole brain and ear preparations were examined using a Leica MZ16 stereomicroscope fitted with a Nikon DS-5M camera. Following examination and photography, tissues and ossicles were stored in 0.1M PBS buffer in a refrigerator.

One relatively large fish (19.7 cm), residing in the previously described animal care facility, died of natural causes. Seizing opportunity, it was placed in a colony of dermestid beetles and left for several weeks until all tissue was cleaned from the skeleton. The skeleton was submerged in water and sonicated for less than ten seconds. Then the fish was rinsed to remove debris and air-dried overnight. Once dry, the head region of the fish was photographed using a Leica MZ16 microscope (at 7.1X) and the Nikon DS-5M camera.

Scanning electron microscopy (SEM)

Following examination with light microscopy, both right and left otolithic organs were opened and the nerve fibers, otoliths, and otolithic membrane were removed and trimmed from the maculae. Upon removal, otoliths were cleaned with a sonicator and air-dried for examination under light microscopy, but were then saved for additional examination under SEM.

Following maculae trimming, tissues were immediately postfixed with 1% osmium tetroxide in 0.1M PBS buffer for 25-35 min following initial fixation. The osmificated tissue was stored in 70% ethanol for at least 12 hours and then was dehydrated through ascending concentrations of ethanol, critical pointed dried (Tousimis Research Corporation Samdri-790 critical point dryer) with carbon dioxide as the transitional fluid, mounted on SEM stubs, sputter coated with gold in a Emscope SC500 sputter coater and viewed with a JEOL JSM-5400LV scanning electron microscope. SEM images of entire maculae from each endorgan were captured using IXRF Systems, Inc. 500 Digital Processing unit and accompanying IXRF software.

Maps of hair cell bundle orientation were created to examine the species-specific pattern found in *P. gibbiceps*. To do this, a series of photographs was taken of the maculae at 50,000X and then a single photograph of the each macula at 5,000X was printed out. By matching obvious landmark features of the highly magnified photograph shared with the entire maculae at low magnification, I was able to observe patterns in positioning of kinocilia at high magnification. Approximate vector arrows were then drawn on the photocopy of each macula to indicate these patterns. By convention, arrowheads pointed from the short stereocilia on one side of the hair cell to the tall kinocilia on the opposite pole of the stereociliary bundle.

In similar fashion to the otolith treatment, SEM photomicrographs were obtained of the conjoined tripus and scaphium (Weberian ossicles). Ossicles were initially attached to the swimbladders but were resected from the animal, and separated from the swim bladder for inspection after the initial dissection. Ossicles used for SEM were cleaned in 80% ethanol using a Blitz sonicator, allowed to air dry, mounted, sputter coated, and viewed using the previously described electron microscope.

Results

Gross Morphology

Similar to other loricariids, *P. gibbiceps* possess a porous fusion of the external pterotic and posttemporosupracleithrum bones (as per Schaefer 1997; Arriata et al., 2003) also labeled pterotic + supracleithrum as per Weitzmann (2005). These bones contain fenestrae (channels) filled with lipids that form a barrier between the swim bladder and the epidermis (Fig. 1). Additionally, they possess two small, laterally-oriented swim bladders encased in bone (Fig. 2). The paired bladders are conical and positioned latero-caudally to the inner ear and separated from the cranial cavity by the pectoral septum that divides the cranial cavity from the abdominal cavity connecting the two pectoral girdles. The conical bladders are positioned with the narrow end medially and the broad end laterally toward the pterotic + supracleithrum.

The outer membrane of each bladder is additionally surrounded by the somatopleural external envelope (i.e. tunica externa). The external envelope is connected on the narrow, medial end to a single ossicle, being the apparent fusion of the tripus and scaphium. This ossicle is loosely attached at its medial surface to the circular hollow structure termed the atrium sinus impar. The atrium sinus impar in turn makes contact with the outer membrane of the lagena endorgan. This creates a mechanical connection from the swim bladder to the fluid of the inner endorgans (Fig. 3). The tripus portion is “L” shaped and very thin. At its lateral-most tip, it is connected to the tunica externa. It then bends through a bony junction thereby limiting its movement strictly to the frontal plane. On its rostral-most tip, it is fused with the nearly circular scaphium which opens its concave surface medially forming a cup (Fig. 4).

The more caudal portions of the ear, the pars inferior, lie in the cranial cavity rostrally adjacent to the pectoral septum. The endorgans are positioned laterally about the medulla (Fig.

2B). The pars inferior designates two pairs of the otolithic endorgans, the saccule and lagena, whereas the pars superior refers to the pairs of utricles and three orthogonal semicircular canals. Both left and right halves of the pars inferior lie in opposing planes approximately 30° from sagittal and are connected via a narrow canal between the ventromedial curvatures of the sacculi termed the transverse canal (Fig. 2B). The saccule and lagena share an outer membrane at the saccule's dorso-lateral surface. A portal exists at this connection allowing the passage of endolymphatic fluid between endorgans (Fig. 3).

While the saccule and lagena share an outer membrane on the saccule's dorso-lateral surface, the utricle is connected to the other portions of the ear only by three mutually orthogonal semicircular canals (anterior, posterior, and horizontal semicircular canals) (Fig. 5). Branches of the auditory nerve (Cranial Nerve VIII) are present on the rostrolateral surface of the lagena and on the ventral surface of the saccule. The pars superior, or the utricular pair, lies in the frontal plane at the level of the cerebellum, connected caudally on its outer membrane to the brain via the auditory nerve (Fig. 2A).

Otolith Structure

The three endorgans contain calcareous "stones" termed otoliths. Each otolith is attached to the sensory epithilum via the otolithic membrane, a porous membrane that surrounds each otolith, through which the ciliary bundles protrude. Saccular otoliths, sagitta, were extremely fragile at their caudal most poles. Most saccular otoliths were broken in handling (Fig. 5) except one which was photographed under SEM (Fig. 6C). In situ, all sagitta resembled the undamaged otolith structure. The otoliths of the other endorgans were less susceptible to damage during handling.

The lapillus (the utricular otolith) is the largest otolith of *P. gibbiceps*. The side of the otolith that faces dorsally is triangular and possesses no defining feature on its surface. However the side that faces the macula, the ventral side, is clearly defined by a small groove, termed the sulcus, that closely resembles the Greek letter sigma (Fig. 6). From the edge of the sigma sulcus to the rostral-most end the surface of the otolith is rough. The rostral region is far narrower than the caudal, which has an extension of approximately 0.25 mm on both sides into the frontal plane (Fig. 6A).

The lagenar otolith, the asteriscus, forms a disk wide in the sagittal plane and narrow in the coronal plane. While the overall form is disk-like, a jagged rostral portion along the median of the asteriscus forms two prongs. The dorsal-most prong extends further rostrally than does the ventral-most prong. The disk is concave medially and bends away laterally at its outer edge, almost taking on the shape of the underside of a saddle (Fig. 6B). Its sulcus follows the caudal curvature around the outer edge of asteriscus forming a crescent beneath which the macular striola lies (Fig. 6B, 7B; see next section for description).

The sagitta had a unique structure. On the ventro-rostral portion of the otolith, a double ala (or wing-like structure) is present. Calcium carbonate is deposited in rays that fan from the end of the otolith creating a concave half cylinder (Fig. 6C). This half cylinder lies adjacent to the window between the saccule and lagena. This double ala is thin and extremely delicate compared to the much more robust ‘spine’ that comprises the body of the sagitta. This spine remains flat in the sagittal plane until it reaches the beginning of the double ala at which point the otolith twists 90° into the frontal plane. The caudal-most end of the sagitta is almost flat and handle-like in appearance.

Ultrastructure

In every endorgan, a patch of sensory epithelium, termed the macula, was observed. Upon this macula thousands of hair cell bundles were present. Each bundle possessed only one true cilium with the 9+2 microtubule configuration. This true cilium, termed the kinocilium, is at one pole of a staircased bundle of stereocilia. Kinocilia were regularly positioned on one side of stereociliary bundles throughout some portion of the macula. Macular tissues were extremely susceptible to shearing and were often damaged in handling. Further, otolithic membranes were tightly held to the macula of all endorgans and some membrane always remained. However, enough otolithic membrane was removed to note distinct alignments of kinocilia orientation on at least three specimens of each endorgan maculae under SEM (Fig. 7). When observed as a whole, the maculae appeared be divided based on kinocilia orientation. The point at which the orientation shifts is termed the striola (Fig. 8).

Utricular maculae were relatively large and trapezoidal in shape. The utricular maculae demonstrated a thumb-like protrusion on the rostro-lateral tip of the macula. The protrusion is bi-planar, wrapping around the rostro-lateral edge of the lapillus leaving solely the frontal plane and bulging into the sagittal plane (Fig. 5, 6A). This structure is relatively removed from the otolith and is positioned adjacent the horizontal semicircular canal (Fig. 5). The trapezoidal shape of the utricular maculae is largest laterally and narrows medially. The striola was observed in the caudal region of the macula and hair cell kinocilia on either side of the striola were oriented toward one another (Fig. 7A).

Unlike the utricular sensory epithelia, lagenar maculae lie only in the sagittal plane. The lagena is spherical in comparison to the rounded elongate saccule. The sensory epithelium is of a peculiar shape resembling the letter ‘P’. The dorso-rostral region is nearly double the width of

the ventro-caudal region of the sensory epithelium. As with the utricular maculae, the lagena possesses a thumb-like protrusion of hair cell bundles in its dorsal-most aspect as if to form the top stroke of the upper portion of the “P”. While the kinocilia of the saccular macula are oriented in opposing vectors, the kinocilia about the striola of the lagenar maculae are orienting facing toward one another. This striolar pattern curves from the dorsal aspect to the ventral in a semicircular form (Fig. 7B).

The elongate and slender saccule lies dorso-medially to the lagena. The saccular maculae followed the otolith from the caudal to the rostral pole twisting 90° medially and dorsally at the rostral-most end. The saccular maculae are elliptical in the caudal region and become more ovoid in the rostral region with a distinct pinched area in the middle. The width of the caudal region in the sagittal plane tended to be greater than the width of the rostral region save for the bulge immediately rostral to the middle pinch which narrowed at the tip. Saccular maculae demonstrated a vertical pattern of kinocilia orientation about the striola as it bisects the dorsal and ventral regions of the saccular surface (Fig. 7C).

Discussion

The paired, encapsulated swim bladders of *P. gibbiceps* are similar to those of other described loricariid species (Bleckmann, 1991; Chardon 1968; Chardon 1999; Lechner & Ladich 2008; Weitzmann 2008). However, *P. gibbiceps* is the most like the genus *Ancistrus,* as represented by *Ancistrus sp.* in Bleckmann (1991), in that their swim bladders tend to be larger than other described loricariid species. Previous studies described separated and reduced swim bladder morphology. Further, they noted that the swim bladders connected at their medial apex to the Weberian apparatus and a porous pterotic + supracleithrum lies external to the swim

bladder. As with the reduction of ossicles, the fenestrations of the pterotic + supracleithrum may have some connection to audition among these fishes, however experimental data are lacking. However, it can be concluded that bi-lobed swim bladders connected medially to the Weberian apparatus and encased in bones opening to fenestrated cranial structures are common among loricariids (Bleckmann et al., 1991).

Weberian ossicle structures of *P. gibbiceps* are much like other members of their order. The "L" shaped tripus and concave cup of the scaphium have been recently described for the loricariid subfamilies Hypostominae, Loricariinae, Hypoptopmatinae (Lechner & Ladich, 2008). However unlike *Ancistrus sp., P. gibbiceps* lacks the ligament connections between Weberian ossicles involved in vibratory transduction. Rather, it possesses only a rigid, single ossicle more limited in terms of directional motion. Gross morphology of the inner ear is likewise similar to the ears of other described Siluriformes (Popper & Platt, 1983; Retzius, 1881). In these previous studies, the saccular endorgans were described to be smaller and more elongate than the larger spherical lagenar endorgan attached at the dorso-caudal surface. However, it should be noted briefly that there is variability among macular shape with some groups possessing larger or smaller macular regions than that of *P. gibbiceps*. Such differences may correspond to otolith shape. However, the true functional significance is unknown.

P. gibbiceps appears to be consistent with the investigations that have taken place on the gross morphology of the inner ears of catfishes (Retzius, 1881; von Frisch, 1936; Jenkins, 1977; Bleckmann et al., 1991). The pars inferior is unconnected to the pars superior save by indirect connection with the semicircular canals. Sacculi are medial and elongate while the lagena tends to be nearly ovoid. Some mention has been made of the portal that exists on the medial wall that the lagena shares with the saccule. It was theorized by von Frisch (1936) that this portal acts as

‘release membrane’ allowing the flux of endolymphatic fluid caused by vibrations from the Weberian apparatus. Jenkins (1977) believed it to be homologous to the round window in mammals. However, neither hypothesis has been experimentally substantiated (Ladich & Popper, 2004).

The utricular otolith, the lapillus, is similar to that found in other otophysans. Yet it should be noted that as compared with the sagitta, few investigations have taken place with the lapillus. However, there is a point of interest concerning the macula. The white outline of the macula in Figure 3 is in contact with the dorsal surface of the otolith at all points except the thumb-like projection. This projection is not actually in contact with the lateral side of the lapillus, but lies adjacent to it on the outer membrane of the utricle.

While the sagitta is morphologically peculiar, the high degree of specialization of otolith structure seems to be common amongst otophysan catfishes (Jenkins, 1979). Additionally, narrow rod-like caudal half found in *P. gibbiceps* is typical of catfish. However, of the five otophysan species Jenkins described, the sagitta of the *P. gibbiceps* is far wider in the rostral portions where the double ala is noted. In fact, the wing-like structure observed was considered merely a “fluted” region in other species (Jenkins, 1979). As for the disk-like asteriscus in *P. gibbiceps*, it appears that this structure is nearly identical, including the crescent shaped sulcus, to that of another ostariophysan, *P. laevis* (Wolfahrt, 1939). The asteriscus present in this species differs only in the roughness of the outer edge of the otolith and the extension of the ventral portion of the sulcus. The sulcus of *P. laevis* appears to be slightly larger.

The fact that two of the three maculae exist outside a single anatomical plane is a striking feature. As opposed to a macula lying in only one plane, saccular twisting at the middle region might allow the saccule to provide more directional acoustic information at the near-field. While

the mechanism of sound localization has yet to be unequivocally determined, recent studies have pointed to the directional orientation of the saccular macula as a possible method of sound source localization (Fay & Edds-Walton 2000). Thus, if the macula is oriented in two planes as with *P. gibbiceps*, a plausible function may be to localize sound sources in two planes. As to the thumb-like appendage of the utricle, it is positioned immediately adjacent the horizontal semicircular canal. This additional portion of the macula may serve as either an auxiliary motion detector similar to the cristae present in the semicircular canals or it may provide precise information concerning the movement of the cranium in the sagittal plane.

Hair cell orientation maps among otophysan fishes are variations on a common theme. The typical saccular macula of otophysan fishes all share a vertical orientation pattern (Popper & Coombs, 1982; Popper & Platt, 1983; Popper & Fay, 1993). The lagenar macula of *P. gibbiceps* is very similar that of a marine catfish *A. felis* (Popper & Tavolga, 1981; Popper & Platt, 1983). However, the macula from *A. felis* appears as though it were the macula from the *P. gibbiceps* rotated on its side. The broad dorsal end that is present in the lagena of *P. gibbiceps* is similar to the wide posterior side of the *A. felis* lagenar maculae. Similarly the macula narrows from the ventral to the dorsal regions, with its widest point at near the ventral apex. Even the crescent shaped orientation pattern is found in both *P. gibbiceps* and *A. felis*. Utricular maculae are similar to that of other otophysan species in shape and orientation pattern (Popper & Fay, 1999). This holds true with present data suggesting that utriculi are relatively similar in pattern except the Clupeomorph fishes. These species tend to have unique orientation patterns similar to the amount of variation found in the sacculi of otophysan fishes (Popper & Platt, 1979; Popper & Coombs, 1982). Thus, in consideration of the unique external modifications of the cranium of

loricariids as well as the paired swim bladders, the maculae are surprisingly similar to those found in other catfishes.

CHAPTER TWO: Figure Legends

Figure 1. Photograph of lateral view of fenestrated pterotic + supracleithrum. orb- eye orbit. Arrow points to a fenestration. White line surrounds the pterotic + supracleithrum. Rostral is to the left. Scale bar, 6 mm.

Figure 2. Outline of *P. gibbiceps* with relative position of inner ear and associated structures showing relative size of the auditory structures including swim bladders and Weberian ossicles as viewed ventrally. Full body outline of fish (A). Enlarged image shown in A of all auditory structures (B). Grayed areas inside endorgans indicate relative position of otoliths. asi- atrium sinus impar; L- lagenar otolith; S- saccular otolith; sb- swim bladder; t-tripus + scaphium; tc-transverse canal; U- utricular otolith. All elements are drawn to scale relative to one another.

Figure 3. Photograph of Weberian ossicles and their mechanical connection to the atrium sinus impar as viewed dorsally. Rostral is towards the top of the image. asi- atrium sinus impar; L-lagena; S-saccule; sb- swim bladder; slp-saccule-lagena portal; tc-transverse canal; t+s- tripus + scaphium; te-tunica externa.

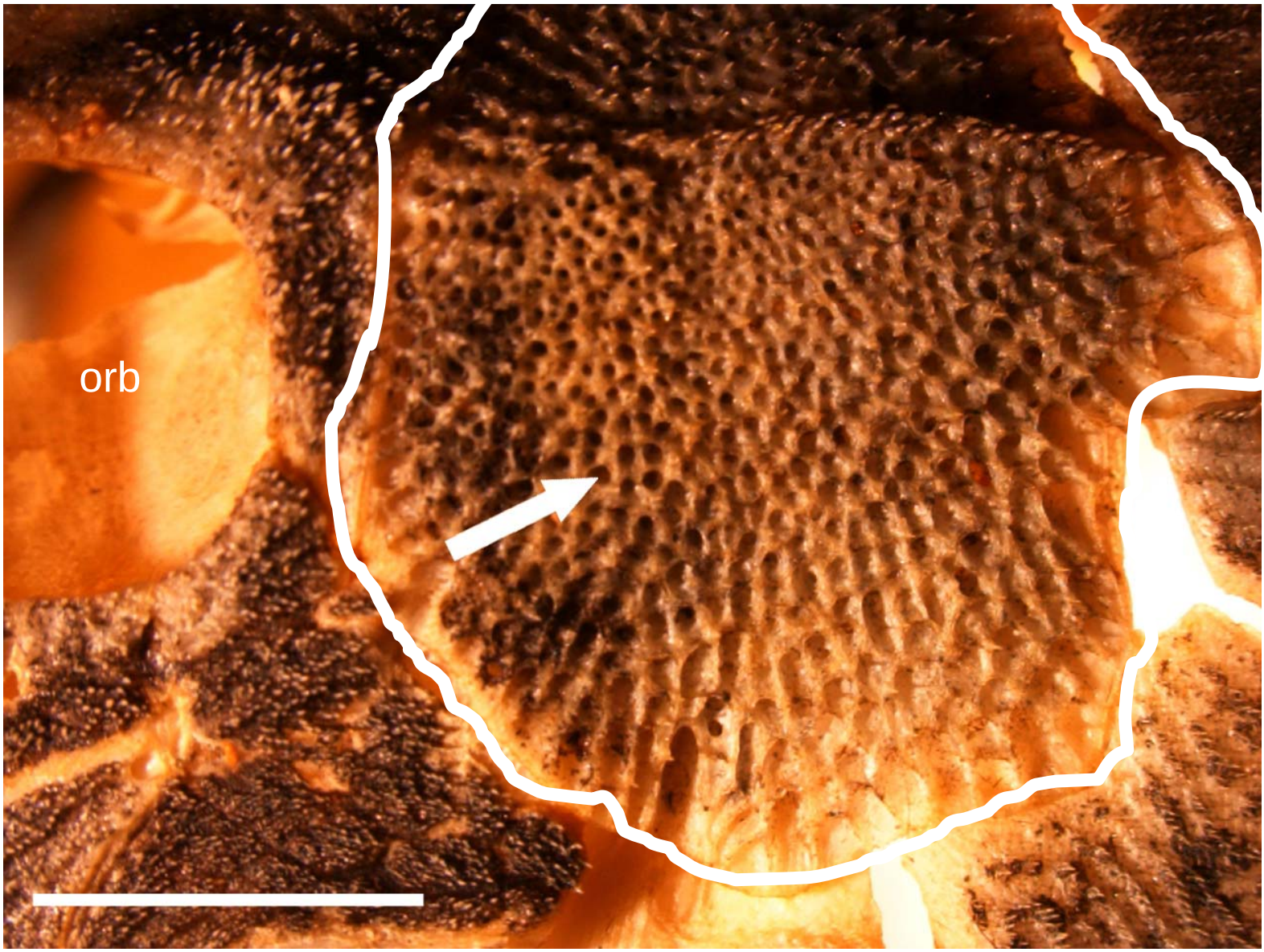
Figure 4. SEM photomicrograph of single Weberian ossicle tripus + scaphium as viewed ventrally. Scale bar, 200µm.

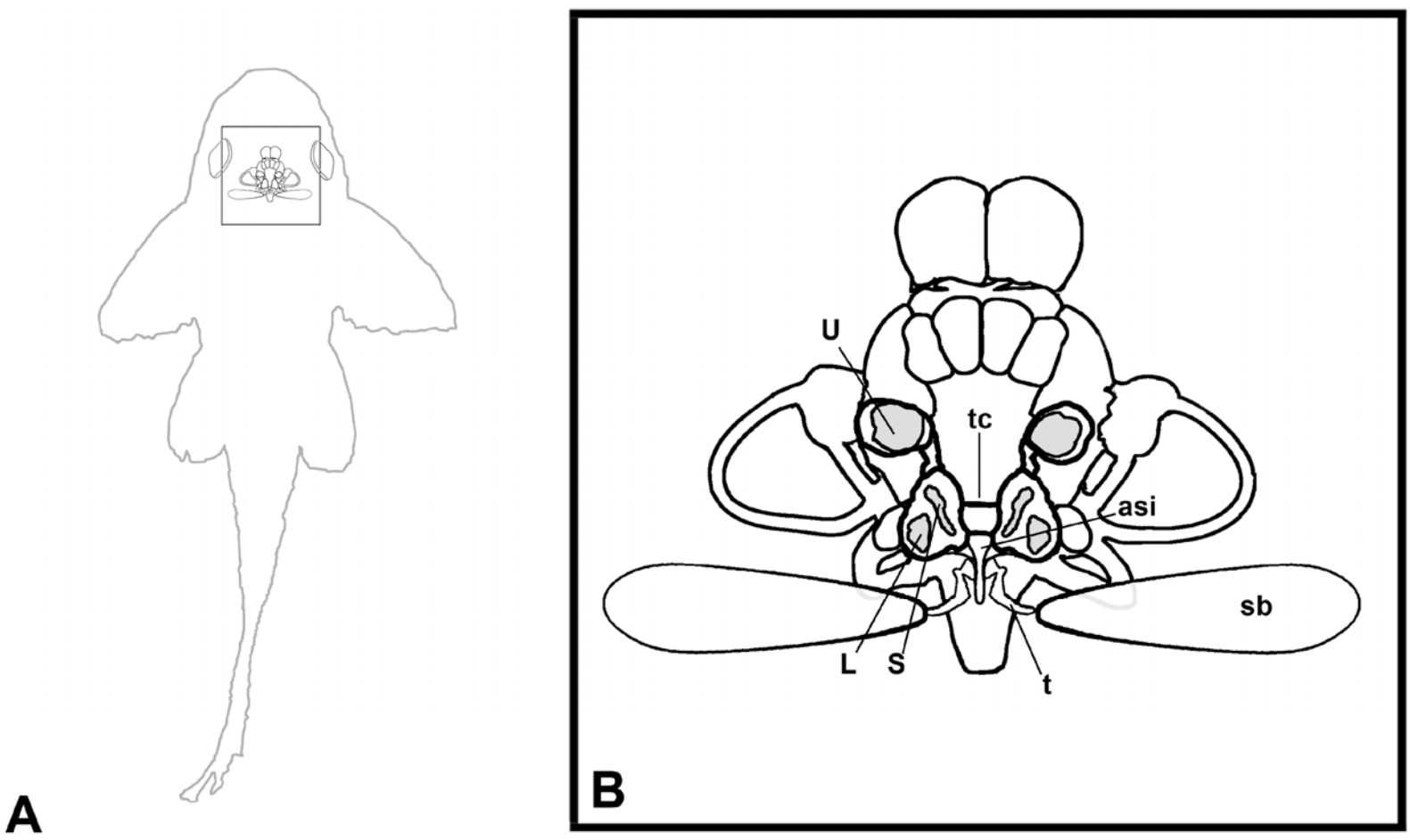
Figure 5. Photograph of ventrolateral view of endorgans with otoliths. ac- anterior semicircular canal; c-cristae; hc- horizontal semicircular canal; L- lagenar otolith; pc- posterior semicircular canal; S- saccular otolith; U- utricular otolith; Arrows indicate opaque broken fragments of the fragile tip of the saccule visible adjacent the caudal portion of the lagena. Asterisk indicates the thumb-like protrusion of the utricular macula. Scale bar, 1.5mm.

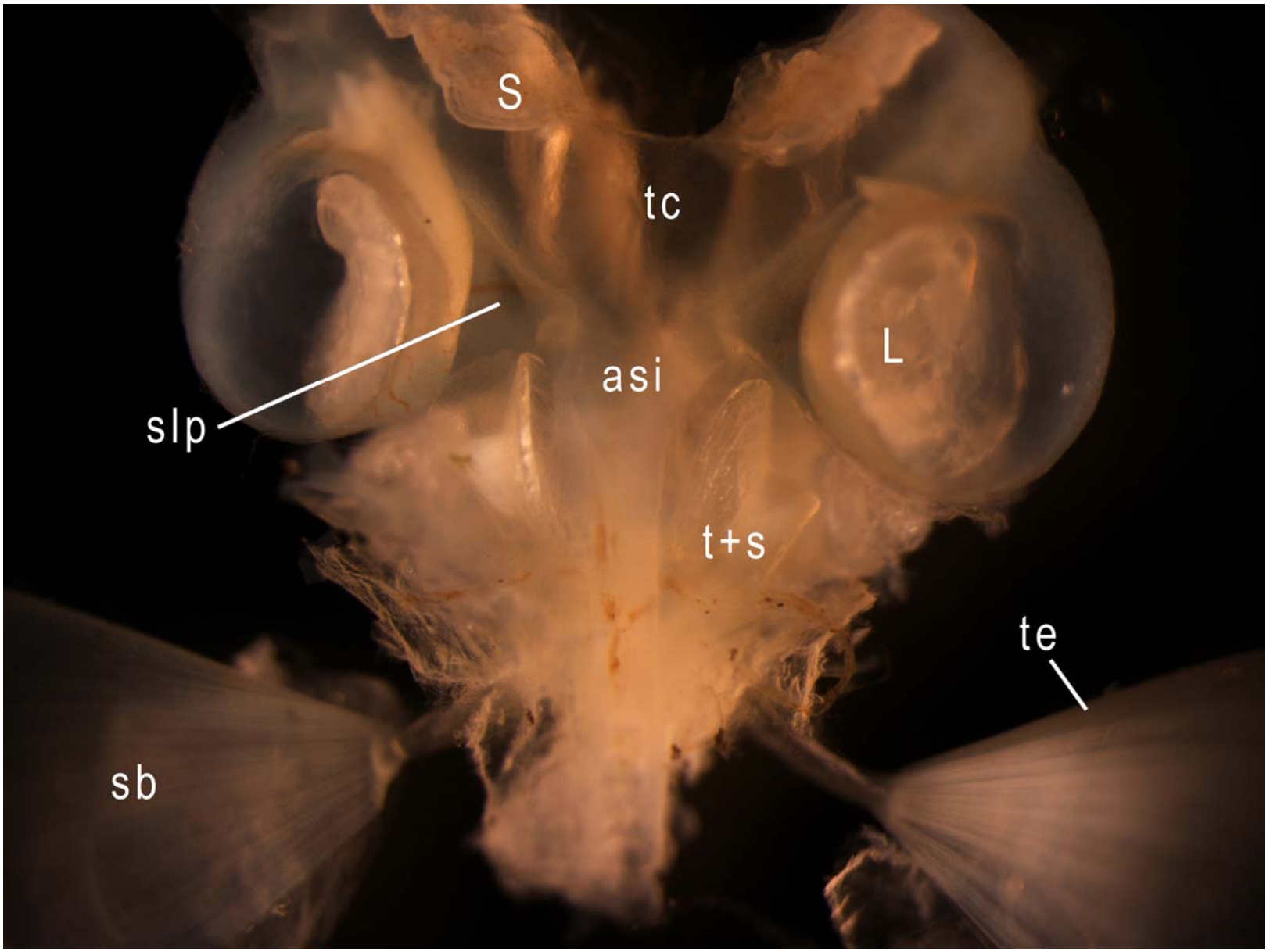
Figure 6. SEM of the lapillus (A), asteriscus (B), and sagitta (C). White outlines represent the relative positioning of the macula on the corresponding otolith. All three otoliths are in a different anatomical plane with the lapillus and the sagitta positioned in two planes each. Scale bar, 0.5 mm.

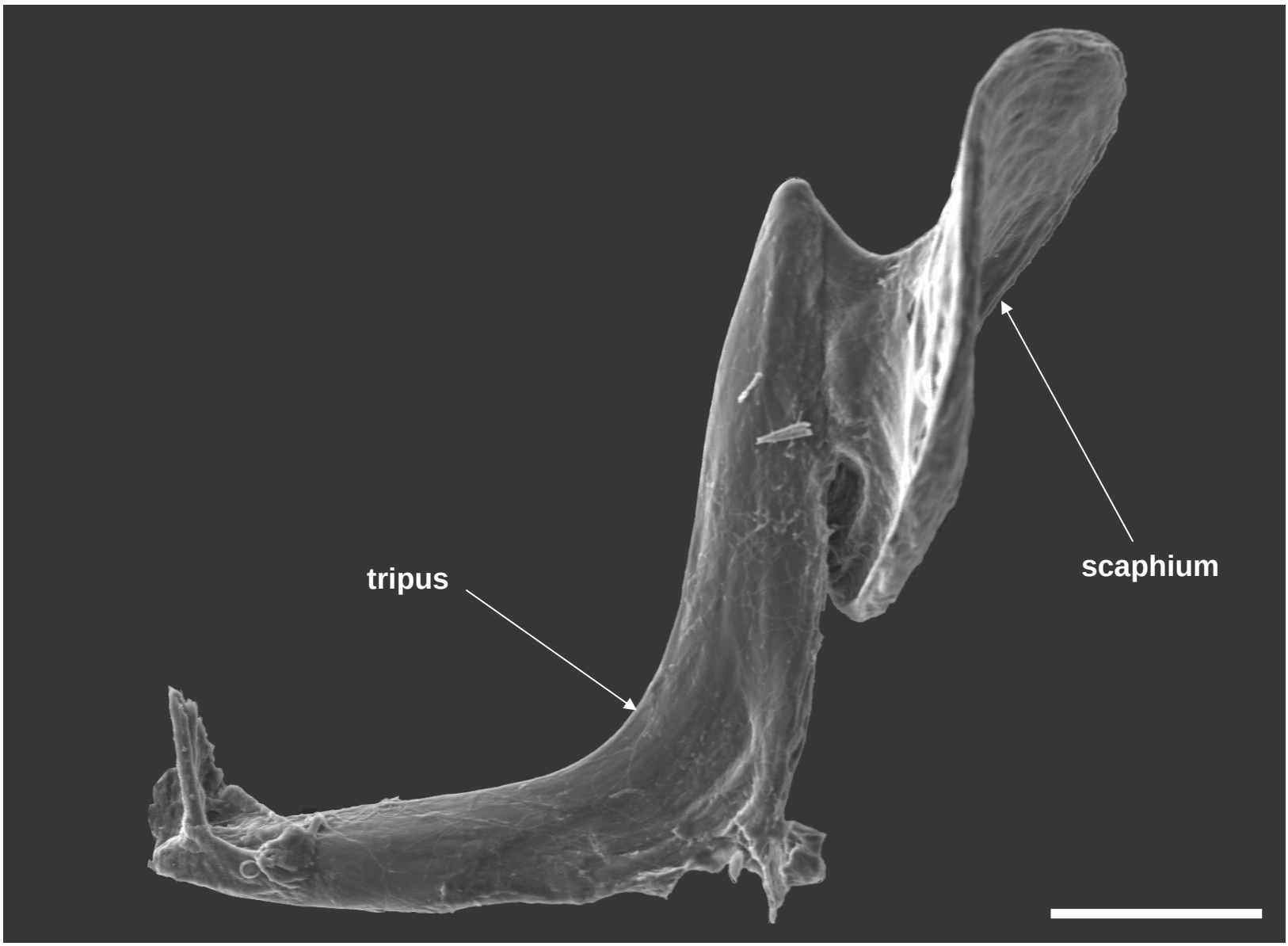
Figure 7. Outlines and hair cell orientation maps of the Utricle (A), Lagena (B), and Saccule (C). Arrows point toward kinocilia orientation. The line between indicates the clear separation between areas of inverse orientation, i.e. the striola. Scale bar, 0.5 mm.

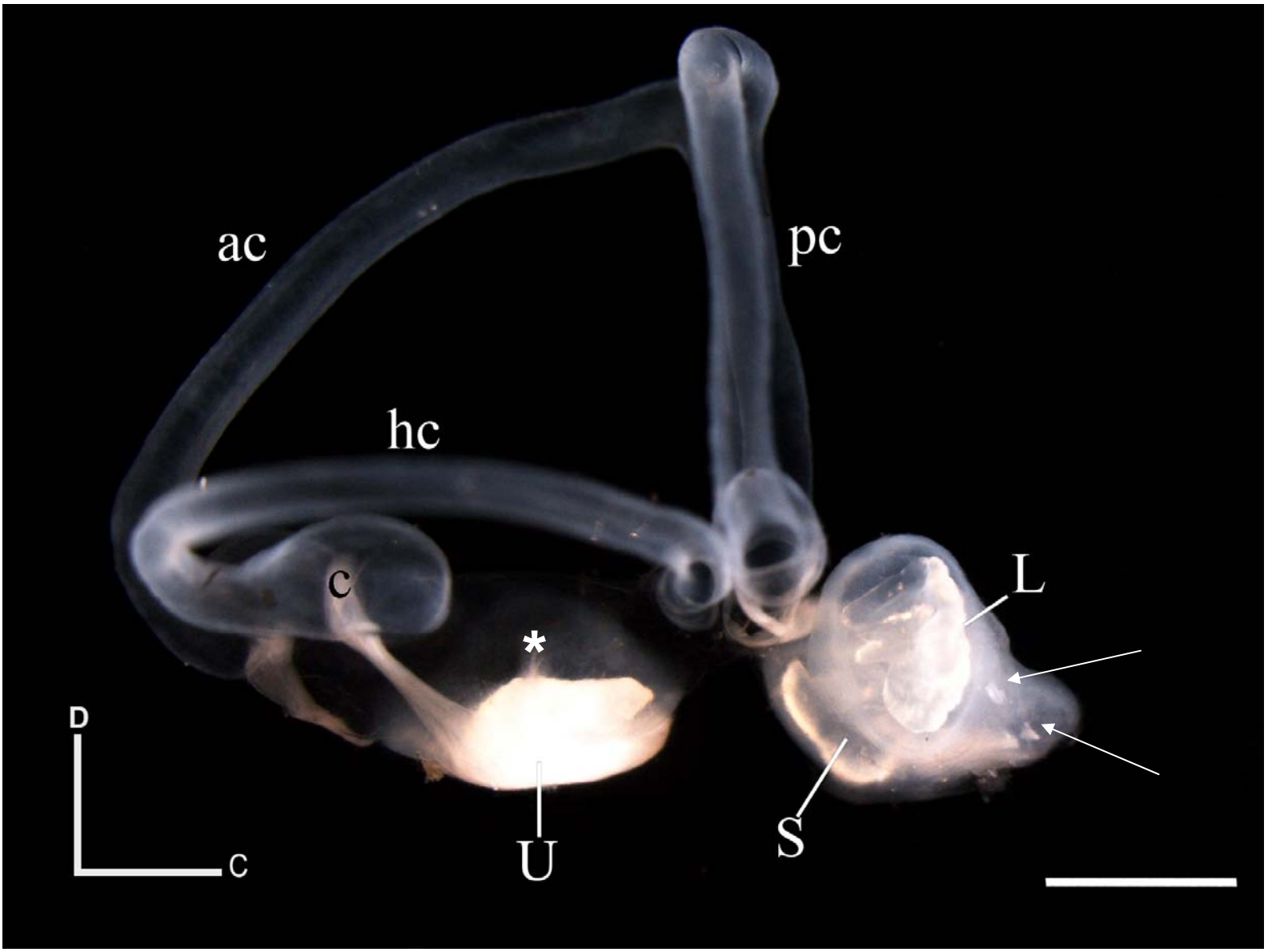
Figure 8. SEM photomicrograph of hair cell bundles on utricular macula. The dotted line indicates the striola.

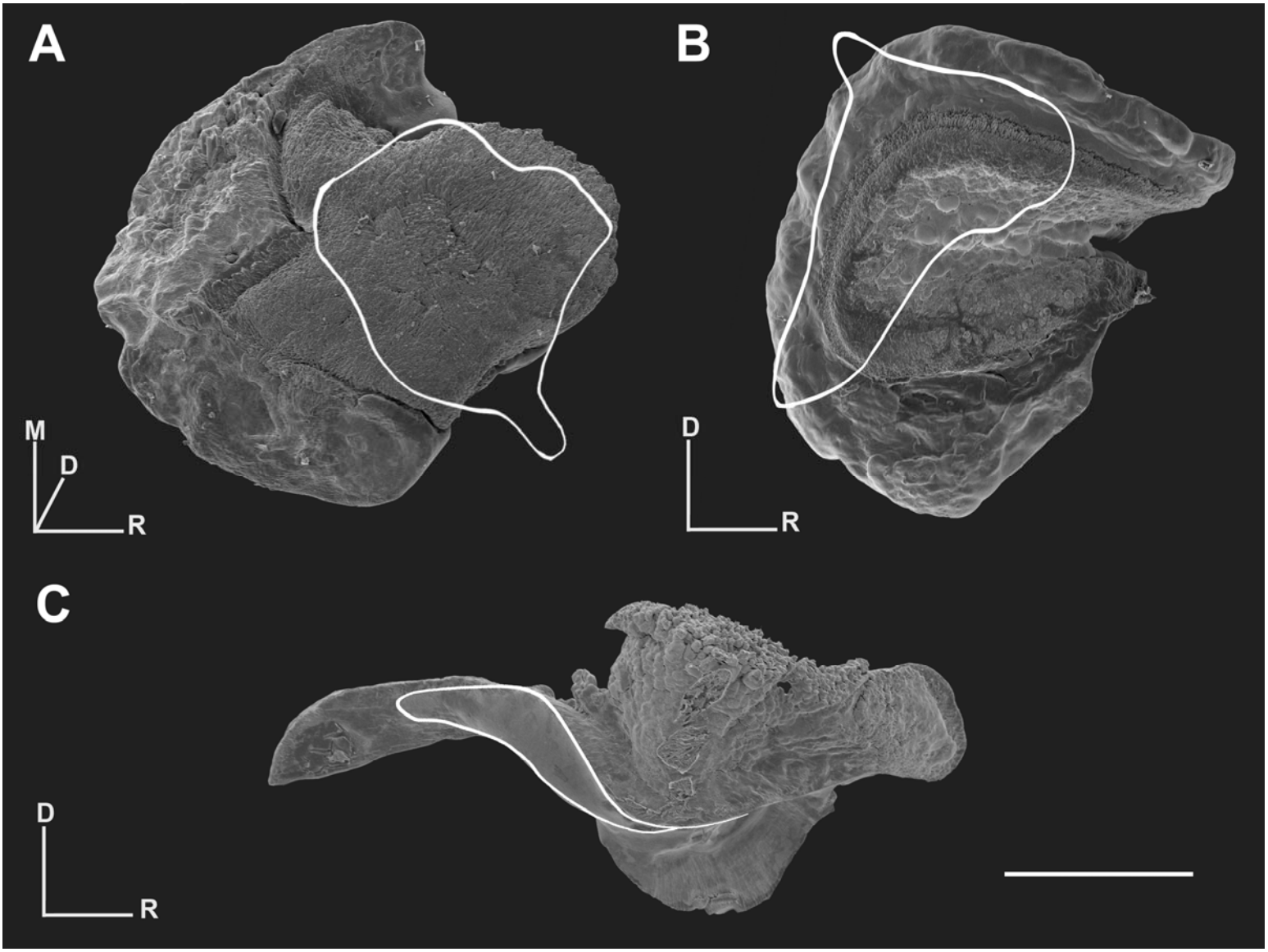

orb

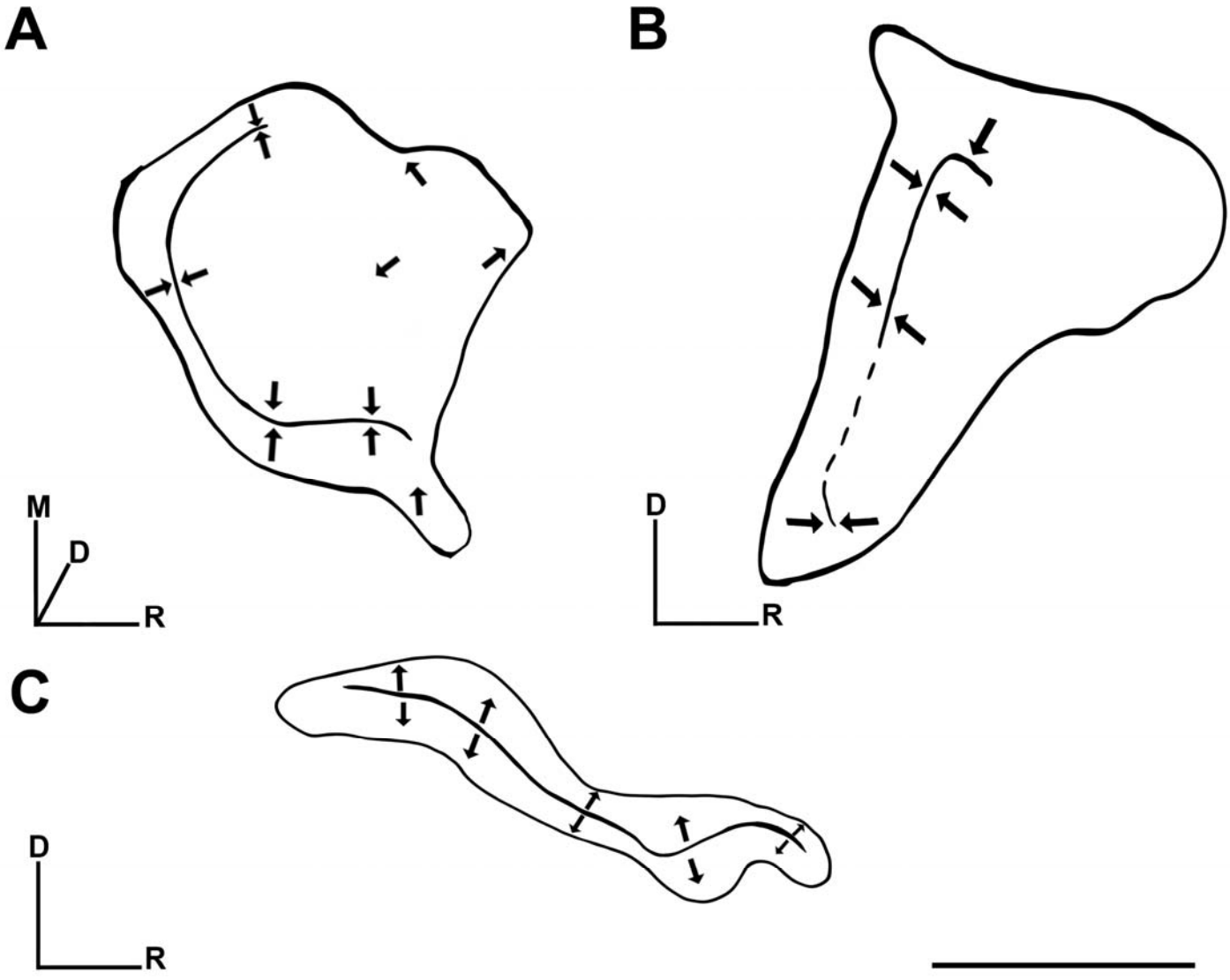

A
U
tc
asi
sb
L
S
t
B

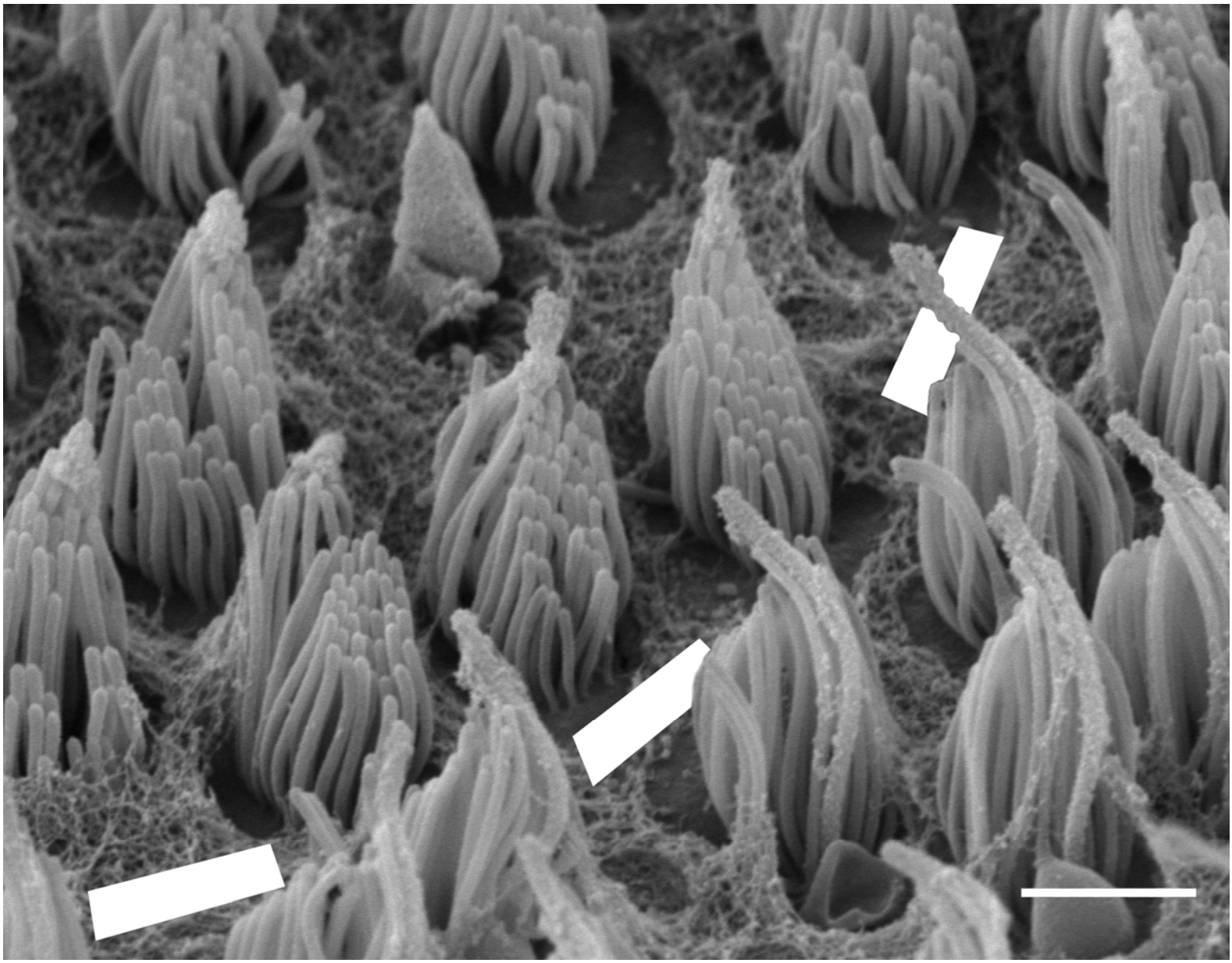

S
tc
L
asi
slp
t+s
te
sb


tripus
scaphium


ac
pc
hc
c
*
L
D
c
U
S


A
M
D
R
B
D
R
C
D
R


A
M
D
R
B
D
R
C
D
R



CHAPTER THREE: Some Insight into Audition

General Conclusions

The close proximity of the conical swim bladders to the external environment is of general interest as they provide a gas-water interface for impinging sound waves. Recent studies have demonstrated that at low frequencies the air-water interface (i.e. the surface) is essentially transparent and most energy is emitted across the interface. Further, more energy is emitted the nearer the source is to the surface. Due to extreme loss of low-frequency sound energy across this interface, long range propagation of low-frequency sound is prevented in shallow water (Rogers & Cox, 1988). However, at high frequencies, the energy is reflected by the surface of the water (Godin, 2007, 2008). Thus in shallow water environments, high frequency sound waves are propagated for much greater distances than low frequency sounds.

It is plausible then that *P. gibbiceps* evolved peripheral auditory structures (such as fenestrations, modified Weberian ossicles, and encapsulated swim bladders) to operate within the physical constraints of freshwater sound wave propagation. The swim bladders provide a necessary gas-water interface to reflect sound, without which sound energies may pass through the fish with little impedance. Then through their Weberian apparatus *P. gibbiceps,* and all otophysans, are able to pass external sound pressure energy onto the fluid of the inner ear. Furthermore, adaptation of the otophysan ear to hear higher bandwidth frequencies as a product of shallow-water environments may potentially explain why Weberian ossicles evolved in shallow-water hearing specialists and not in deep-sea generalists. Otophysans, in fact, are rarely found in marine environments (Briggs, 2005). Detecting sound signals at lower thresholds and over broader bandwidths could potentially improve predator avoidance, foraging, and

conspecific recognition. Thus the Weberian ossicles, a trait which unites all otophysans, may have played an important role in the evolutionary success of this taxonomic group of fishes.

While most agree that an otophysic connection between the Weberian apparatus and the atrium sinus impar exists in loricariids, some discrepancy remains concerning the morphological details of the connection. It is not only difficult to observe the precise connection of the Weberian apparatus with the endorgans, but it is especially difficult to diagram. One diagram produced in Aquino & Schaefer (2002) (Fig. 9A), portrays the connection of the Weberian ossicles to be directly at the outer membrane of the lagena, altogether negating the atrium sinus impar. In contrast, Bleckmann (1991) depicts the Weberian apparatus connecting indirectly to the pars inferior via the sinus atria impar, rather than the directly to the outer membrane of the lagena (Fig. 9B). This slight difference would alter the physics of audition among *P. gibbiceps* from detection of fluid motion within the saccule-lagena complex to detection of physical vibrations from the outer membrane of the lagena.

While no other study to date has provided photographs of the sinus impar, *P. gibbiceps* appeared to exhibit the morphology illustrated by Bleckmann (1991). The scaphium is clearly not in contact with the lagena; rather it serves only to push endolymphatic fluid from the atrium sinus impar to the outer wall of the lagena.

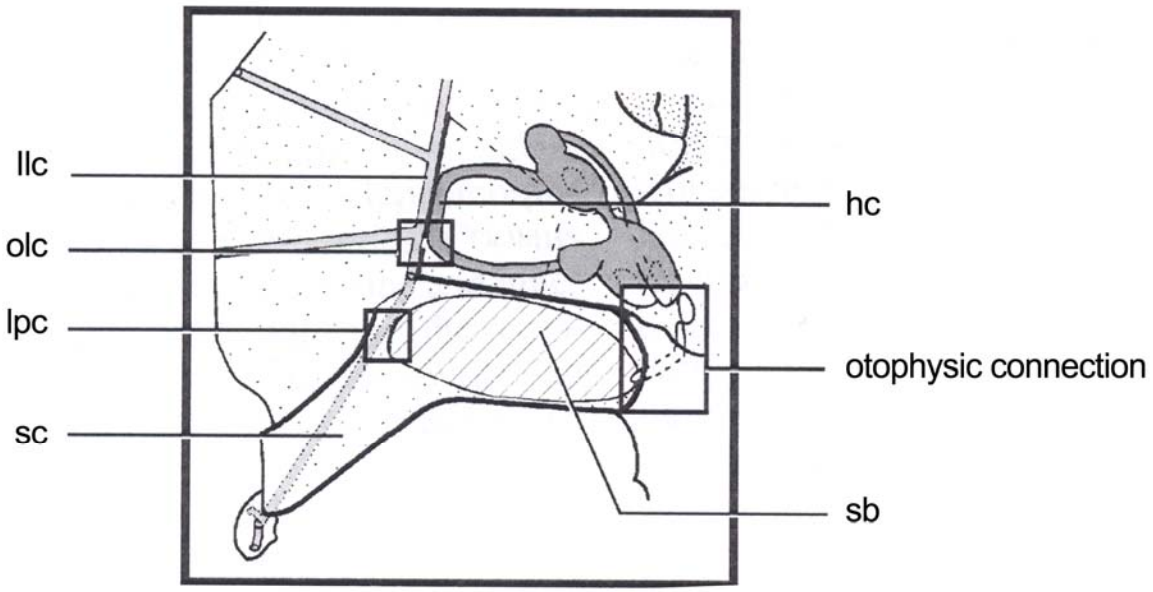
The inner ears of *P. gibbiceps* are similar to past studies of other otophysan fishes and illustrations provided by Retzius (1881) of *Phoxinus laevis* are remarkably like that found in the present study. Of notable interest in all data available thus far is the position of the transverse canal and the portal between the saccule and lagena. With the transverse canal positioned posteriorly and the portal positioned anteriorly, a directional flow of endolymphatic fluid would be established.

Further, the transverse canal is much larger in terms of area than the lagena-saccule portal. By constricting portal size, the velocity of endolymphatic fluid would be dramatically increased as the it passes beneath the double ala of the sagitta, through the narrow opening, and past the concavity of the asteriscus. Theoretically, the sagitta would rock mediolaterally with impinging pressure waves and the asteriscus would oscillate rostrocaudally. As the shape of the sagitta suggests a rocking motion, otolith morphology becomes extremely important to maintain appropriate fluid dynamics necessary to elicit sensory response. This rocking motion was similarly theorized by Van Bergeijk (1967) and Jenkins (1979) when discussing audition in the Ostariophysan superorder. Therefore, the morphology of the asteriscus and sagitta of *P. gibbiceps*, in addition to previous studies, indicates that the overall shape of otoliths may be a correlate of variable ear morphology and fluid dynamics.

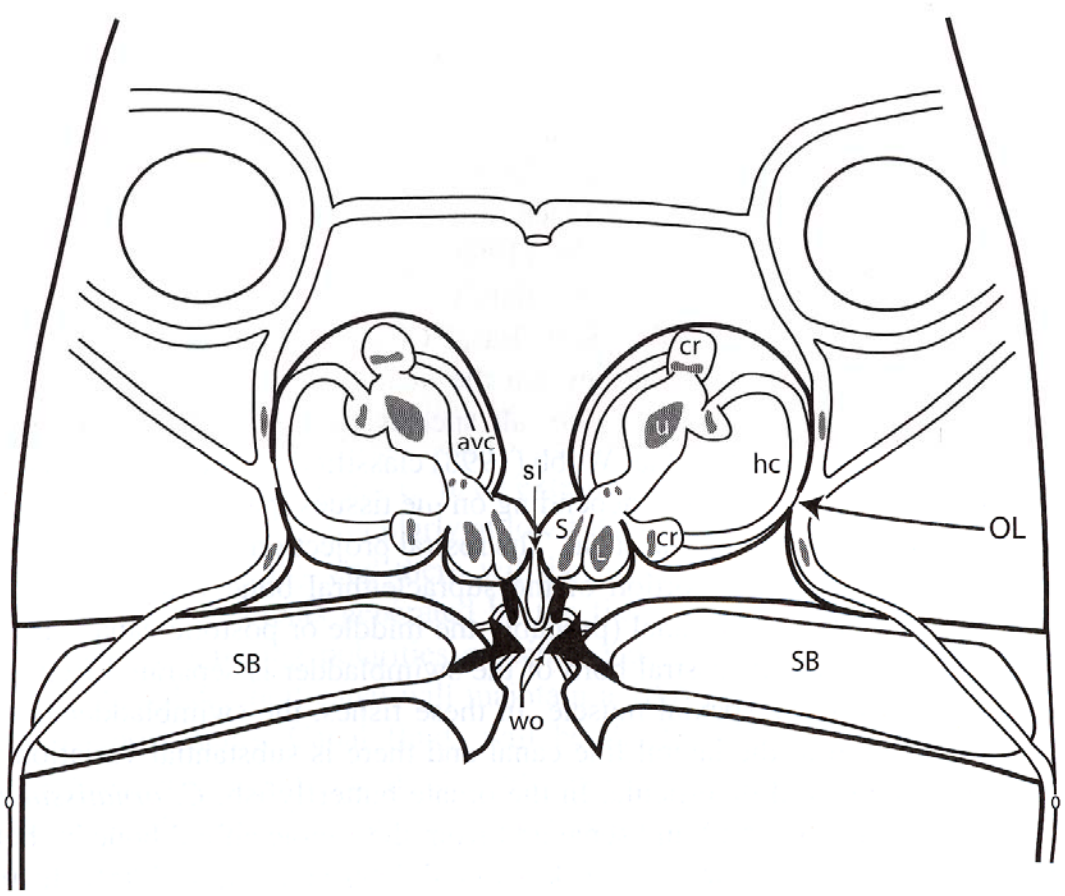
While the functional significance of variable otolith shape is presently unproven, its density is believed to play a role (Fay & Popper, 1999). It is thought that the denser otolith lags out of phase as the macula and body of the fish oscillate as sound pressure displaces the body of the fish. This results in the mechanical bending of ciliary bundles on the macula and is believed to be responsible for the transduction of motion to electrical signals sent to the brain (Yost, 1994; Popper, 1995; Braun & Grande, 2008). However, in *P. gibbiceps* and other previously discussed catfishes, the theoretical fluid dynamics of the inner ear would cause the otolith to rock rather than lag. Thus, both experimental data and modeling are needed to state precisely how the otolith aides in the process of acoustical signal transduction.

CHAPTER THREE: Figure Legend

Figure 9. Figures modified from Aquino & Schaefer 2002 (A) and Bleckmann et al. 1991 (B). A. llc- lateral-line canal, olc- otolateralic connection, lpc- laterophysic connection, sc-swim bladder capsule, hc- horizontal canal of the inner ear, sb- swim bladder. B. avc- anterior vertical canal, cr- cristae, hc- horizontal canal, L- lagena, OL-otolateralic connection, S-saccule, SB-swim bladder, si- sinus impar, WO- Weberian ossicles.


llc
olc
lpc
sc
hc
otophysic connection
sb

A


cr
avc
si
hc
S
cr
OL
SB
SB
wo

B

LITERATURE CITED

Arratia G. 2003. *Catfishes*, Vol 1. Enfield, NH: Science Publishers, Inc.

Armbruster JW. 2003. Phylogenetic relationships of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. Zoo J Linn Soc

Bleckmann H, Niemann U, Fritzch B. 1991. Peripheral and central aspects of the acoustic and lateral line system of a bottom dwelling catfish, Ancistrus sp. Jour Comp Neur 314:452-466.

Braun CB, Grande T. 2008. Evolution of Peripheral Mechanisms for the Enhancemen of Sound Reception. In: Webb JF, Popper AN, Fay RR, editors. Fish Bioacoustics. New York, NY: Springer. p 99-144.

Bridge TW, Haddon AC. 1889. Contribution to the anatomy of fishes volume I. The airbladder and Weberian ossicles in the Siluridae. Proc Roy Soc Lond 46: 209-227.

Bridge TW, Haddon AC. 1892. Contribution to anatomy of fishes volume II. The airbladder and Weberian ossicles in the Siluridae. Proc Roy Soc Lond 52:139-157.

Briggs J. 2005. The biogeography of otophysan fishes (Ostariophysi: Otophysi): a new appraisal. J Biogeogr 32: 287–294.

Chardon M, Vandewalle P. 1997. Evolutionary trends and possible origin of the Weberian apparatus. Neth J Zoo 47 (4): 383-403

Chardon M. 1968. Anatomie comparee de l'appareil de Weber et des structures connexes chez les Siluriformes. Ann Mus R Afr Centr Sci Zool 169:1-273.

Coburn MM, Grubach PG. 1998. Ontogeny of the Weberian apparatus in the armored catfish Corydoras paleatus (Siluriformes: Callichthyidae). Copeia 1998: 301-311

Fay RR, Popper AN. 1980. Structure and function in teleost auditory systems. In: Popper AN, Far RR, editors. Comparative studies of hearing in vertebrates. New York: Springer. p 3-42.

Dijgraaf S. 1960. Hearing in Bony Fishes. Proc Roy Soc Lond. Series B. Vol. 152. 946: 51-54

Fink SV, Fink WL. 1981. Interrelationships of ostariophysan fishes (Teleostei). Zoo J Linn Soc 72 (4): 297-353.

Godin OA. 2007. Transmission of low-frequency sound through the water-to-air interface. Acous Phys. Vol. 53 (3). p 305-312.

Godin OA. 2008. Sound transmission through water–air interfaces: new insights into an old problem. Contemp Phys. Vol. 49. (2) p105–123

Jenkins D. 1977. Light microscopic study of the saccule and lagena in certain catfishes. Am J Anat 150: 605-630.

Jenkins D. 1979. A transmission and scanning electron microscopic study of the saccule in five species of catfishes. Am J Anat 154: 81-102.

Ladich F, Bass AH. 2003. Audition: in Arratia et. al 2003 Catfishes Vol. 2: 701-730

Ladich F, Popper AN. 2004. Parallel evolution in fish hearing organs. In: Manly GA, Popper AN, Fay RR, editors. Evolution of the vertebrate auditory system. New York, NY: Springer. p 95-127.

Ladich F, Wysocki LE. 2003. How does tripus extirpation affect auditory sensitivity in goldfish? Hear Res 182:119-129.

Lechner W, Ladich F. 2008. Size matters: diversity in swimbladders and Weberian ossicles affects hearing in catfishes. Jour Exp Biol 211: 1681-1689

Lombarte A, Popper AN. 2004. Quantitative changes in the otolithic organs of the inner ear during the settlement period in European hake Merluccius merluccius. Mar Ecol Prog Ser 267: 233-240.

Nelson JS. 1994. Fishes of the World. 3rd Edition. New York: John Wiley.

Popper AN, Coombs S. 1982. The morphology and evolution of the ear in actinopterygian fishes. Amer Zool 22: 311-328.

Popper AN, Fay RR, 1999. The Auditory Periphery in Fishes. In: Fay RR, Popper AN, editors. Comparative hearing: fish and amphibians. New York: Springer. p 43-100.

Popper AN, Fay RR. 1993. Sound detection and processing by fish: critical review and major research questions Brain Behav Evol 41:14-38

Popper AN, Platt C. 1983. Sensory suface of the saccule and lagena in the ears of ostariophysan fishes. J Morph 176: 121-129.

Popper AN, Schilt CR. 2008. Hearing and acoustic behavior: basic and applied considerations. In: Webb JF, Popper AN, Fay RR, editors. Fish Bioacoustics. New York, NY: Springer. p 17-48.

Popper AN, Tavolga WN. 1981. Structure and function of the ear in the marine catfish, Arius felis. J Comp Physiol A 144: 27-34.

Popper AN. 1995. The teleost octavolateralis system: Structure and Function. Mar Fresh Behav Physiol. Vol. 27 (2-3). p 95-110.

Retzius G. 1881. Das Gehororgan der wirbelthiere. Vol. 1. Stockholm: Samson and Wallin.

Rogers P, Cox M. 1988. Underwater sound as a biological stimulus. In: Atema J, Fay RR, Popper AN, Tavolga WN, editors. Sensory Biology of Aquatic Animals. New York, NY: Springer-Verlag, p 131-149.

Rosen DE, Greenwood PH. 1970. Origin of the Weberian apparatus and the relationships of the ostariophysan and gonorynchiform fishes. Amer Mus Nov 2428: 1-25

Sagemehl M. 1885. Beiträge zur vergleichenden Anatomie der Fische. III. Das Cranium der Characiniden nebst allgemeinen Bemerkungen über die mit einem Weber'schen Apparat versehenen Physostomen-Familien. Gegenbaurs Morphol Jahrb 10: 1-119.

Saidel WM, Popper AN. 1983. Spatial organization in the saccule and sagena of a teleost: hair cell pattern and innervation. J Morph 177: 301-317.

Schaefer S. 1987. Osteology of Hypostomus plecostomus with a phylogenetic analysis of the loricariid subfamilies (Pisces: Siluroidei). Contrib Sci 394:1-31

Schaefer S. 1997. The Neotropical cascudinhos: Systematics and biogeography of the otocinclus catfishes (Siluriformes: Loricariidae). Proc Acad Natl Sci Philad 148: 1-120

Stetter H. 1929. Untersuchungen uber den Gehorsinn der Fische besonders von *Phonxis laevis* und *Amiurus nebulosus*. Raf Z vergl Physiol 26: 740-752.

Tavolga WN. 1977. Mechanisms for directional hearing in the sea catfish Arius felis. J Exp Biol 67: 97-115.

Van Bergeijk WA. 1967. The Evolution of Vertebrate Hearing. In: Neff WD, editor. Contributions to Sensory Physiology. Vol. 2. New York, NY: Academic. p 1-49.

von Frisch K. 1923. Ein Zwergwels, der kommt, wenn man ihm pfeift. Biol Zentralbl 43: 439-446.

von Frisch K. 1936. Über den gehörsinn der Fische. Biol Rev 11:210-246.

von Frisch K. 1938. The sense of hearing in fish. Nature 141: 8-11.

Weber EH. 1820. De Aure et Auditu Hominis et Animalium. Pars I. De Aure Animalium Aquatilium. Leipzig: Gerhard Fleischer.

Weitzmann SH. 2005. Hearing in catfishes especially that of the family Loricarridae. In: Hans-Georg E, Seidel I, editors. Catfish Atlas. Vol. 1. Washington DC: Baensch. P 31-39.

Wolfahrt TA. 1939. Untersuchungen uber das Tonunterschidungsvermogen der Elritze (Phoxinus laevis Agass). Z vergl Physiol 26: 570-604.

Yost WA. 1994. Fundamentals of hearing. San Diego, CA: Academic Press.

ACKNOWLEDGMENTS

Before all others, I thank my God for having given me the strength and peace to see this thesis to completion. All is owed to Him. As to the people who guided me, I owe my parents my deepest loving gratitude for their support and encouragement, without which I may have not completed this project. Especially considering that much of the document was produced on their computer. While they couldn't help by describing the images for me, they gave an ear when I wrestled with the problems and they mercilessly nagged me about finishing. I should also thank my little brother, Barrett who picked up the slack on the farm while I worked on this thesis. Thanks Burt.

To my mentor, friend, and walking encyclopedia of ichthyology, Dr. Michael Smith, I wish to offer my sincerest appreciation and gratitude. He not only helped me to apply scientific rigor in research, but also showed me the necessity of sympathy in an otherwise cold, impersonal pursuit of information. Thank you for being willing to postpone research progress for a semester so that I wouldn't be personally smothered by the demands of it.

It is a pleasure for me to thank Dr. John Andersland for teaching and assisting me in SEM—all the while making me enjoy the very tedious task. Rarely have I ever met a man whose exploration of the world around him causes him such delight and who so seamlessly integrated that search into his personality. One couldn't help but become excited with him as his face often enlivened at the mention of electron beams, travel, politics, or any other matter of discussion. If only the rest of the field could enjoy a life of investigation as he has!

Many thanks are owed to the Honors College at Western Kentucky University. The staff has been excellent in accommodating my unusual time frame and never failed to guide me when I had questions about the project. Particularly I am grateful for my scholastic superior who always treated me as though he were my equal, Dr. Craig T. Cobane. He too, oversaw my personal and intellectual growth constantly filling my ear with possibilities. Thank you for telling me most often what I could do and never mentioning what I couldn't.

I also wish to gratefully acknowledge the funding and resources provided for my project through NIH INBRE P20 RR-16481 and NSF EpSCOR grants, and the Western Kentucky University Biology Summer Undergraduate Research Experience Program. I additionally and especially acknowledge the Biotechnology Center at Western Kentucky University, who allowed me to occupy their fume hood for hours on end, all the while offering assistance and information. I would finally like to thank Dr. Steve Huskey and his lively dermestid beetle colony for having cleaned a few specimens for me to observe.