ABSTRACT

Title of Document: COMPARATIVE STUDIES ON THE STRUCTURE OF THE EARS OF DEEP-SEA FISHES

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Many deep-sea fishes have sensory adaptations for living at great depths with very limited light. While such adaptations are best known in the visual system, it is likely that there are also adaptations in the auditory system that enable deep-sea fishes to use the "auditory scene." However, there are few data on the inner ear of deep-sea fishes. The purpose of this study was to add to those data. Since deep-sea fishes are rarely taken alive, this study was done through comparative anatomical investigations. Three families were chosen from two major deep-sea fish fauna: benthopelagic and mesopelagic.

In *Antimora rostrata* (family Moridae, deep-sea cods), the inner ear structure and its coupling to the swim bladder were analyzed and compared with similar systems found in shallow-water fishes. Part of the membrane labyrinth is thick and rigid. The elaborate structure of the saccular epithelium and the close contact between the ear and swim bladder suggests enhanced hearing sensitivity.

In the family Melamphaidae (bigscales and ridgeheads), five species from three genera show broad interspecific variation in the saccular otolith shapes, including having

a long otolithic “stalk” in two genera. The presence of this “stalk” corresponds with a gradual change in the saccular maculae. A special type of ciliary bundle on the saccule may have enhanced sensitivity to bundle displacements.

Ears were compared between six species of Macrouridae (grenadiers and rattails) that live at different depths. The saccule/lagena size ratio seems to increase with depth, especially between a mesopelagic and a benthopelagic species in the genus *Nezumia*, in which the benthopelagic species has an enlarged saccule associated with sound production.

These findings support the hypothesis that some deep-sea fishes have evolved specializations for inner ear function. While it is not possible to test hearing in deep-sea fishes, the various adaptations found suggest that at least some such species have evolved specialized structures to enable them to use sound in the deep-sea. Some features in the ears of deep-sea fishes that have never been seen in the ears of other vertebrates, which further reveals the structural diversity of fish inner ears in general.

# COMPARATIVE STUDIES ON THE STRUCTURE OF THE EARS OF DEEP-SEA FISHES

by

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# Dedication

**This dissertation is dedicated to my beloved parents**

**for their love, support, and care.**

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# Chapter 1: Introduction

## SCOPE OF RESEARCH

The main goal of this study is to test the hypothesis that there are structural and morphological specializations in the ears of deep-sea fishes that have evolved during adaptations to the deep-sea environment.

Three families of deep-sea fishes from different deep-sea environments are included in this study. The inner ear structures of the species studied are compared within the same family, across different families, and with morphological data from fishes that inhabit shallower waters.

Fishes make up the most diverse group of vertebrates on Earth. They inhabit all aquatic environments with an enormous number of species and individuals (Nelson, 2006). Different species have evolved diverse specializations to enable them to live in all kinds of extreme environments, including in the deep ocean.

Two extreme ecological features of the deep ocean are considered in this study. One is the depletion of sunlight, while the second is very low biomass. Generally, the "deep-sea" starts from the lower limit of the Euphotic Zone, which is at approximately 200 meters depth (Fig. 1.1). From 200 meters to 1000 meters is the Twilight Zone, in which live the mesopelagic fauna. From 1000 meters, which is below the thermocline and where sunlight is totally depleted and the temperature is mostly below $4^{o}$ C, is the bathypelagic zone (Marshall, 1971; Angel, 1997). Deep-sea fishes living in these environments have to deal with darkness, low temperature, high pressure, scarce food, and difficulties in finding mates due to low population density (Herring, 2002).

Previous studies have documented adaptations and specializations in sensory systems of deep-sea fishes (Marshall, 1980). Many studies have demonstrated that deep-sea fishes have evolved highly adaptive and sensitive sensory organs for survival in the dim or lightless deep sea. Such adaptations are found in vision (Locket, 1977; Douglas et al., 1998; Wagner et al., 1998), olfaction (Herring, 2002), and in the lateral line systems (Marshall, 1996).

However, while we know a considerable amount about other sensory systems, we know very little about any structural and functional specification in the inner ears of any deep-sea fishes except for some gross morphological description from Marshall (1971, 1980) and the scanning electron microscope (SEM) study of ultrastructure in ten deep sea species (Popper, 1977, 1980).

In the sunlight-depleted environment of the deep-sea where visual cues are scarce, it is possible to hypothesize that the auditory system plays a very important role in the lives of fishes. This is suggested since hydrodynamic stimulation is continuously presented to fishes; acoustic signals provide a larger coverage area in terms of distance as compared to light, and a better directionality as compared to olfactory signals (Tavolga, 1971; Fay, 1988; Popper et al., 2003).

The acoustic environment in the deep ocean is different than the surface water because it is far away from the noise generated by wind and surface breaking waves. Although low frequency sound can propagate a long distance in the deep-sea, the “deep sound channel” below 1000 meters usually causes the propagation path of sound to bend upwards. This is due the change of sound velocity with pressure. The may cause the acoustic signals from some shallower sources to never reach to the bottom (Kuperman

and Roux, 2007). Thus there may be a higher signal to noise ratio for biologically relevant sounds in the deep-sea. There the sound environment may contains signals from many difference sources, some of which are from the interaction between currents and bottom structures; some are biogenic sounds from fishes, mammals, and invertebrates; some are generated by human activities that may still reach the deep. The mixture of acoustic signals forms a complicated "auditory scene" and must be undone by the animals to achieve an understanding of the acoustic environment. The process is call "auditory scene analysis" (Bregman, 1990, 2008). The function of hearing in fishes may help to gain an acoustic image of the surrounding world (Popper and Fay, 1993; Fay and Popper, 2000). This is likely to be especially useful for deep-sea fishes since they live mostly in the dark.

**My hypothesis is that, in order to obtain the best accuracy and detail in perceiving the environment, some deep-sea fishes have evolved enhanced sensitivity and directionality in their hearing organs.**

Since deep-sea fishes are rarely taken alive, I will test my hypothesis through a comparative anatomical study. Two major groups of fishes were chosen for this study, one belongs to the mesopelagic deep-sea fishes which live between 200 and 1000 meters and have no contact with the sea floor. The other group belongs to the benthopelagic deep-sea fishes, which live below 1000 meters and are close to the sea floor.

Three lines of studies will be carried out using these deep-sea fishes.

**The first line of study is to analyze the inner ear structure of *Antimora rostrata* and its connections to the swim bladder, and compare these structures to shallow-water hearing specialists that have similar ear-swim bladder connections.**

Shallow-water fishes are those living in the euphotic zone of the ocean or in fresh waters. This species were chosen because it is one of the dominant species in the benthopelagic fauna and it is well studied for its biochemical and physiological adaptations to deep-sea life. The connection between the ear and the swim bladder in this species is potentially useful for inner ear function and it hasn't been studied in detail. The goal of this study is to determine if there are indications of specialization in the ear based upon the inner ear structures of this deep-sea species when comparing it with some shallow-water species

**The second line of comparison is of the inner ear structure between five species in the mesopelagic family Melamphaidae** whose member species have extremely broad variations in their otolith shapes and sensory epithelia structures. The goals of this comparison are two-fold: One is to find out if the variation in inner ear structure is related to the fishes' ecological status and life style (food, prey, or predator); The other is to try to correlate the structural variation with the phylogenetic history of these species in order to understand the evolution of these varying traits in their inner ears.

**The third line of comparison is of the inner ear structure between six species in a benthopelagic family (Macrouridae)** whose member species live at different ocean depths ranging from 200 meters to 5000 meters. The goal of this comparison is to find out if there are structural differences in the inner ear that are potentially correlated with the depth at which each species lives.

The significance of the investigations of the characteristics and specializations in deep-sea fish inner ear morphology and ultrastructure has many aspects. It will help us to hypothesize about the hearing ability of deep-sea fishes, thus providing data to help understand their life style. Comparison of inner ear structures between different deep-sea

fish species as well as with other shallow water fish species can provide insight into the adaptation and evolution of the inner ear in fishes. This research also presents many structures only found in deeps-sea fishes so far, which serve as representations of the diversity in fish ears in general, as well as a representation of the unique evolution pathways in deep-sea fishes.

The positions of the three selected deep-sea fish families in the phylogenetic relationship of teleosts (bony fishes) are shown in a cladogram (Fig. 1.2). The sequence of superorders and orders is based on Nelson (2006) and Helfman et al. (1997). Representations of the most commonly seen fish groups are listed for each order. The orders containing deep-sea fishes or fishes with hearing specializations are highlighted in different colors. Some of the families or species will be discussed throughout this manuscript.

## BACKGROUND

### Hearing Organs in Fishes

The inner ear of all bony fishes has three semicircular canals and three otolithic end organs, the saccule, lagena, and utricle. Figure 1.3 shows the gross structure of the inner ear from one fish in this study, the deep-sea cod *Antimora rostrata*. This figure shows the main components of a teleost fish ear. The semicircular canals are vestibular organs. However, the functional division of the three otolithic end organs is not as clear. For many fishes, the saccule may be primarily hearing, the utricle may be primarily vestibular, and the lagena may have dual functions (Platt and Popper, 1981; Platt, 1983). However, many fish species have different specializations in some of the end organs.

Clupeid fishes (herrings and shads) are likely to use their utricles for ultrasound detection (Mann et al., 1997; Mann et al., 1998; Plachta and Popper, 2003), while the saccule and lagena of *Carassius auratus* (goldfish) both showed sensitivity to sound (reviewed by Popper and Fay, 1999).

Each of the otolithic end organs is a sac that has a sensory epithelium on the medial wall. The sensory epithelium, or macula, contains sensory hair cells and supporting cells. A calcium carbonate otolith lies next to the macula and attaches to it via a gelatinous otolith membrane (Fig. 1.4) (Dale, 1976; Popper, 1977).

**Sensory Epithelium and Hair Cells**

The sensory hair cells on the three sensory epithelia have their cell body embedded in the epithelium, with their ciliary bundles pointing towards the otolith and embedded in the gelatinous otolith membrane (Popper, 1977; Platt and Popper, 1981).

A typical fish sensory hair cell has a cylindrical cell body with hair bundles on its apical surface. The hair bundle is made up by a kinocilium and a graded bundle of stereocilia, with the tallest stereocilia standing closest to the kinocilium (Fig. 1.5A,B). The direction of the maximum response lies along the axis from the shortest stereocilia to the tall kinocilium (Hudspeth and Corey, 1977) (Fig. 1.5C). Usually we use an arrow pointing from the shortest stereocilia to the kinocilium to indicate a hair cell bundle's orientation.

**Hair Bundle Orientation Patterns**

Hair bundles with the same orientations form groups on the epithelia. The groups form a certain pattern on the maculae. Figure 1.6 shows a map of hair bundle orientation pattern of utricle from a deep-sea fish *Melamphaes acanthomus* (shoulderspine bigscale) with scanning electron microscopic (SEM) photos of part of the macula and some hair bundles that are oriented in opposite directions.

There are similarities as well as variations in the hair bundle orientation patterns. Figure 1.7 shows some typical orientation patterns of teleost saccular epithelia. The standard pattern has four hair bundle orientation groups, which is a very common pattern in non-ostariophysan fishes (Popper and Coombs, 1980; Popper et al., 2003). The term "ostariophysan" refers to fishes that have a series of bones, called the Weberian ossicles, connecting the swim bladder and the inner ear (e.g. goldfish and carps, Weber, 1820; reviewed by Popper, 1983); and non-ostariophysan fishes are those without such connections. The vertical pattern is found in the saccule of ostariophysan fishes, which has two hair bundle groups, one oriented dorsally and the other ventrally. More on the differences in hearing between the ostariophysan and non-ostariophysan fishes will be covered in later sections.

Besides these two basic patterns, there are many other orientation patterns found in saccules of different fish species: dual, opposing and alternating pattern are the ones that have been categorized by Popper and colleagues (Popper and Coombs, 1982; Popper and Fay, 1999; Popper et al., 2003). However, not all hair cell orientation patterns on fish saccules can be described by these patterns, including the ones in my study.

The hair cell bundle orientation patterns are believed to play a role in directional hearing. The direction of sound may be coded by the orientation patterns (Wersäll et al., 1965; Flock, 1971; Platt and Popper, 1981; Schellart and Popper, 1992). This suggestion was strengthened by physiological studies that recorded from nerve fibers from hair cells oriented in different directions (Lu et al., 1998).

Hair cell bundles also show variations in shapes and heights. Dale (1976) studied the ears of *Gadus morhua* (Atlantic cod) and found that shorter bundles are primarily found in the center of saccule and taller bundles are close to the edge. Figure 1.8 shows the distributions of cilia bundle heights on the *Carassius auratus* (goldfish) saccule. It is also hypothesized that these variations can help in detecting different aspects of sound signals (Platt and Popper, 1984; Popper and Fay, 1993). SEM data in goldfish saccule have shown that the area in the sensory epithelium responsive to low frequency sound has long hair bundles (Platt and Popper, 1984).

**Underwater Sound Detection**

Fishes live in an environment in which the body has the same density as the water. When sound propagates through the water, the body is transparent to the sound except for the otolith and the swim bladder. The otolith is three times denser than the rest of the fish body. When sound reaches the fish body, the fish oscillates together with the water, while the otolith lags behind this oscillation due to its higher density. This lag during oscillation causes a shearing movement between the otolith and the surface of the macula.

The hair cell bundles are connected to the otolith through the otolith membrane. The hair cell bundles bend as a result of the relative movements between the otolith and the sensory epithelium. Thus the sound signal is transformed to a mechanical stimulation that can be detected by the hair cells (Popper, 1983).

When a hair bundle bends to the direction of its best sensitive axis, the cell produces a maximum response, a maximum depolarization. When it bends to the opposite direction of its most sensitive axis, maximum hyperpolarization occurs (Flock, 1971; Hudspeth and Corey, 1977). When a hair bundle bends in directions other than the last two, different degrees of depolarization or hyperpolarization occur. During a depolarization, bending of the stereocilia to the responsive direction opens ion channels called transduction channels, which allow the influx of $Ca^{2+}$ and $K^{+}$, causing depolarization of the hair cell (Lewis and Hudspeth, 1983). Thus the mechanical stimulation is transformed into electrical energy during the response, which is called mechanoelectrical transduction.

There are two components to sound, pressure fluctuations and particle motion. Particle motions are hydrodynamic flows caused by the vibration of the sound source and attenuate very rapidly with distance from the source. The pressure component of the sound, on the other hand, attenuates much less with distance, thus the sound pressure can travel farther than the particle motion and be detected at a greater distance from the source.

The two different components of the sound reach the fish ear through different pathways. The stimulation from the relative movement between the otolith and the hair bundle is called the direct pathway and it is involved in hearing in all fishes. In this

pathway, the particle displacement component of the sound stimulates the ear. Since particle motion attenuates greatly with distance, it is thought that only a sound source near the fish can be detected through this pathway (Popper and Fay, 1999).

For fishes that have swim bladders or any gas chambers close to their ear, there is a second pathway, the indirect pathway. The swim bladders and other gas chambers have much lower density than the water; the compression and expansion of the walls of these gas chambers is caused by sound that is then reradiated by the swim bladder to the ear. The swim bladder can radiate not only the particle displacement, but also the pressure component of the sound. Since pressure wave travels greater distance than particle movement, the hearing range of fishes that are able to detect pressure signals is extended much further than fishes without this capability. These fishes have been called hearing "specialists." Fishes that primarily hear via direct stimulation have been called hearing "generalists" since they have poorer sensitivity and a narrower bandwidth than the "hearing specialists" (Popper and Fay, 1999).

**Specializations in Shallow Water Fish Ears**

One problem of studying the hearing of deep-sea fishes is that it is very difficult to obtain live specimens to conduct physiological experiments. The alternate approach is to compare the anatomical structure with the structure of shallow water fishes that have known physiology and anatomical data to demonstrate their hearing capabilities. Such comparisons may give insights that may help us understand the specializations in deep-sea fish ears. Here I give some examples of some specializations in shallow water fish ears.

Some teleost fishes, known as the hearing specialists, have evolved specializations in their peripheral structures that can enhance hearing as compared to those hearing generalists that do not have such structure. Figure 1.9 shows the audiograms of some fish species for a comparison between hearing specialist, represented by *Carassius auratus* (goldfish) and *Alosa sapidissma* (American shad), and non-specialists, represented by *Salmo salar* (Atlantic salmon), and *Gadus morhua* (Atlantic cod). The audiograms of hearing specialists show a lower threshold in sensitivity and extended range in frequency as compared to non-specialists.

In fishes that have specializations in hearing, a gas-filled chamber near the ear is one common strategy to enhance the sound signal that reaches the fish ears by radiating the pressure component of the sound to a displacement that can detected by the inner ear, expand the fish's hearing range in distance and bandwidth. Some examples are found in the Ostariophysan fishes (Fig. 1.10A), as represented by goldfish and carps (von Frisch, 1938; van Bergeijk, 1967; Popper, 1983) and *Arius felis* (marine catfish) (Popper and Tavolga, 1981). This connection greatly enhances the hearing ability of ostariophysans.

Another major specialization is seen in the clupeid fishes (herrings and shads). *Alosa sapidissima* (American shad) is able to detect sound up to 180 kHz (Mann et al., 1997; 1998; Plachta and Popper, 2003). Its specialization for ultrasound detection lies in a bulla membrane and elastic thread apparatus near the utricle (Fig. 1.10B). The bulla is subdivided into a gas-filled and a fluid-filled portion by a flexible bulla membrane. The sensory epithelium of the utricle, unlike the single epithelium in most vertebrate utricles, is divided into three distinct parts. The bulla membrane is directly connected to the anterior portion of the middle macula of the utricle by an 'elastic thread' (Higgs et al.,

2004). The extremely thin connection between the middle epithelium (arrow in Fig. 1.10B) and the rest of the utricle is essentially a spring with miniscule mass that would require little energy to stretch, thus making this epithelium more sensitive to vibrations at ultrasonic frequencies (Higgs et al., 2004).

A sound producing fish *Micropogonias undulates* (Atlantic croaker; Family Sciaenidae) is found to have better hearing sensitivity than some of the other members in the family (Ramcharitar and Popper, 2004; Ramcharitar et al., 2006). In species with better hearing sensitivity in this family, the specializations in their inner ear structure may involve an enlarged part in saccular macula. Figure 1.11 shows a schematic drawing of saccular maculae of four different species from the family Sciaenidae (croakers and drums). Note that although these four saccular maculae have similar orientation patterns, there is a gradual change in the "head" (anterior) area. The enlargement of the "head" corresponds to a change in the distance between the swim bladder to the ear, with the largest "head" found in the species that have the swim bladder placed closest to the ear. Furthermore, acoustic brainstem response (ABR) study shows that these species also have the best hearing ability among the four species (Ramcharitar et al., 2001; Ramcharitar and Popper, 2004).

**Adaptations in Deep-Sea Fish Sensory System**

In most oceans, the depth of 200 meters is the lower limit of euphotic zone, the upper, illuminated zone of aquatic ecosystems that allows effective photosynthesis. From 200 meters to 1000 meters is the twilight zone, in which live the mesopelagic fauna. This is a transition layer between the surface and the deep water where the temperature drops

rapidly (Fig. 1.1). A mesopelagic family Melamphaidae is one of the groups used in this study.

Below 1000 meters, where sunlight is totally depleted, is the bathypelagic zone. Further down from the continental rise to the abyssal plain is the Abyssopelagic Zone (Angel, 1997). Within the bathypelagic and abyssopelagic regions, fishes resting on the sea floor are called benthic fishes, whereas fishes that hover just above the sea floor are called benthopelagic fishes (Fig. 1.1). The latter fauna primarily includes Macrouridae (rattails and grenadiers), Moridae (deep-sea cods), and Brotulidae (brotula, relatives of cusk eels) (Marshall, 1971). Two Gadiform families (Macrouridae and Moridae) in this benthopelagic fauna are of interest in this study.

Although very little is known about the lives of deep-sea fishes, some aspects of their sensory systems are of special interest and have been the subject of extensive study, providing us with some information about aspects of sensory system adaptations in deep-sea fishes. For example, the visual system of many mesopelagic fishes is adapted to the low light environment by means of having very large eyes and tubular lenses to condense light (Munk, 1966; Wagner et al., 1998). Many deep-sea fishes have a pure-rod retina, while some even have multiple banks of rods with up to 30 to 40 layers. In comparison, humans have only one layer of rods in the retina (Locket, 1977; Wagner et al., 1998). The increased number of receptors gives the eyes a better sensitivity. A group of dragon fishes (for example, *Malacosteus niger*) can detect far-red light using a chlorophyll-derived substance in the retina. Similar to the mechanisms in photosynthesis, the chlorophyll-derived photosensitizer captures the energy from the far-red light and

transfers it to the other visual pigments (Partridge and Douglas, 1995; Douglas et al., 1998).

Unlike mesopelagic deep-sea fishes, benthopelagic fishes do not have such dramatic specializations in their eyes. Although large eyes are still found in many species living below 1000 meters, regressed eyes become more common at greater depths (Munk, 1964). In bottom dwellers such as benthopelagic and benthic fishes, the olfactory, gustatory, hearing, and lateral line senses may take over vision (Marshall, 1971).

Similar to the visual system, the lateral line system of deep-sea fishes also varies with habitat and life style (Marshall, 1971; reviewed by Coombs et al., 1988; Coombs et al., 1992). The lateral line system is a series of mechanosensory hair cell patches that are distributed on the body surface of the fish. These hair cell patches are called neuromasts. There are two kinds of neuromasts: superficial neuromasts are located on the surface of the skin exposed to the water flow; canal neuromasts lie inside fluid-filled canals below the skin surface, with openings between the neuromasts. A gelatinous cupula covers the sensory epithelium of the neuromast and transfers the water flow movements to the hair cells (Coombs and Montgomery, 1999).

Many fishes have mixed lateral line organs, with both superficial neuromasts and canal neuromasts, such as those found in most benthopelagic and mesopelagic fishes (Marshall, 1980). A trend of widened neuromasts in the cephalic canal organs (the lateral line organs on the head) is also found in macrourids (rattails and grenadiers) and halosaurs (a family of eel-like deep-sea fishes). These widened neuromasts (4 mm in diameter) may be very sensitive, but they may be also well buffered against the noise by a large mass of viscous fluid (Marshall, 1971, 1980).

For some slow swimmers living in quiet waters, such as cavefish and bathypelagic fishes, their lateral line organs are composed mostly of superficial neuromasts that are directly exposed to the hydrodynamic environment (Marshall, 1971). Some of these fishes even have superficial neuromasts set on long stalks, for example, *Amblyopsis* (cave-dwelling blindfish; Poulson, 1963), the bathypelagic *Caulophryne* (fanfin anglerfish), and *Neoceratias* (toothed seadevils; Bertelsen, 1951). These cavefish and deep-sea fishes appear to have increased sensitivity in their lateral lines (Montgomery and Plankhurst, 1997); they are also able to decrease self-generated noise through slower motions and reduced respiration movement with low metabolic rate (Denton and Marshall, 1958).

**Studies on Ears of Deep-Sea Fishes**

The structure and function of the inner ear in deep-sea fishes is yet to be explored broadly. The earliest data might be the drawing of the ear from *Lophius piscatorius* (angler, monkfish) by Retizius (1881) and ears from three mesopelagic species and a rattail fish (Fig. 1.12) by Bierbaum (1914). During the 1960s and 1970s, N. B. Marshall described some of the gross morphological features of the inner ears of a few species of deep-sea fishes. Having compared inner ears of fishes from different depths, Marshall concluded that mesopelagic and bathypelagic fishes have large utricles and small saccules, whereas benthopelagic fishes (macrourids, deep-sea cods, and brotulids) tend to have very large saccules and saccular otoliths, which suggests that they might have sensitive hearing (Marshall, 1980). Such observations are yet to be confirmed by further studies.

It has been noticed that some deep-sea fishes have larger ear capsules relative to the size of their brain (Marshall, 1971; Fine et al., 1987) as compared to shallow water fishes. Fine et al. found in a deep-sea benthopelagic fish *Acanthonus armatus* (bony-eared assfish) the largest ear dimension relative to its brain of any other known fish species. This fish also has very large saccule sacs that contain very large saccular otoliths (Fine et al., 1987).

The only data we have so far on the ultrastructure of the ears of deep-sea fishes are Popper's (1977, 1980) SEM studies of saccules and lagenae in ten species of deep-sea fishes. These ten species covered some of the major groups from mesopelagic to bathypelagic fishes: Myctophidae (lantern fishes), Stomiiformes (dragon fishes), Osmeriformes (barreleyes), Lophiiformes (anglerfishes), Scorparniformes (scorpion fishes), and a pelagic Gadiform (codlet). A typical four quadrant bundle orientation pattern was found in most of these species except for the Gadiform *Bregmaceros* (codlet), with the bundles in the rostral part of the macula oriented horizontally and bundles in the caudal part of macula oriented vertically. *Bregmaceros* has six hair bundle orientation groups, a finding that is consistent with our present study results in Gadiform macrourids.

Among these deep-sea macrourids, one type of hair cell bundles appears more frequently than in shallow water fishes. These bundles have very long kinocilium, and a few long stereocilia that are almost as long as the kinocilium (Popper, 1980). Other features found in these deep-sea fishes were teeth-like sculpted saccular otolith in *Opisthoproctus soleatus* (barreleye) and large extra-macular cells in the ventral region of macula in *Gonostoma* (bristlemouth) and three species of myctophids (lanternfishes). Most of these features were also found in the deep-sea fishes that are currently being

studied. The functional significance of these characters needs further consideration and discussion.

**FIGURES**

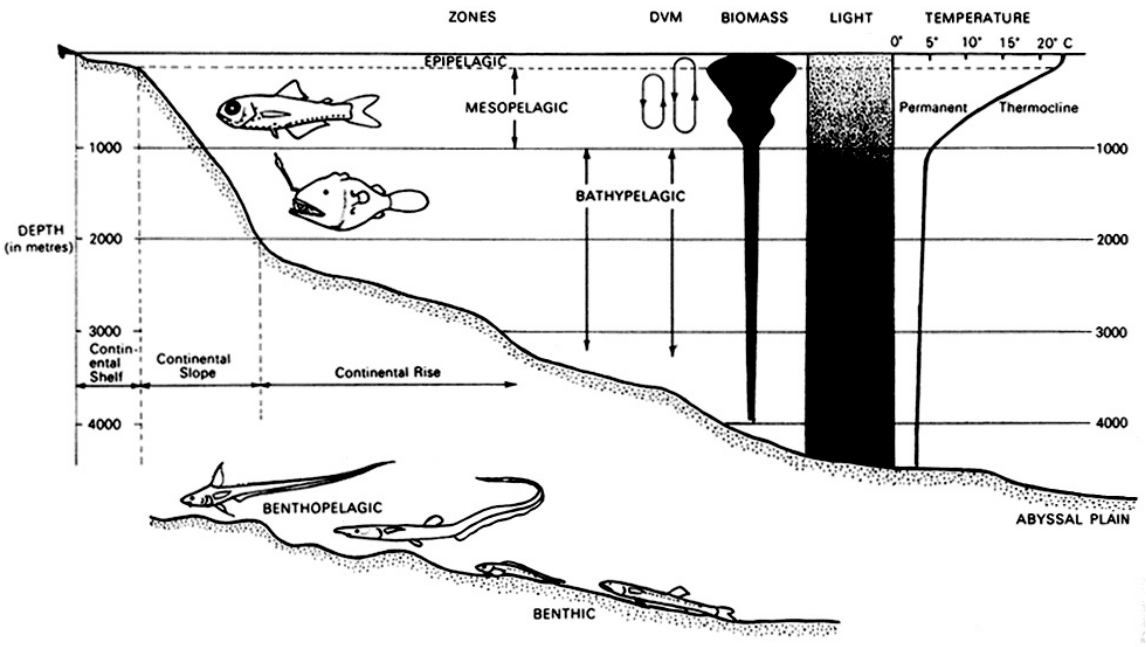


**Figure 1.1.** Ocean profile showing contrasting living environments of deep-sea fishes (from Marshall, 1971)

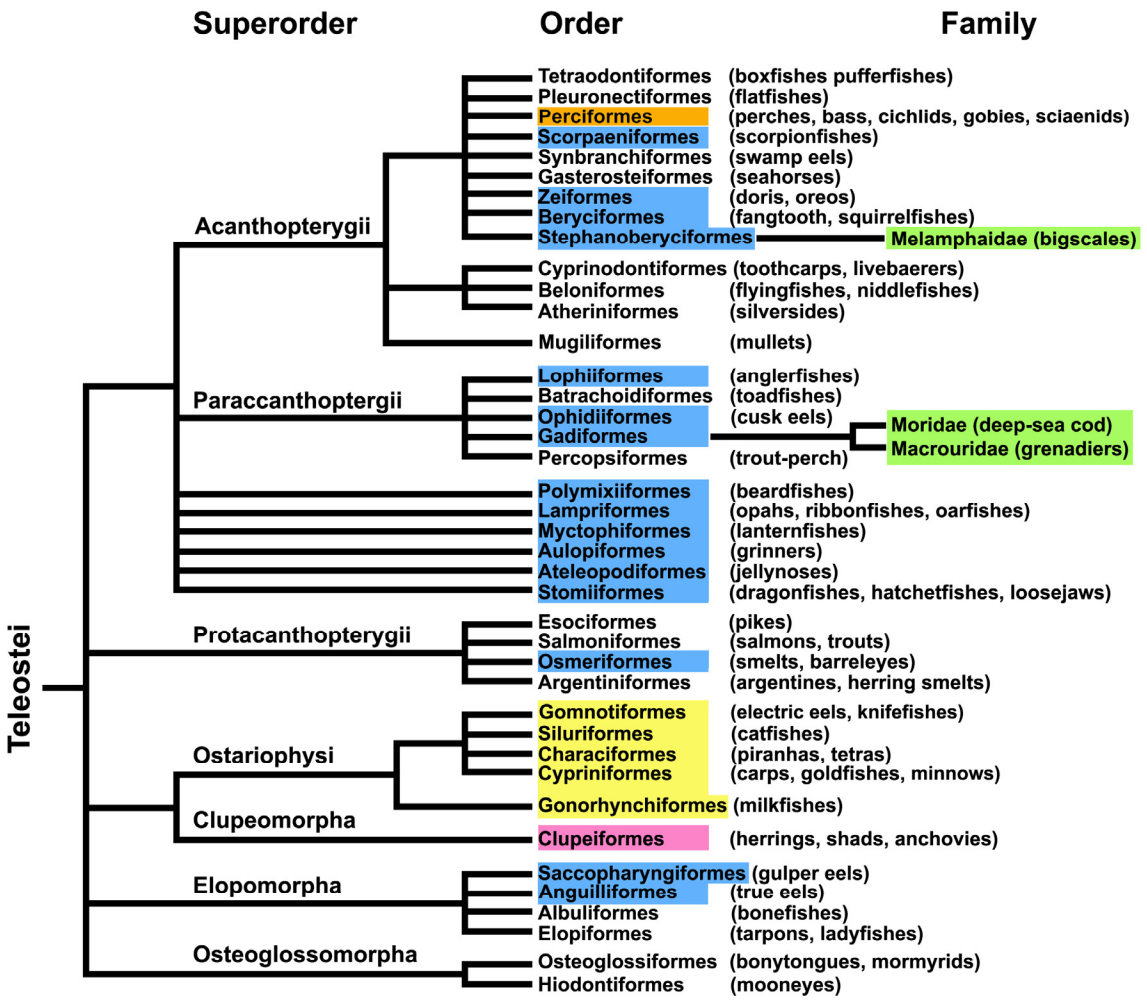


**Figure 1.2.** Cladogram of teleost fishes showing the relationship of different fish groups. The lengths of branches do not represent time. The sequence of orders is based on Nelson (2006). Representations of the most commonly seen fish groups are listed for each order. The orders containing deep-water living fishes are highlighted in blue. The three deep-sea fish families used in this study are highlighted in green. The fish orders highlighted with orange, yellow, and pink contain species with specialized structures introduced in later sections of this Chapter.

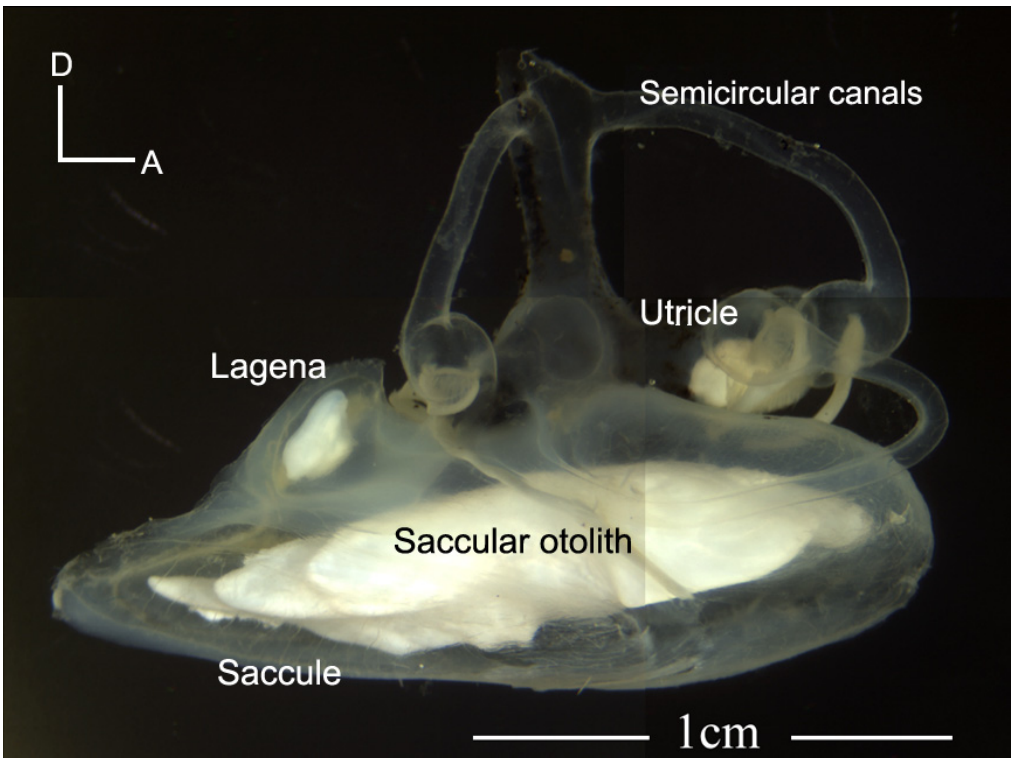


**Figure 1.3.** Lateral view of a right ear from *Antimora rostrata* (deep-sea cod). A, anterior; D, dorsal.

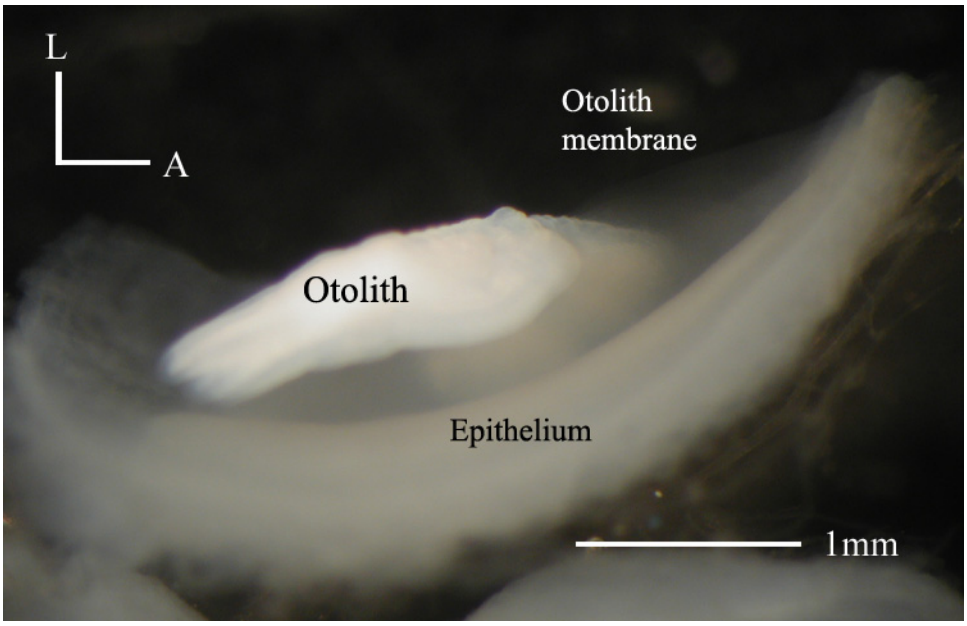


**Figure 1.4.** Ventral view of an opened lagena from *Caelorinchus occa* (swordsnout grenadier) showing the relationship between the epithelium, otolith membrane, and the otolith. A, anterior; L, lateral.

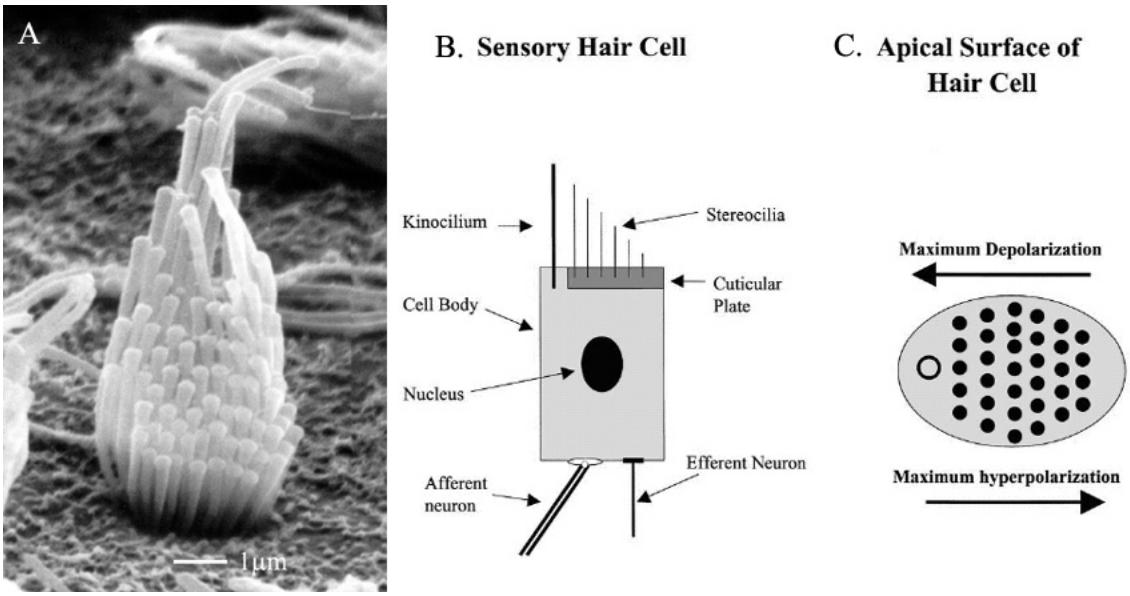


**Figure 1.5.** Hair bundle structure. **A:** SEM photo of a fish hair cell bundle from the lagena of *Coryphaenoides rupestris*. **B:** Lateral view of a hair cell showing the cell body, kinocillium and stereocilia. **C:** Dorsal view of the hair cell with the open circle indicates the kinocilium and the closed circles indicate the stereocilia (from Popper and Lu, 2000).

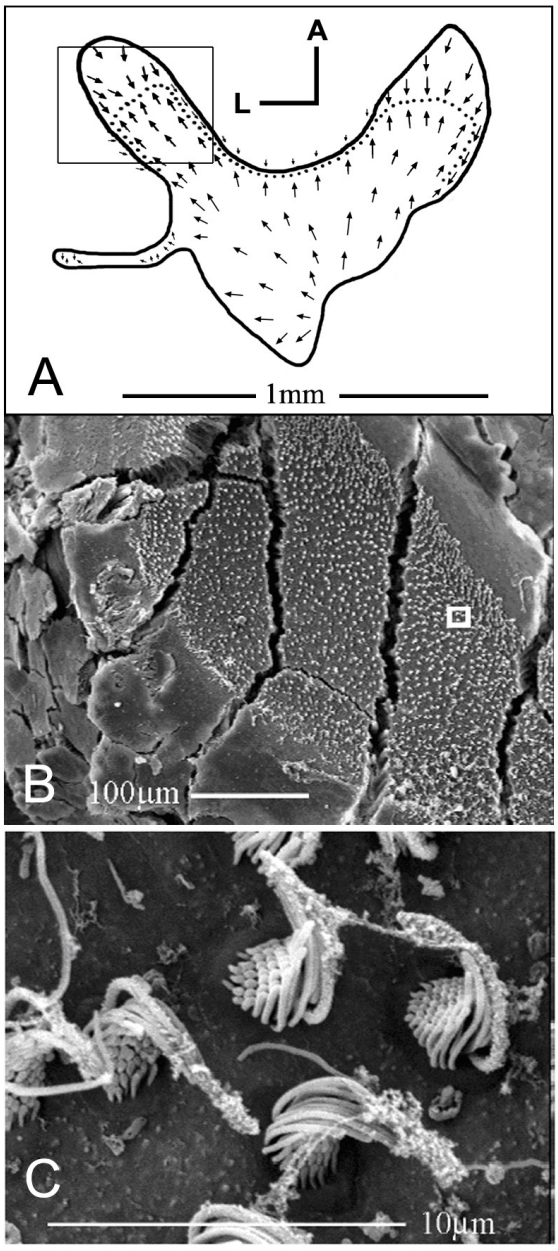


**Figure 1.6.** Utricle of *Melamphaes acanthomus.* **A:** Map of hair bundle orientation pattern. **B:** SEM photo of the maculae area in box from **A**. **C:** Hair bundles oriented in opposite directions in box from **B**.

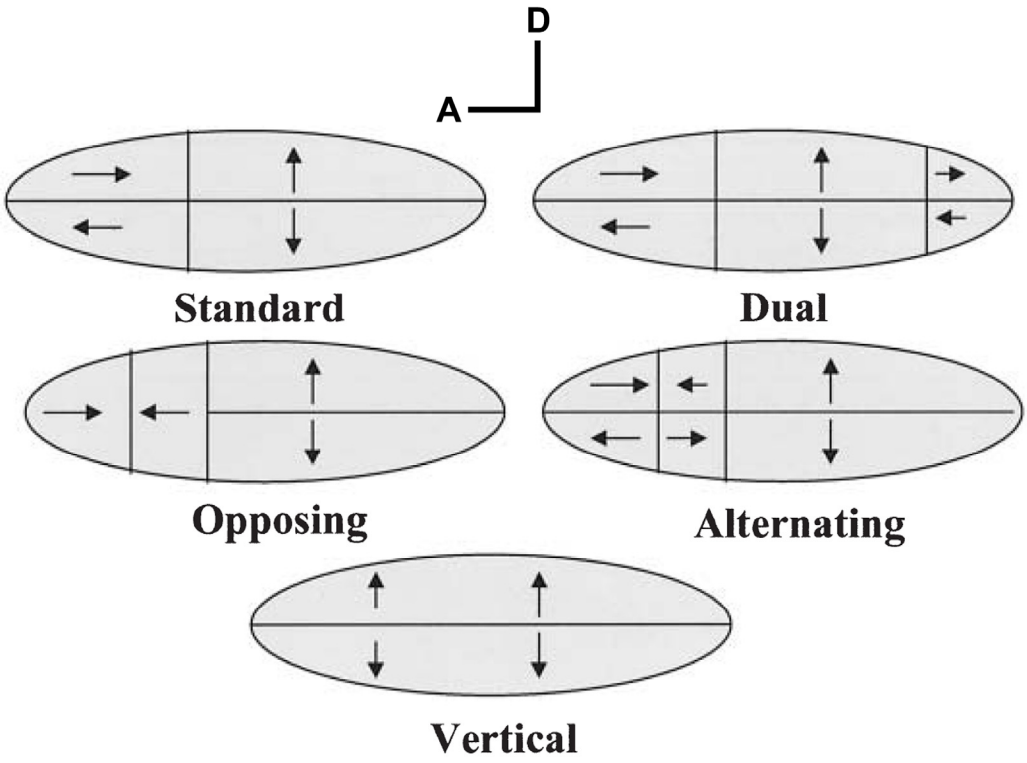


**Figure 1.7.** Schematic diagram of typical saccular hair bundle orientation patterns (from Popper et al., 2003). Each arrow indicates a group of hair cell bundles that has the same direction of the kinocilium. The standard pattern has four directional groups which is typical for many fishes. The vertical pattern has saccular hair cells oriented in two directions; this is typical for ostariophysan fishes (Popper and Lu, 2000). The dual pattern is also found in *Gadus morhua* (Atlantic cod) (Dale, 1976).

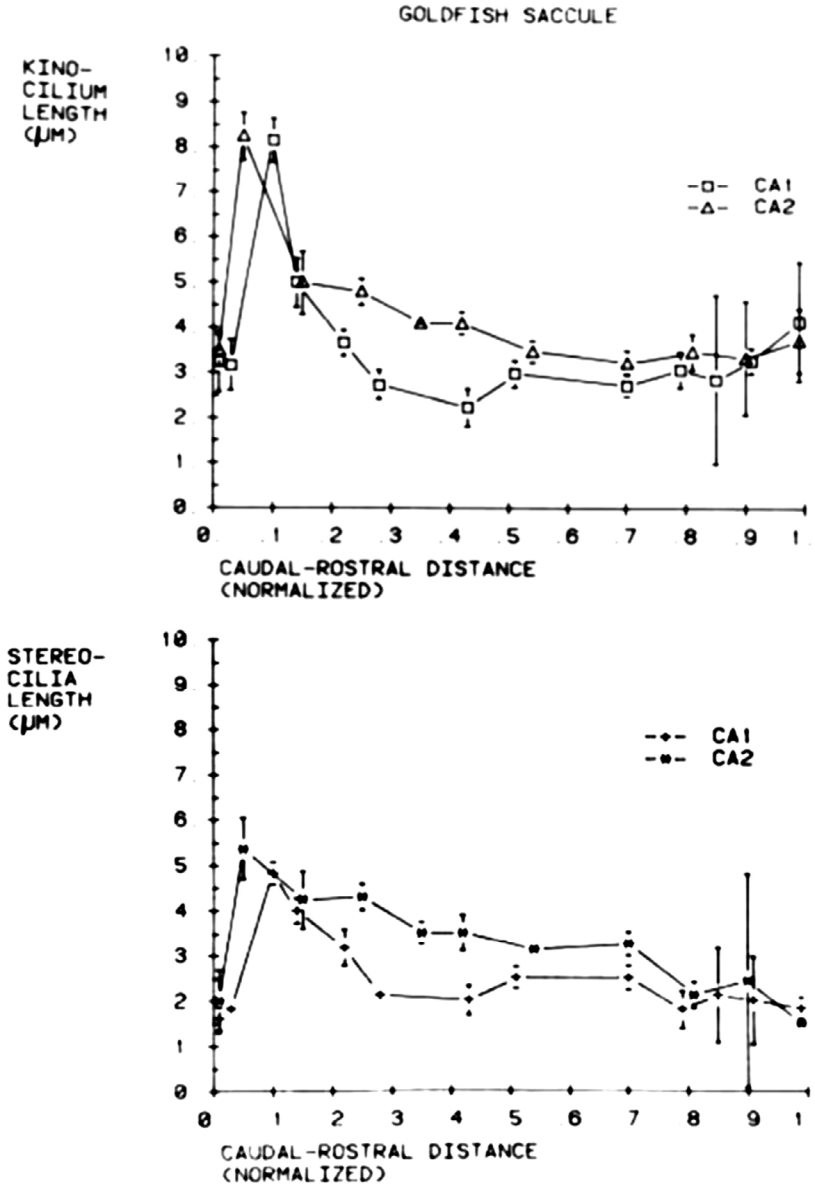


**Figure 1.8.** Distribution of cilia bundle heights on the goldfish saccule (from Platt and Popper, 1984). Length of kinocilia (left) and the longest stereocilia (right) are plotted against the caudal-rostral distance of the saccule. CA1 and CA2 label two different fish samples.

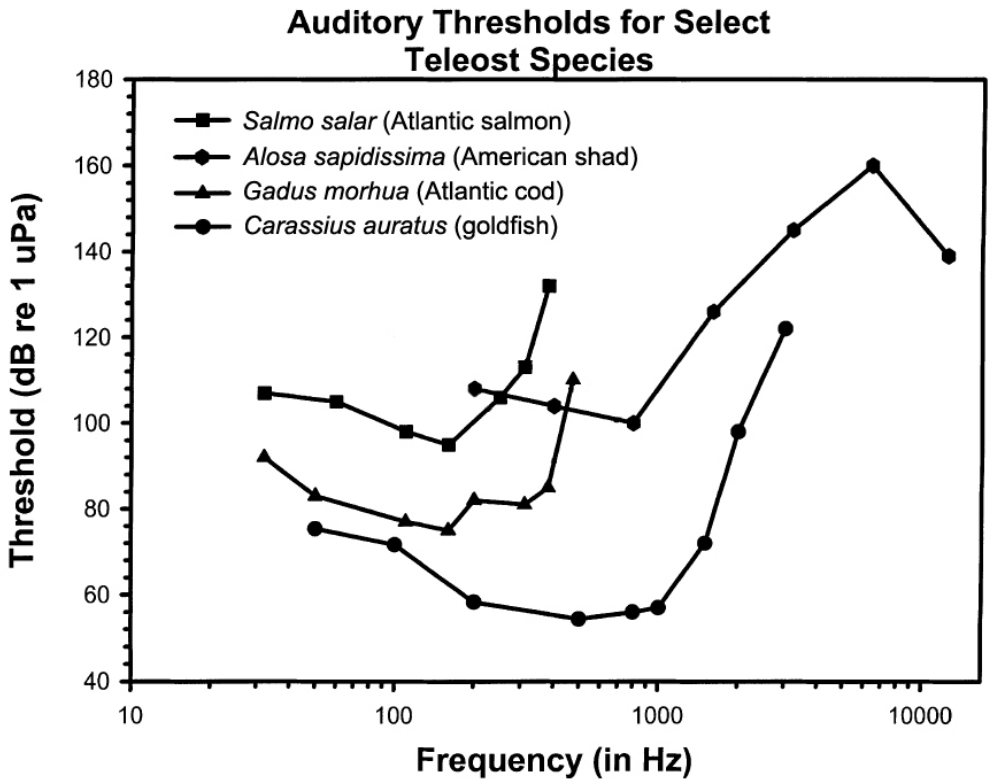


**Figure 1.9.** Auditory thresholds of select group of teleost fishes to illustrate the range of hearing capabilities determined with behavioral methods. *Carassius auratus* (goldfish) is a hearing specialist (Jacobs and Tavolga, 1967). *Salmo salar* (Atlantic salmon; Hawkins and Johnstone, 1978) and *Gadus morhua* (Atlantic cod; Chapman and Hawkins, 1973) do not have any known specializations to enhance hearing, and they are considered "generalists". *Alosa sapidissima* (American shad) is considered a specialist with regard to its very broad hearing range (to over 180 kHz), but sensitivity at low frequencies is not nearly as good as that of a hearing specialist like goldfish (modified from Mann et al., 1998).

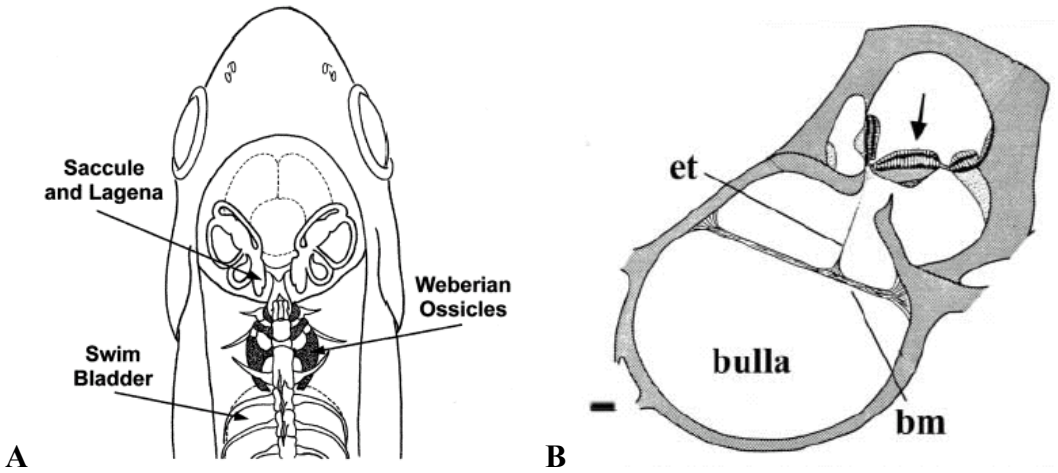


**Figure 1.10.** Specialized structures in fish ears. **A:** Relative positions of the ear, the Weberian ossicles, and the swim bladder within the body of the goldfish (Popper and Coombs, 1980; from von Frisch, 1936). **B:** Diagram (Higgs et al., 2004) showing the relationship of the prootic bulla to the utricle in adult American shad. BM, bulla membrane; ET, elastic thread; arrow points to the middle macula.

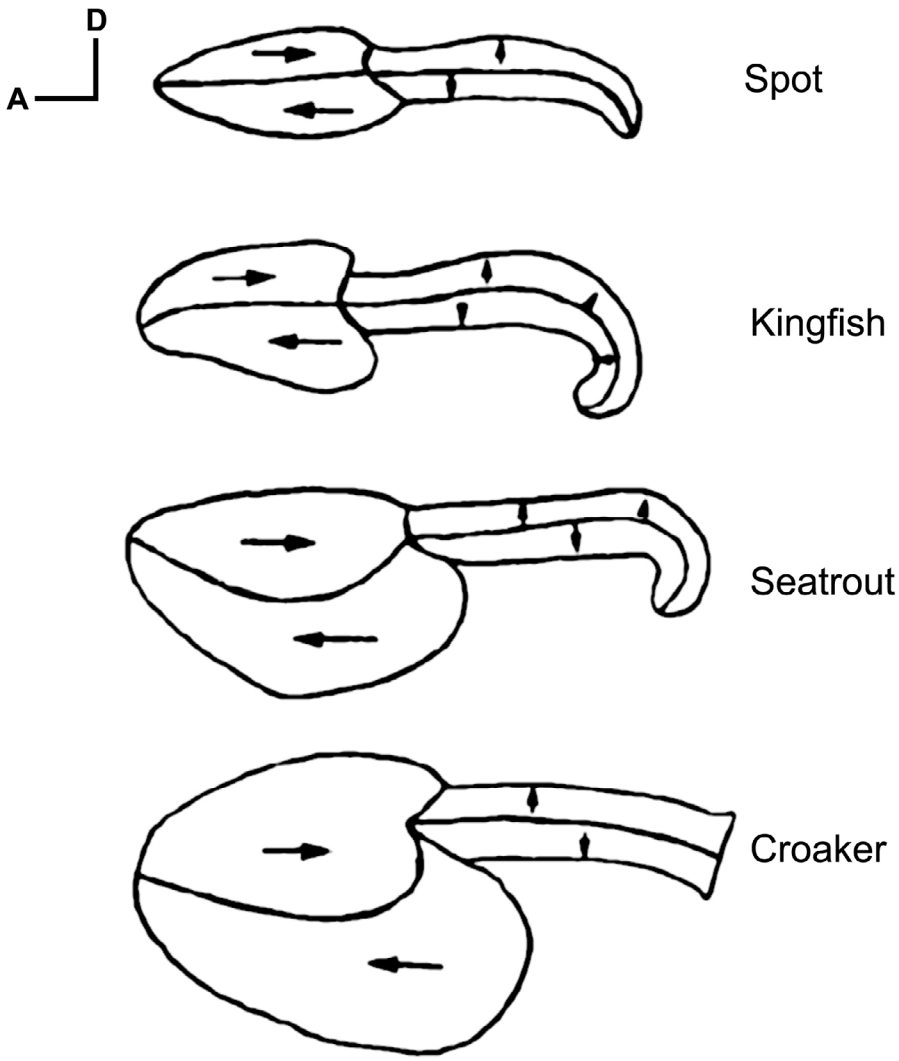


**Figure 1.11.** Schematic drawing of saccular maculae from four different species of sciaenids (from Ramcharitar et al., 2001). A, anterior; D, dorsal.

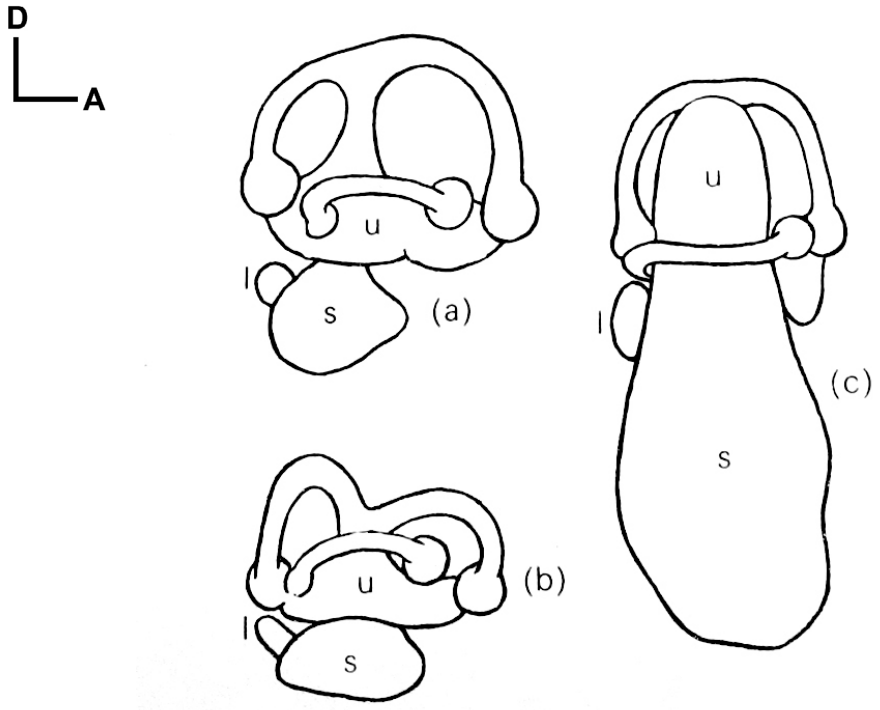


**Figure 1.12.** Redrawing of three deep-sea fish ears from Biebaum (1914) (from Marshall, 1971). (**a**) *Vinciguerria lucetia*, a mesopelagic fish; (**b**) *Dolopichthys*, a ceratioid anglerfish from the bathypelagic zone; and (**c**) a macrourid, *Hymenocephalus*. U, utricle; l, lagena; s, saccule; A, anterior; D, dorsal.

# Chapter 2: The Inner Ear and its Coupling to the Swim Bladder in the Deep-sea Fish *Antimora rostrata*


## ABSTRACT

The inner ear structure of *Antimora rostrata* (family Moridae, deep-sea cods) and its coupling to the swim bladder were analyzed and compared with ears of some shallow-water hearing specialists. The ear of *Antimora* is exceptionally big, with a very long saccular otolith and macula. The elaborate structure of saccular macula and its anterior enlargement may imply increased hearing sensitivity, as may the close contact with the swim bladder. The ciliary bundle types on the sensory hair cells are heterogeneous on the epithelia of the three otolithic end organs. Bundles were classified into different categories and mapped on the maculae to provide an overview of the bundle type distributions. Part of the saccular maculae is rich in multiple bundle types. The rigid part of the inner ear membrane and the attachment between the end organ walls and the surrounding bones may help the whole ear move together with the membrane attached to the bony capsule so that stimulation from the swim bladder is directly transmitted into the ear.


## INTRODUCTION

In most oceans, sunlight is totally depleted below 1000m and the temperature is mostly below 4$^{o}$ C. Bottom-dwelling deep-sea fishes living in these environments have to deal with darkness, low temperature, scarce food, and difficulties in finding mates due to low population density (Herring, 2002). At the same time, many studies have

demonstrated that some deep-sea fishes have evolved highly adaptive and sensitive sensory organs for survival in the light scarce deep-sea. These include adaptations for enhanced sensitivity in vision (Munk, 1964, 1966; Locket, 1977; Douglas et al., 1998; Wagner et al., 1998), olfaction (Herring, 2002), and lateral line systems (Marshall, 1996). However, we still know very little about the auditory system of deep-sea fishes except for some gross morphology (Marshall 1971, 1980) and the ultrastructure of ten deep-sea species by (Popper 1977, 1980).

The auditory system is likely to be especially useful in the darkness. Underwater sound travels great distances and provides directional information about the source (Tavolga 1971; Fay, 1988; Popper et al. 2003), thereby having the potential of providing fishes with very wide acoustic "view" of the world around them. This is particularly useful at great ocean depths where fishes need to overcome the lack of vision, and so they need to glean as much information as they can from other senses. In fact, there is evidence that there are structural and functional specializations in several sensory systems in many deep-sea fishes, but it is not yet clear if these specializations extend to the auditory system.

We have begun a set of studies on anatomy of the inner ear in some deep-sea fishes to fill major gaps in our knowledge about the auditory systems of deep-sea fishes (cf. Deng et al., 2002, 2003; Buran et al., 2005). We hypothesize that some deep-sea fishes may have evolved enhanced hearing sensitivity and directionality in their inner ears which may include the evolution of acoustic communication. Since deep-sea fishes rarely can be taken alive, the only way at present to study their hearing is to infer function from anatomical studies.

*Antimora rostrata* (Günther, 1878), commonly referred to as the blue antimora, blue hake or flatnose codling, has a broad distribution in the Atlantic, Pacific, and Indian Oceans, and also in Antarctic waters. The species lives at depths ranging from 300 to 3000 meters (Cohen et al., 1990). It belongs to the deep-sea cod family Moridae (the Morid cods). As a benthopelagic fish, *Antimora* lives close to the ocean bottom and may be the dominant scavenging species in some areas (Iwamoto, 1975; Wenner and Musick, 1977). It is well-studied for its biochemical and physiological adaptations to many aspects of deep-sea life (Small, 1981; Siebenaller and Murray, 1990).

Deep-sea fishes are thought to have a lower metabolic rate than their shallow water relatives at similar temperatures. This may result from food limitation and reduced interaction between animals due to low abundance in the deep-sea community (Childress, 1995). However, in situ studies of *Antimora*'s metabolic rate, swimming speed, and muscle performance show that its performance is more similar to animals living in similar temperatures in shallow regions, such as the Antarctic, than to other deep-sea species (Collins et al., 1999; Bailey et al., 2003).

In adaptation to the low-light environment in the deep sea, *Antimora* has a multibank rod retina in its eye (Fröhlich and Wagner, 1996, 1998) with four layers of rods stacked together rather than having a single layer of rod cells as in most vertebrate eyes. This structure in deep-sea fishes may increase the sensitivity of the retina to light, or may serve as spectral filters for wavelength discriminations (Denton and Locket, 1989).

*Antimora*'s swim bladder is also highly adapted to overcome high pressure in the deep-sea with a substantive oxygen pumping ability under great hydrostatic pressure

through its gas glands supplied by elaborated arterial and venous capillaries, the rete mirabile. Its hemoglobin also has very low oxygen affinity (Noble et al., 1975, 1986).

The auditory capsule in *Antimora*, like other Moridae, is connected to the swim bladder. Marshall (1966) demonstrated the anatomical coupling between swim bladder and ear capsule in one morid species, *Lepidion eques* (North Atlantic codling), and discussed the possible sonic relationship between pharyngeal-mill muscles and swim bladder in another related species, *Salilota australis* (tadpole codling). Iwamoto (1975) demonstrated the relative position between the anterior chamber of the swim bladder and the skull in *Antimora*, but the anatomical connections with the ears has yet to be shown in detail.

This paper analyzes the morphology of the inner ear and its relationship to the swim bladder in *Antimora*. The ultrastructure of the sensory epithelia of the inner ear are analyzed with scanning electron microscopy (SEM) and compared with other ears of other shallow-water species that have swim bladder-ear connections. The comparison to shallow water fishes with functional data may help to understand the significance of a swim bladder-inner connection in *Antimora*. Some features in the ear of *Antimora* appear unique among vertebrates, so how such structures may reflect the fish's adaptation to deep water living is also considered.

## MATERIALS AND METHODS

The specimens of *Antimora rostrata* used in this study were collected using semi-balloon-otter-trawls from the Porcupine Seabight in the northeast Atlantic Ocean near Ireland during Discovery cruises D252 in April 2001, D255 in August 2001, and D260 in

March 2002. The area of the stations during these cruises covered 48–52°N, 11–16°W, trawling at bottom depths of 1000–4200m with an average temperature of 4°C.

All fish were dead when they came to the surface as a result of the two to three hour retrieval of the trawl from the ocean bottom. As soon as the fish came to the surface they were put on ice. Identifying (Whitehead et al., 1984) and measuring the catch usually took three to seven hours, during which time the fish were distributed to the labs and dissected. Shortly after the catch came in, heads of *Antimora* were fixed in cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer with 0.05% $CaCl_2$. Other, unfixed, specimens were dissected on board to analyze the otoliths and the relationship between the ears and the swim bladder. All tissues were stored in the 4°C cold room on board.

After transfer to the lab at the University of Maryland (about one to three months after capture) the fixative was replaced with 0.1 M cacodylate buffer (pH 7.4) and specimens stored in a 4°C refrigerator until further analysis. The long-term fixation did not noticeably degrade the inner ear tissues, and it hardened the brain tissue very well. Most otoliths did not seem to have noticeably changed between fixed and fresh specimens, particularly in cacodylate buffer instead of phosphate buffer.

In the study of swim bladder-ear relationships, one fixed specimen was used and two others were dissected freshly on board without fixation for observation of swim bladder contents. In the study of inner ear structure, several fresh specimens were dissected on board for observation and preservation of the otoliths; three fixed specimens were used for the gross morphology and SEM of ultrastructure.

Four ears from the three fish were used for SEM study. One was a left ear from a 602 g specimen with a total length of 440 mm; one was a right ear from an 1100 g specimen with a standard length of 484 mm. Two ears were from a 370 g specimen with a standard length of 354 mm. The fixed specimen for anatomical drawing of swim bladder was 375 mm in standard length and weighed 390 g. Body measurements were made before fixation.

Ears were dissected under a binocular microscope in 0.1 M cacodylate buffer. Photographs were taken during the dissection. The ears were then post-fixed in 1% $OsO_4$ with 0.1 M cacodylate buffer or PIPES buffer at room temperature for 30 to 60 minutes. After three buffer rinses followed by three double distilled water rinses, the ears were dehydrated in 30%, 50%, and 75% ethanol at 10 minute intervals. The whole ears were then further dissected into individual end organs during the dehydration step at 75% ethanol; the otolith and all otolith membrane were removed. The dehydration continued at 85%, 95% and three ×100% ethanol at 10 minute intervals immediately before critical-point drying.

Critical-point drying was done using $CO_2$ as the intermediary fluid (maximum pressure was 2000 psi). Tissues were then mounted on aluminum stubs using silver paste to preserve the natural curve of the lagenar and utricular epithelia. The stubs were coated with about 4–6 nm thickness of Au-Pd from a filament/vacuum using a Denton Vacuum DV 503, and viewed with an AMRAY 1820D scanning electron microscope.

The relative measurements of hair bundles were taken using SEM Digital Imaging System provided by SEMtech solution INC during the operations on AMRAY 1280D, as well as on SEM images taken during the operations. All measurements are relative

because of shrinkage of samples during dehydration and the tilting and bending of hair bundles.

Mapping of the hair cell ciliary bundle orientation was done by scanning up and down at high magnification across the entire sensory epithelium. Orientations of sensory hair cell ciliary bundles were marked on photos of the maculae. The shape of sensory epithelia and hair ciliary bundle orientation patterns did not differ between the four ears.

## RESULTS

### Gross Morphology of the Ear

The inner ear of *Antimora rostrata*, like that of other bony fishes, is a membranous labyrinth that can be divided into upper and lower compartments. The upper labyrinth includes the utricle and three semicircular canals, the anterior, posterior, and horizontal (or lateral) semicircular, with an ampulla attached to the end of each canal (Figs. 2.1A, B). The lower labyrinth includes two otolithic end organs, the saccule and lagena (Figs. 2.1A-B, 2.2). No macula neglecta have been found in the ear. Each otolithic end organ is a membranous pouch enclosing a calcareous otolith, the saccular otolith (Figs. 2.1C, D), lagenar otolith (Fig. 2.2), and utricular otolith (Figs. 2.3C, D). The upper and lower labyrinths are joined together at base of the common crux (Fig. 2.1B). Inside each otolithic end organ, the otolith overlays a sensory epithelium (or macula) which contains numerous sensory hair cells. A gelatinous otolith membrane (Figs. 2.2B, C) connects the otolith with the sensory epithelium. Hair cells are innervated by branches of the eighth cranial nerve that leave each macula and enter the brain stem (Fig. 2.1A, 2.4 B).

The ears of *Antimora* fill a very large portion of the volume of the cranial cavity; one ear alone is taller, wider, and longer than the brain. Figures 2.4A and C show the relative position and proportion between the two ears and the brain. The saccule is long and large as compared to the other end organs. The lagena sits on top of the saccular sac, approximately one-third of the way forward from the posterior end of the saccule, and the utricle is above the saccule at one-quarter of the distance back from anterior end, at the junction between anterior and horizontal semicircular canals (Figs. 2.1A, B).

The posterior and horizontal semicircular canals are completely enclosed in cartilage, while the anterior semicircular canal and the utricle are partially enclosed in cartilage. The anterior and lateral wall of the saccular sac is tightly attached to the cranial bones and the bones have to be peeled off carefully during dissection. The medial wall of the saccular sac is attached to the cranial bone at a point located just anterior to the macula. The stubs of a ligament-like connection on the skull are shown in Figures 2.4A, B (see circular part); the other end of the ligament connection on the saccular sac is shown in Figure 2.5A.

The posterior part of the saccular sac, including part of the dorsal wall and the lagena, is not surrounded by bones, but is thick and rigid with a cartilaginous texture. The rigid parts of saccular and lagenar sacs are exposed in Figure 2.4A and B. The substance of the wall looks like soft cartilage that can support the weight of the heavy otolith and sustain its own shape even out of water. The anterior and posterior semicircular canals in Figure 2.4A are also semi rigid and can retain their natural shape and position after removing the supporting bony labyrinth. The observation in fresh sample on board was similar to this so the rigidity was not a result of fixation.

The saccular otolith, the sagitta, fills up most of the space in the saccular pouch (Figs. 2.1A, B). This elongated otolith has a very complicated three-dimensional structure which is thicker at the rostral end and pointed at the caudal end with bumps and concavities all over. A deep groove, the sagittal sulcus, carves into the center of the medial side of the otolith, in which the otolith membrane connects the otolith to the sensory epithelium (Figs. 2.1C, D).

The part of the eighth nerve innervating the saccule does not appear as a bundle. Instead, it fans out as an array of nerve fibers from the hindbrain and projects along the length of the saccular macula (Fig. 2.5B). Some parts of the sac walls are as thick as 0.6mm and the eighth nerve has to penetrate this cartilage-like wall to reach the macular side (Fig. 2.1A).

The lagena of *Antimora* has a very thick wall around the macula where the nerves penetrate about 0.6 mm to reach the medial side of the epithelium (Fig. 2.1A). In the lagena of a fish 440 mm total length, the thickness of the dorsal lagenar sac ranges from 0.4 to 0.8 mm, measured in pictures taken under a stereoscope with a stage micrometer (Fig. 2.2A). The lagenar otolith, the asteriscus, is convex on the medial side and only covers two-thirds of the sensory epithelium (Fig. 2.2A). Most of the anterior one-third and a little part of the caudal end of the lagenar macula is covered only by otolith membrane that extends beyond the otolith (Fig. 2.2B, C).

The utricle of *Antimora* does not look very different from the utricle of most other teleost species (Fig. 2.3A). The sensory side of the utricular sac is shaped like a bowl with the utricular otolith, the lapillus, sitting on top of it. The vertebrate utricular epithelium has a striolar region populated with hair cell ciliary bundles and it continues

laterally into an elongated tail-like region, the lacinia (Fig. 2.3B). The utricular striola is visible even under a light microscope (Figs. 2.3A, B), and it is evident that this striola-lacinia region is not covered by the utricular otolith. The utricular otolith *of Antimora* is dome-shaped with a bumpy ventral surface attached to the epithelium via otolithic membrane; and a smooth surface on the domed dorsal side (Figs. 2.3C, D).

**Coupling between Inner Ear and Swim Bladder**

The swim bladder of *Antimora rostrata* has anterior and posterior chambers (Figs. 2.4C, D). The interior of the gas chamber is filled with a foamy substance which is a mixture of gas and lipid bi-layer membranes as confirmed by many earlier authors (Patton and Thomas, 1971; Josephson et al., 1975).

Figure 2.4D presents the relative position of ear and swim bladder with respect to the fish's head and body in a lateral view. Figure 2.4C shows the relative position and connection of the ears and swim bladder in a dorsal view. The anterior chamber of the swim bladder has two rostral horns which attach to the lateral part of the bony capsule of each saccule via ligament-like connections (Figs. 2.4A-C). On the interior side of the medial part of the bony capsule, another ligament-like structure links the membranous capsule of the ear to the bony capsule at a location just before the anterior tip of the sensory epithelium (macula) (Fig. 2.5A). Thus there is an intimate mechanical connection between the anterior chamber of the swim bladder and the saccule. The rostral end of the anterior chamber is also attached to the fourth vertebra which is also the root of the two muscle bundles that move the upper pharyngeal teeth (Fig. 2.4D).

## Sensory Epithelia

### *Hair cell ciliary bundles*

Hair cells are the sensory cells on the macula of each end organ of the ear. The apical surface of the hair cell contains a graded bunch of stereocilia and a single kinocilium that is generally longer than all of the stereocilia. The kinocilium and stereocilia on top of one hair cell is referred to as a ciliary bundle (Fig. 2.6). The orientation of a ciliary bundle is defined by the direction of sensitivity which is from the shortest stereocilium to the kinocilium. The hair cell ciliary bundle orientation patterns are shown in diagrams of the sensory epithelia with arrows representing groups of ciliary bundles that are oriented in the same direction (Figs. 2.7, 2.8); the tip of arrow indicates the position of the kinocilium.

Hair cell ciliary bundles are found with different shapes and heights. The shape of a bundle can be defined by the height of the kinocilium, the height of the stereocilia, and/or the gradient of the staircase of the stereocilia array. The length of the stereocilia array (from the kinocilium to the shortest stereocilia) can also reflect the size of the bundle.

Some bundle shapes often appear on similar locations on different maculae or species while other bundle shapes are rare or unique. Most ciliary bundles appearing on the three end organs of *Antimora* were classified into six different categories according to their shape characters and sizes as described in Table 2.1. Figure 2.6 shows representative bundles of all six categories from the maculae of *Antimora*; each category is represented by different color on the corresponding diagram (Fig. 2.7). The hair cell ciliary bundles on each macula were then classified according to these categories and

mapped on different areas of the maculae along with the corresponding color codes on the contour map (Figs. 2.7, 2.8).

*Saccule*

The saccular macula of *Antimora* has a long and narrow shape in three segments like a spatula with an elongated blade, a stem, and a handle (Fig. 2.7A). SEM results of the bundle orientation are shown by arrows in Figure 2.7B. The three distinct segments on the saccule have different hair cell ciliary bundle orientation patterns, and can be further divided into eight regions according to the bundle orientation (Fig. 2.7B).

The rostral segment of the saccular epithelium is much wider than the other two segments. It can be horizontally divided into four ciliary bundle groups; from dorsal to ventral they are a horizontal-posterior oriented group, a vertical-dorsal oriented group, a vertical-ventral oriented group, and a horizontal-anterior oriented group (Fig. 2.7B). The very rostral end of the rostral segment does not have clear boundaries between bundles oriented in different directions, but instead has ciliary bundles that gradually change in direction (enlarged inset in Fig. 2.7B).

The middle segment of the macula has all ciliary bundles oriented dorsal-ventrally, while the caudal segment is horizontally bi-directional. At the junction of the middle and caudal segments, a small group of ciliary bundles form a vortex with counterclockwise orientations (Fig. 2.7B).

The ciliary bundle shapes and heights vary considerably on the sensory epithelium of the saccule (Fig. 2.7C), especially on the caudal segment of the macula which has four categories of ciliary bundles (category 1, 4–6 in Table 2.1 and Fig. 2.6;

enlarged inset in Fig. 2.7C). The majority of anterior and middle segments consists of short bundles from category 5 (coded orange) and 6 (coded pink), many bundles are only 3 μm long (category 5, 6 in Table 2.1 and Fig. 2.6).

*Lagena*

The lagenar macula is concave and shaped like a "boomerang" with wider angle (Figs. 2.8A, B). The curvature of the "boomerang" shape bends upward and backward against the saccule, which is not common among know structures of fish lagena.

The dorsal one-third of the lagenar macula has two opposing vertically oriented hair cell ciliary bundle groups; the directions of the bundle orientation axes are parallel to the length of the macula. The ventral one-quarter of the macula also has two opposing vertically oriented bundle groups. The direction of the ciliary bundle orientation axes, however, is almost perpendicular to the length of the macula. The middle part of the macula has two oppositely oriented bundle groups giving a 45° direction relative to vertical, and they are perpendicular to the length of the macula (Figs. 2.8B). The very posterior tip of the macula is only covered by the otolithic membrane and not by the otolith.

Four categories of bundles are mapped on the lagenar macula (Fig. 2.8C). The ciliary bundles at the ventral edge of the lagenar macula have a very long kinocilium (around 10 to 15 μm) that is more than five times longer than the tallest stereocilium (2.3 μm) (coded blue, category 1 in Table 2.1 and Fig. 2.6). The bundles from the middle to the dorsal region of the macula are mostly short but with more number of stereocilia than the bundles at the ventral edge, the kinocilium is about twice as long as the longest

stereocilium (coded orange, category 5 in Table 2.1 and Fig. 2.6). The oppositely oriented bundles along the dividing line (the striola region) are much bigger than those in the other region of the macula, with the kinocilium and all the stereocilia arranged in a steep gradient (coded red, category 3 in Table 2.1 and Fig. 2.6). There is a dip along the very center of the striolar region with bundles that contain shorter kinocilium (coded pink, category 6 in Table 2.1 and Fig. 2.6).

*Utricle*

The ciliary bundle orientation pattern of the utricular macula in *Antimora* (Fig. 2.8E) is similar to other fishes. The axes of posterior to anterior oriented ciliary bundles spread out radially from the narrow posterior region towards the broader anterior border. The bundles in the middle of macula are short and the kinocilium is about twice as long as the longest stereocilia (coded orange, category 5 in Table 2.1 and Fig. 2.6) until they are close to the dividing line (Fig. 2.8F). At that point the bundles become longer and larger in stub diameter (coded red, category 3 in Table 2.1 and Fig. 2.6) and meet with their opposing bundles, anterior to posterior oriented bundles in the striolar region (Figs. 2.8E, F). The bi-directional striolar region continues into a lacinia region that extends laterally with similar bundle types (category 3 and 6). The two rostral-caudal oppositely oriented bundles, however, shift directions gradually to two medial-lateral opposite orientations (Figs. 2.8E, F). The bundles at the anterior edge of the macula and the tip of the lacinia have the longest kinocilium (coded blue, category 1 and coded yellow, category 2 in Table 2.1 and Fig. 2.6).

## DISCUSSION

Anatomical and SEM study show that *Antimora rostrata* has some unique inner ear structural features that have not been described in other fishes. These include a rigid and cartilage-like end-organ wall in the lagena and part of the saccule, a complex hair bundle orientation pattern in the saccular macula with more orientation groups than previously described for any vertebrate, and a direct saccule-swim bladder connection. While a tight direct connection between the anterior projections of the swim bladder and the saccule is not unique, in *Antimora* there are some unique features of this connection. Some of these characters may reflect adaptation to living in deep water.

### Bigger and Thicker Ears

The ears of *Antimora*, especially its saccule, are very large as compared with its brain (Figs. 2.4A-C). For comparison, in the shallow water gadiform fish *Gadus morhua* (Atlantic cod), the length of ear is only half of the length of brain (from forebrain to medulla oblongata); while in *Antimora rostrata*, the length of ear is slightly longer than the same brain region (Dale, 1976). A large ear is an interesting phenomenon in some deep-sea fishes. Fine (1987) reported a deep-sea fish *Acanthonus armatus* (bony-eared assfish) with the mass of the two ears being several times larger than that of the brain. Large ears were also found in a deep-sea gadiform family Macrouridae (Deng et al, 2002; and Chapter 3), and in other deep-sea macrourids (*Hymenocephalus*) by Bierbaum (1914).

Although large ears in fishes may not necessary related to deep-water living, flying fish, for example, has huge semicircular canals as compare to its brain, however, its lower labyrinth is very tiny (Retzius, 1881). The large ear observed in deep-sea

gadiform fishes may be simply a fact that many gadiform fishes have large saccules in general, For example, *Raniceps raninus* (tadpole fish ) and Atlantic cod both have large lower labyrinths (Retzius, 1881). Nevertheless, none of these ears are comparable to *Antimora* with a saccule that extends 175% of the span of upper labyrinth. This has not been found in any other group of fishes so far. Other members in the Moridae family, like *Brosmichlus* (*Gadella*) *imberbis* (beardless codling), *Laemonema barbatula* (shortbeard codling), also have large saccular otoliths with similar sculpted features to those found in *Antimora* (Campana, 2004). It would be worthwhile to see if the inner ear structures are similar through out this deep-water living family.

The rigidity and thickness of the end organ walls in *Antimora rostrata* appear to be rare features among fishes. These thick and cartilage-like features of the labyrinth wall have never been reported in other fishes except for one study of five very large bluefin tuna (Song et al., 2006). These bluefin tuna (*Thunnus thynnus*) were close to three meters long and weighed about 230 to 380 kg, which is about six times longer and several hundred times heavier than the *Antimora* specimens studied here. The maximum recorded length of *Antimora* was 75cm standard length (Chiu, 1990) and for bluefin tuna it was 458cm total length (Claro, 1994). Interestingly, the length of saccule in a 255 cm tuna is about the same as that of the saccule in an *Antimora* only one-fifth of its size. It was speculated that the cartilaginous wall of bluefin tuna ear may be an adaptation to protect its ear during fast swimming and deep diving, or it may be simply a feature of very large fish ear (Song et al., 2006). Since the size and life style of these tuna are very different from *Antimora*, the implication of the cartilaginous labyrinth wall may be very different. In the large bluefin tuna, the whole membrane labyrinth including semicircular canals are

all cartilaginous , where in *Antimora*, only the lagena sac and the posterior part of the saccular sac is extra thick and rigid.

Considering the fact that the thin portion of the *Antimora* saccule sac is tightly attached to the bony capsule, and the other parts of the ear sacs are rigid, the whole membranous structure of the ear may be able to move synchronically with the oscillation of swim bladder via the ligament connections. This may provide distinct stimulations to the sensory epithelia in the saccule with a large and dense otolith, and can be considered as a mechanism that may improve inner ear sensitivity in *Antimora*.

**Lagena and Utricle**

The curvature of the lagenar macula bends upward and backward against the saccule, which is opposite to one of the commonly described shapes of lagenae in many other fishes including some deep-sea fishes (Popper 1977, 1980; Buran et al,. 2005) in which the macula bends downward and forward to embrace the posterior end of saccule. It is also different from the lagena of another gadiform fish *Merluccius merluccius* (European hake) which is bent towards the saccule (Lombarte and Popper, 1994, 2004). The lagenar macula of *Gadus morhua* (Atlantic cod) reported by Dale (1976) is shaped like a narrow tongue with an anterior-ventral process.

The shape of utricle is similar to that in most other fishes. The hair bundle orientation pattern is similar to the two studied gadiform fishes *Gadus morhua* (Atlantic cod) (Dale, 1976) and *Merluccius merluccius* (European hake) (Lombarte and Popper, 1994, 2004). It is also similar to those seen in many other fishes studied to date (Buran et al, 2005; Platt and Popper, 1981; Platt, 1993; Lu and Popper 1998).

**Ciliary Bundle Types**

Ciliary bundles of various shapes are found the in the end organs of *Antimora*. Combining with studies from other deep-sea fish families in the subsequent Chapters, the variations in bundle shapes in these deep-sea fishes are greater than usually seen in other fishes that have been studied (e.g., Popper, 1977; Platt, 1977, 1983; Platt and Popper, 1984).

Some types of bundles consistently appear on certain part of the macula, while other bundle types are not common. Categorizing these various bundles in to groups and mapping the locations on different end organs is an attempt to get an overview of the bundle type distribution.

Different types of bundles in the fish otolithic end organs are very difficult to classify due to the variations in bundle heights and different ratio between kinocilium and stereocilia length. The bundle categories in Table 2.1 and Figure 2.6 are classified according to the overall structure comprised by bundle heights, array length and the character of stereocilia staircase. Some of the categories may be separations or combinations of certain bundle types classified by previous authors.

Popper (1977) described two common types of bundles in fish ears; F1 may include the category 3 and 6 bundles in this study, F2 may include category 1 and 5 bundles. In two other less common bundle types described by Popper, F3 is similar to category 4, and it happens to appear in a deep-sea lantern fish. F4 is similar to the longer members in category 1. Platt (1983) proposed a different kind of classification using "K" to represent kinocilium and "s" as stereocilium, and numbers to represent cilia length in

μm. In the seven types of bundles described by Platt, K10s3 is comparable to some of the F1 bundles and can be put into category 1 in this study. K6s3 and K8s4 are long and short version of F1 and category 5 bundles. K6s5 and K8s7 are long and short versions of F2, but may be included in category 6 or 3 in this study depending on their locations. This K-s nomenclature is very precise in describing the bundle length. However the number of types would have increased dramatically if similar bundles of various heights were encountered; and this might make analysis of bundle distribution more difficult than the more qualitative description provided here.

The rationales of classification in this study are in steps. The first consideration is the characteristic of the kino-stereocilia relationship: **a)** Categories 1, 2 and 5 are clearly different from 3 and 6 in that the former have a kinocilium that is at least twice the length of the tallest stereocilium, the latter's kinocilium join together with all stereocilia to form an even staircase. **b)** Category 4 stands out by having a few stereocilia as long as the kinocilium.

The second consideration is the length or size of the bundle, with location taken into account in some cases: **a)** Category 3 is different from 1 and 2 are in that 3 has more stereocilia than most of 1and 2; the kinocilium in 3 usually does not reach several times longer than the tall stereocilium as in 1 and 2. **b)** Categories 1 and 2 are basically the same type that usually appears on the edge of macula, or in regions only covered by gelatinous membranes; Category 2 is different from 1 because bundles longer than 15 μm are rare and are restricted in certain regions on the macula; this discrimination is to increase the resolution of distribution map. **c)** Categories 3 and 4 differ mostly by height and size. Category 3 is usually the biggest of bundle group on a macula, and they often

line up along the reversal line or striola region. Category 6 bundles are smaller and vary in size. They may appear in large areas in the center of a macula, or as intermediate bundle type between striola bundle and other shorter bundles.

This classification for mapping is not perfect and is a compromise between generalization and resolution. The size ranges given in Table 2.1 are wider than the actual bundles in *Antimora*, so as to accommodate mappings in the subsequent Chapters. Overlapping can occur in some area, and the variations in sizes and heights of bundles within each type can not be reflected in the map.

The list of bundle types in this study is subject to revision with further investigations. It is expected that with the exploration of more fish species, new categories will be added to the list. On the other hand, the number of categories may be reduced with further analysis of the bundle type characteristics, by grouping similar bundle types together and using secondary codes to indicate the bundle heights or sizes.

Hair bundle types in the saccule and utricle of other vertebrates may be similar or different from the bundles in fishes (Lewis et al, 1985). Further discussions will continue in the next Chapter.

**Swim Bladder to Ear Connection**

The swim bladder of *Antimora* and its connection to the skulls or ear capsules have been described by Iwamoto (1975) and Paulin (1988). However, these investigators did not show the relative position between the swim bladder and the ears. In the diagrams of the lateral view of the swim bladder, the authors depicted a relatively ventrally pointed anterior chamber, which is the opposite of the current observation (Fig. 2.4D). The

anterior chambers are actually pointed slightly dorsally, thus they reach upwards to connect with the lateral wall of the ear's bony capsules.

The left and right projection of the anterior chamber of the swim bladder each make a ligament-like connection to an anterior lateral spot at the bony capsule around the respective saccule (Figs. 2.4A-C ); inside the interior medial wall of the bony capsule, another ligament located just anterior to the macula affixes the saccule to the bone (Fig. 2.5A). A compressible gas chamber in close vicinity to the ear is considered to provide an auditory advantage for the fish (see review in Popper et al., 2003); *Antimora* may receive direct stimulus from sound pressure since the swim bladder has a direct mechanical connection with the anterior macula of the saccule, which is often associated with enhanced hearing sensitivity and frequency range in fish (Popper and Fay, 1999). Although the connection seen in *Antimora* is different from the Weberian ossicle connection in the hearing specialist otophysan fishes (Weber 1820; Poggendorf 1952), the ligament-like connection from the swim bladder to the ear in *Antimora* may serve as a direct link connecting the motions of the swim bladder to the cranial bone, and then in turn to the saccule since the wall of the sac is tightly attached to the bony capsule.

Otophysic (ear to swim bladder) connections without a Weberian apparatus are found in many families of fishes (Schellart and Popper, 1992). Different species of the squirrelfish family Holocentridae have different configurations between the swim bladder and the ear capsule; from no direct contact to intimate contact. The latter is represented in *Myripristis argyromus* (synonym of *Myripristis amaena*, brick soldierfish) , whose anterior swim bladder chamber projects as two narrow horns to make lateral contacts with the auditory capsule (Nelson, 1955). Hearing studies in the squirrelfish family

(Coombs and Popper, 1979) showed that the species with a direct ear-swim-bladder connection (*Myripristis kuntee*, shoulderbar soldierfish) has a much broader hearing range (100–3000 Hz) than the species that has no such connection (*Adioryx xantherythrus*, synonym of *Sargocentron xantherythrus*, Hawaiian squirrelfish, 100–800 Hz), as well as much better hearing sensitivity, with a 30 dB difference in the threshold at best frequency.

Another species that has non-Weberian otophysic connection is *Notopterus chitala* (synonym of *Chitala chitala*; clown knifefish) from family Notopteridae. Clown knifefish has a saccule that is roughly two-thirds "covered" by the anterior projection of the swim bladder (Dehadrai 1957; Coombs and Popper, 1982). The coupling is also via the attachment from the elongated extensions of the swim bladder to the bony capsule, and from the saccular membrane to the bone. Similar to *Antimora*, the membranous wall of the saccular sac in clown knifefish is tightly attached to the surrounding bony capsule. Thus the "sandwich" attachments of swim bladder-extensions/bony-capsule/saccular-sac in these fishes represent a pattern of otophysic connection in fishes without Weberian ossicles.

With an otophysic connection similar to some of the fishes described above, it is possible that *Antimora* has enhanced hearing sensitivity or a broader frequency range via this acoustic coupling. However, such enhancement may be limited because the wall of *Antimora*'s deep-sea adapted swim bladder is less elastic than that of the goldfish and other hearing specialists, and the *Antimora* swim bladder is filled not entirely by gas, but by a mixture of gas and lipid bi-layer membrane foams (Josephson et al., 1975).

**Complex Saccular Hair Bundle Orientation Pattern and a Convergence Implication of Non-Weberian Otophysic Connection**

The highly complicated saccular hair bundle orientation pattern in *Antimora* also suggests specialization in its hearing. One character of some hearing specialist is the elaboration in the hair bundle orientation pattern on the saccular epithelium, which has been observed in many fishes (Popper and Coombs, 1982; Popper and Fay, 1999). The pattern in *Antimora* (Fig. 2.7B), with eight orientation groups, has not been seen in other fishes that have been looked at and adds a new category to the saccular hair bundle orientation patterns summarized by Popper and Coombs (1982). Such a complicated pattern may imply a differentiated signal processing of the direction of sound at the periphery level.

The "fan-like" innervation of the saccular branch of the eighth nerve in *Antimora* occupies a relatively longer region along each side of the hindbrain (Fig. 2.4B, Fig. 2.5A) as compared with many studied fishes, in which the eighth nerve forms discrete bundles, and the insertion into the hindbrain is not as spread out as in *Antimora.* This anatomical feature in *Antimora* may imply a larger representation of input from the saccule in the hindbrain or a larger auditory nucleus.

The swim bladder's anterior projection overlaps with two thirds of the length of the saccular pouch, and it almost embraces the entire length of the saccular macula, so it may provide signal enhancement to the whole length of the macula. Although the main gas chamber is closer to the posterior portion of the saccule (Fig. 2.4C), where there are only two horizontally oriented bundle groups and variable bundle types (Fig. 2.7B), the direct connection to the swim bladder via the ligament is at the anterior part of the macula

(Fig. 2.4A–C, Fig. 2.5) which has four or five bundle groups and all four orientation directions in that area, but with relatively homogenous bundle types (Fig. 2.7B). Thus it is not easy to determine which part of the macula is under stronger stimulation from the swim bladder and how the different parts work. It may be speculated that the posterior portion of the saccular macula is for frequency analysis with its rich bundle shapes and heights and the anterior portion is for directional detection with its relative similar bundle heights and rich bundle orientations.

Similarly, complex saccular hair bundle orientation patterns have also been noted in two groups of fishes that are not related to morids, but which have similar coupling between the swim bladder and the ear. For example, in clown knifefish the swim bladder projections cover two-thirds of the saccule with a sandwich attachment of swim-bladder/bony-capsule/saccular-sac (Coombs and Popper, 1982). Intriguingly similar to *Antimora*, the saccular macula of clown knifefish is also elongated and can be separated into three parts by narrow bridges (Coombs and Popper, 1982). The orientation pattern on the saccular macula is also elaborated in the anterior portion; however, this area is not covered by the swim bladder as in *Antimora*. The hearing range of clown knifefish is slightly wider than non-otophysic fishes (from 100 to 1000 Hz in behavioral tests), but the sensitivity is not as great as in other fishes with swim bladder-ear connections (Coombs and Popper, 1982).

The saccular macula of *Myripristis murdjans* (pinecone soldierfish), another species from the squirrelfish family that has otophysic connection, also has separate parts with narrow linkages between them (Popper, 1977) like *Antimora*. The shape and length of the saccular macula in this fish is very different from Hawaiian squirrelfish, the non-

otophysic member in the same family; and the hair bundle orientation pattern in the anterior portion of the macula in pinecone soldierfish is more complicated than in Hawaiian squirrelfish. As mentioned in the previous section, the hearing sensitivity and frequency range in soldierfish are much better than in squirrelfish (Coombs and Popper, 1979).

On the other hand, the swim bladder of a gadiform fish from shallow water, *Gadus morhua* (Atlantic cod), does not have any direct connection with the ears, instead, it only has very thin dead end ducts that extended from the swim bladder and stop at a distance of 6 to 7mm from the saccule (Dale, 1976). The structure and hair bundle orientation pattern of Atlantic cod's saccular macula is not as complicated as in *Antimora*, with no enlargement in the anterior part and only six differently oriented bundle groups (Dale, 1976). The European hake (*Merluccius merluccius*), another non-otophysic gadiform fish, also has different saccular structure than the *Antimora*, but is similar to the cod (Lombarte and Popper, 1994, 2004). Interestingly, the saccule in the Atlantic cod and European hake are very similar to those in a non-otophysic deep-sea gadiform family Macrouridae, which ecologically coexist with *Antimora* on the deep-sea slope (Deng et al., 2002 and personal observations). With these data, it seems that at least in the Order Gadifomes, the enlarged and elaborate saccular macula is not necessarily related to deep-water living, but to an otophysic connection.

The complexity of the saccular hair bundle orientation pattern in *Antimora* may be related to its connection between ear and swim bladder. However, it is very different from the saccule in freshwater otophysan fishes, such as goldfish and zebrafish, which has a direct connection from the swim bladder to the saccule via the Weberian apparatus.

In those otophysan fishes, the saccular macula is small and narrow and is not overlapped by the swim bladder, and has only two vertical hair cell orientation groups; the lagena, however, is much enlarged compared to other fishes that do not have Weberian ossicles (Platt, 1977, 1993). In contrast, *Antimora* and clown knifefish have an enlarged saccule, thus the specialized auditory function in *Antimora* and clown knifefish may have evolved differently than the fishes with Weberian apparatus.

It is difficult to imply any physiological characteristics of *Antimora*'s saccule from data of clown knifefish and soldierfish, but the similar "eccentric" organization in bundle orientation in the saccules of these three species may give a clue for such non-Weberian swim bladder-saccule relationships. Cross-taxon comparisons suggest that saccular complexity associated with an otophysic connection is a convergent evolutionary feature in many groups of fishes.

**Conclusion**

A number of unique features in the ear structure of *Antimora rostrata* support our hypothesis of enhanced sensitivity and directionality in some deep-sea fishes' inner ears. The large ears and extra long saccular otolith and macula, especially the enlarged anterior part saccular macula, may imply increased sensitivity. The complicated eight-way bundle orientation pattern may facilitate enhanced directional coding at the peripheral level. The suggestion of enhanced sensitivity is also supported by a close connection between the saccule and the swim bladder. The rigidity and thickness of end organ walls may suggest an adaptation for deep-water living. The membrane wall of the sensory epithelium is several times thicker than in other fishes and is partially sclerotic, which may help the

whole ear move together with the membrane attached to the bony capsule; and the attachment between the end organ walls and the surrounding bones implies that the chambers may oscillate exactly with the fish body movements while the otoliths are fully suspended via the otolith membrane and hair bundles.

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## TABLES

**Table 2.1.** Categorization of hair bundle shapes.

| Categories | Character Description | Range of Bundle Length | Bundle Diagrams |
|---|---|---|---|
| Category 1 | Small but long bundles located on the edge of an epithelium. The kinocilium is three to ten times longer than the tallest stereocilium. The stereocilia staircase is short (2-3 μm) and the array length is small (1-2 μm). | 5-15 μm |  |
| Category 2 | Long bundles located in the lacinia of utricle or other areas that are not covered by an otolith. The bundle is similar with Category 2 except that the kinocilium is often more than ten times longer than the tallest stereocilium. | 15-30 μm |  |
| Category 3 | The bundles that are often found in the striolar region of a macula. The bundle is usually long and the array length is the longest among all categories. The kinocilium and all the stereocilia are arranged in an even and steep slope. | 10-20 μm |  |
| Category 4 | The bundles have a long kinocilium and a few long stereocilia that are almost as long as the kinocilium, the other stereocilia form a short staircase. | 10-15 μm |  |
| Category 5 | The bundle is shorter than Category 3 and the array length is larger than Category 1 and 2. The kinocilium is at least twice as long as the longest stereocilium as compare with Category 6 bundles. The stereocilia are arranged in an even gradient. | 3-12 μm |  |
| Category 6 | Bundles with a short kinocilium. The entire bundle is in an even and shallow slope. | 4-10 μm |  |

## FIGURES

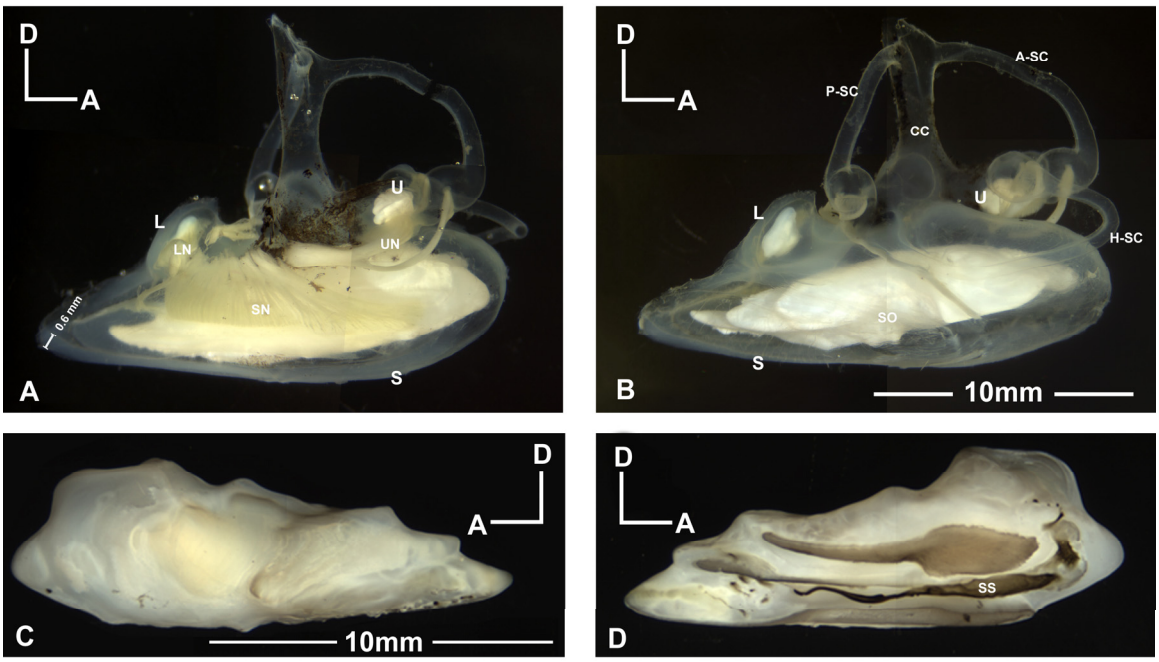



**Figure 2.1.** Left and right ears of *Antimora rostrata*. **A:** Medial view of a left ear showing the three end organs and eighth nerve branches that innervate them. A measurement bar indicates the thickness of the posterior wall of the saccular sac. **B:** Lateral view of a right ear with all three otoliths clearly present in their pouches. **C:** Lateral view of a left saccular otolith. **D:** Medial view of a left saccular otolith. The otolithic membrane is visible (brown) after staining with osmium; a darker otolithic membrane is still inside the sagittal sulcus (SS). A-SC, P-SC, H-SC, anterior, posterior, and horizontal semicircular canals; CC, common crux; L, lagena; LN, nerves to lagena; S, saccule; SN, nerves to saccule; SO, saccular otolith (sagitta). SS, sagittal sulcus; U, utricle; UN, nerves to utricle.

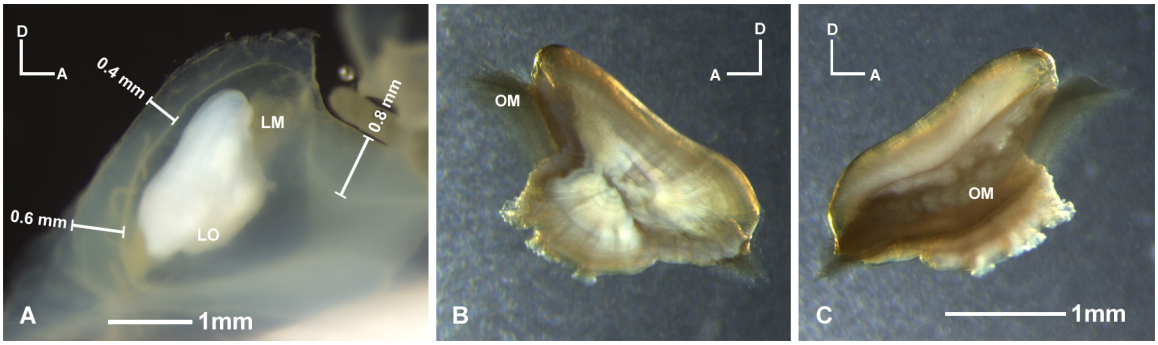



**Figure 2.2.** Lagena of *Antimora rostrata*. **A:** Lateral view of a right lagena. The thick walls of the sac are from 0.4 to 0.8mm thick. **B:** Left lagenar otolith and otolithic membrane taken out from lagenar sac after osmium fixation. **C:** Medial view of the same otolith. The brown veil-like substance is the otolithic membrane (OM) stained by osmium. Note that the otolithic membrane extends out from two ends of the lagenar otolith. Dissections show that the otolithic membrane covers the regions of the sensory epithelium that are not covered by the otolith itself. LM, lagenar macula; OM, otolithic membrane.

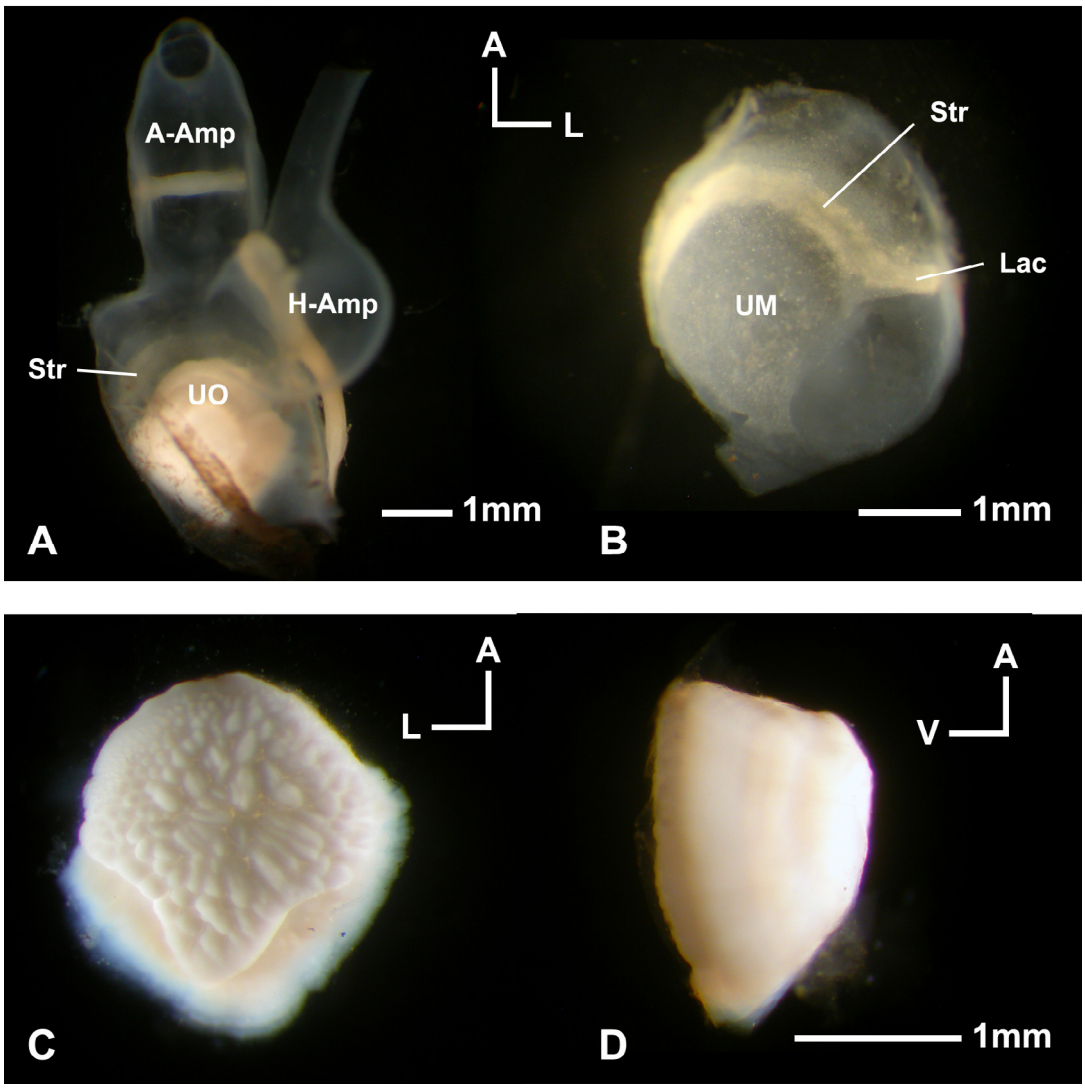



**Figure 2.3.** Utricle of *Antimora rostrata*. **A:** Dorsal view of a right utricle with the otolith inside the pouch and the ampullae of the anterior (A-Amp) and horizontal semicircular canals (H-Amp) still attached. **B:** The utricular epithelium isolated from **A**, note that the striola (Str, more opaque yellowish region) and lacinia (Lac) is not covered by the otolith. **C:** Ventral view of the otolith taken from **A**, revealing the bumpy surface that is connected to the otolith membrane that lies between the otolith and the sensory epithelium. **D:** Side view of the same otolith to show the dome shape. UO, utricular otolith.

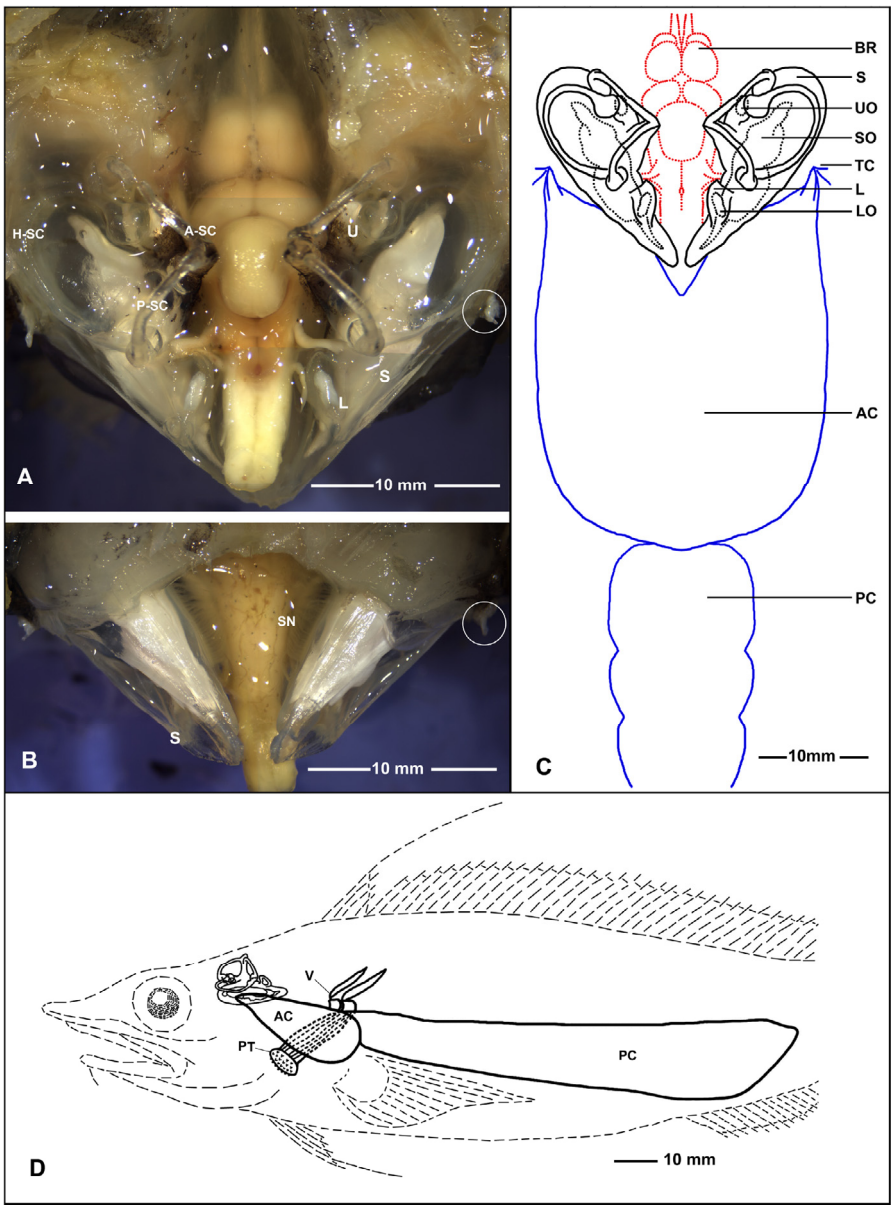



**Figure 2.4.** Ears, brain, and swim bladder of *Antimora rostrata* (anterior to the top for A, B, and C). **A:** Dorsal view of the brain and ears after removing part of the skull and cartilage; note that the rigid semicircular canals and the lagenar sac are able to retain their natural shapes even when exposed to the air. **B:** Ventral view of the ears and the brainstem after removing part of the bottom of the cranium, with a clear indication of the rigidity of the saccular sacs without the support of water. After removing the two anterior chambers of the swim bladder from where they attached to the ear's bony capsules, two ligament stubs (indicated by white circles) are still seen on either side of the ears' bony capsules. **C:** The relationship between brain, ears, and swim bladder in *Antimora*; note that the size of brain is relatively small compared with the size of the ears. **D:** Lateral view of the relative position between the ear and the swim bladder with respect to the head and the body. Also shown in **D** are two vertebrae (V) and a muscle bundle of the upper pharyngeal teeth (PT). AC, PC, anterior and posterior chamber of swim bladder; A-SC, H-SC, P-SC, anterior, horizontal, and posterior semicircular canals; BR, brain; L, lagena; LO, lagenar otolith; PT, pharyngeal teeth; S, saccule; SN, nerve to saccule; SO, saccular otolith; TC, ligament connection; U, utricle; UO, utricular otolith.

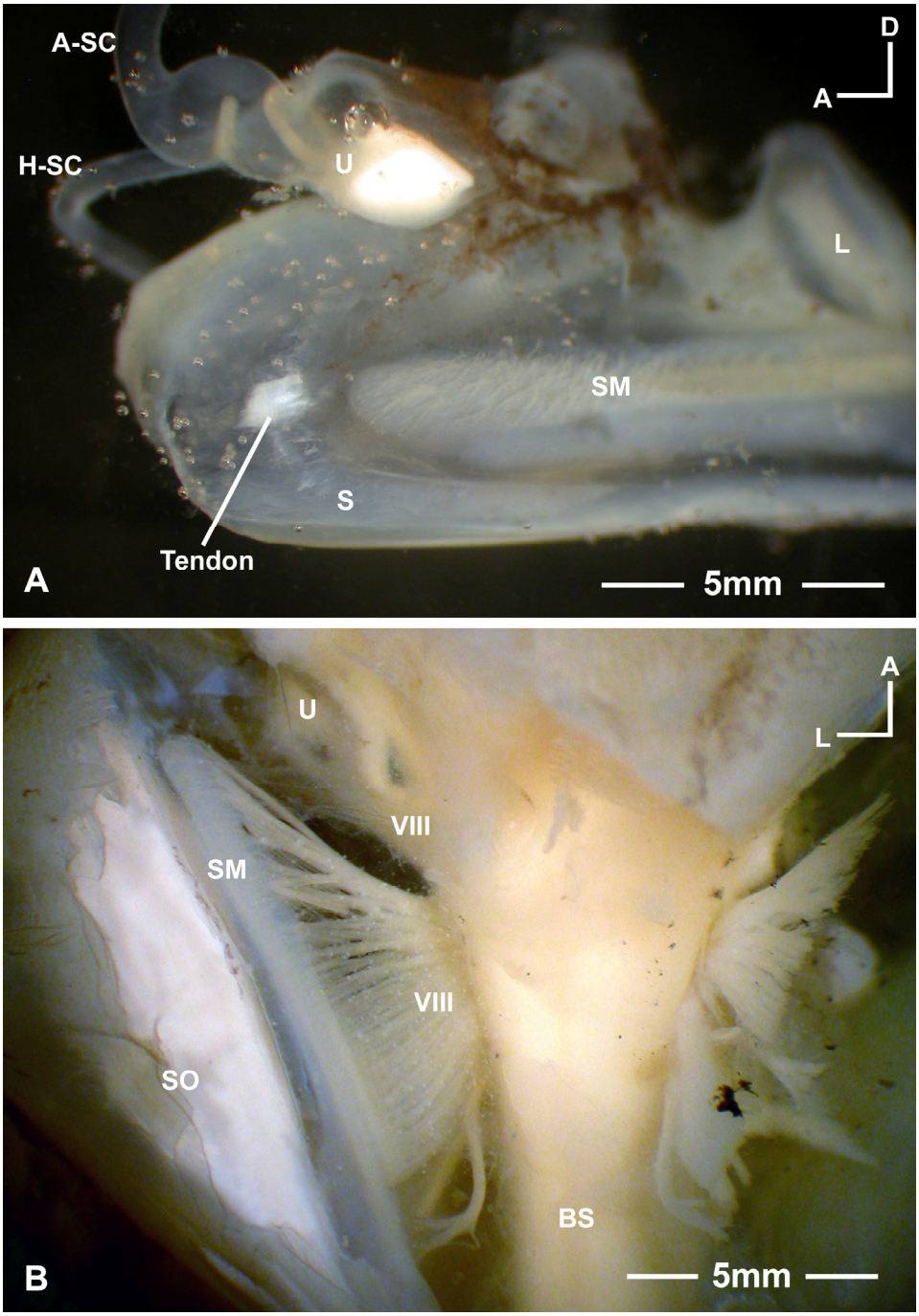



**Figure 2.5. A:** The ligament-like connection on the medial wall of a saccular sac. This is a medial view of part of a right saccule. The ligament-like structure is located just anterior to the saccular macula (SM) and is connected to the interior wall of the cranial bone. **B:** Ventral view of the brainstem (BS) and right ear of *Antimora*, showing the array of auditory nerve fibers (VIII, eighth cranial nerve) spreading along either side of the brain stem. Refer to Figure 2.4 for other abbreviations.

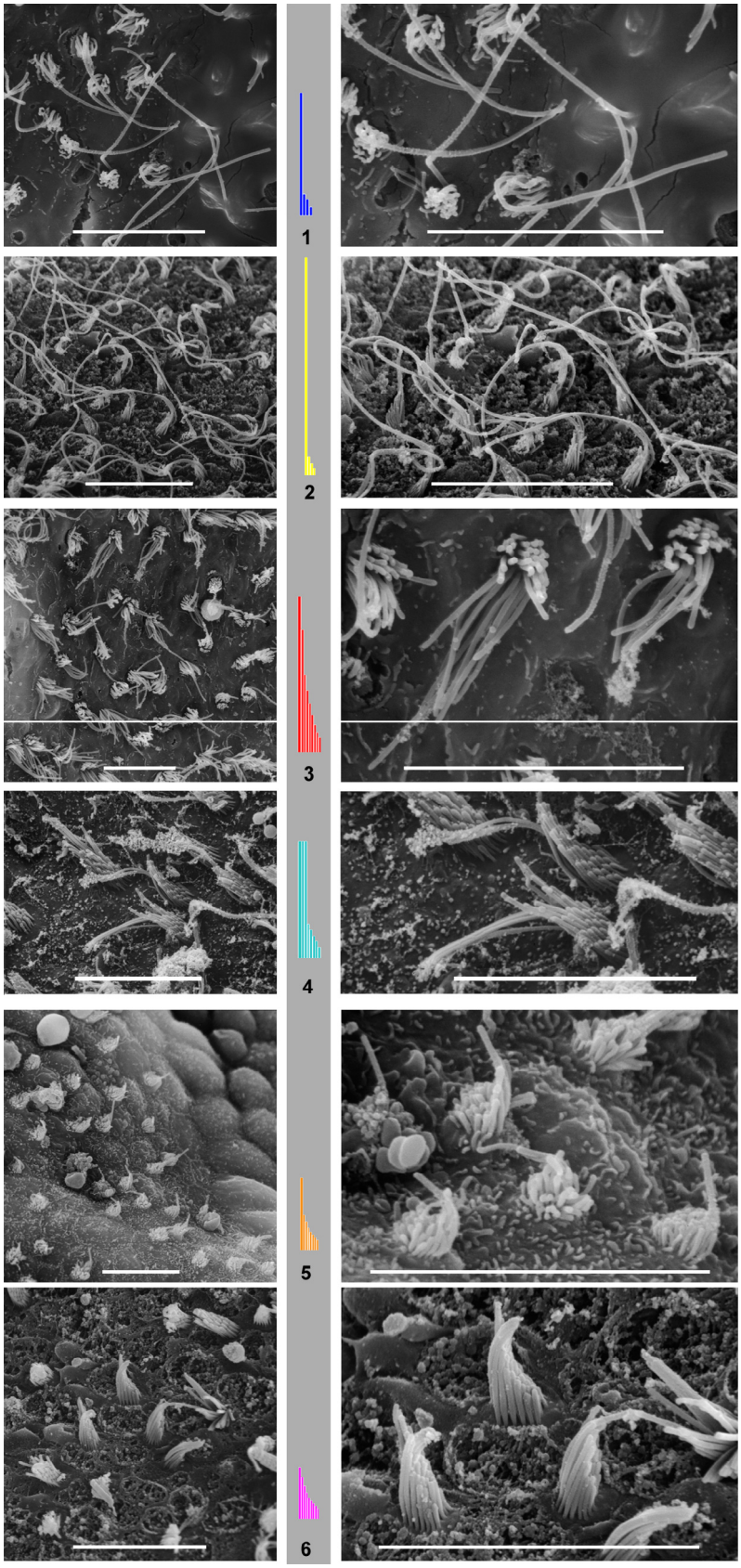



**Figure 2.6.** Examples of hair bundles from the end organs of *Antimora rostrata*. Numbers **1-6** correspondent to the category numbers in Table 2.1. Each category is represented by a SEM photo of a patch of hair bundles with an enlarged view on the right and a color coded schematic drawing in the center. This color scheme is used in Figures 2.7 and 8 to show the location of different types of bundles on each sensory epithelium.

**1:** Category 1 bundles from ventral edge of lagena (Fig. 2.8C).
**2:** Category 2 bundles from the tip of lacinia on utricle (Fig. 2.8F).
**3:** Category 3 bundles from the striola of lagena (Fig. 2.8C).
**4:** Category 4 bundles from the caudal segment of saccule (enlarged inset in Fig. 2.7C).
**5:** Category 5 bundles from ventral tip of utricle (Fig. 2.8F).
**6:** Category 6 bundles from the center of striola in utricle (Fig. 2.8F).

Scale bars = 10 μm.

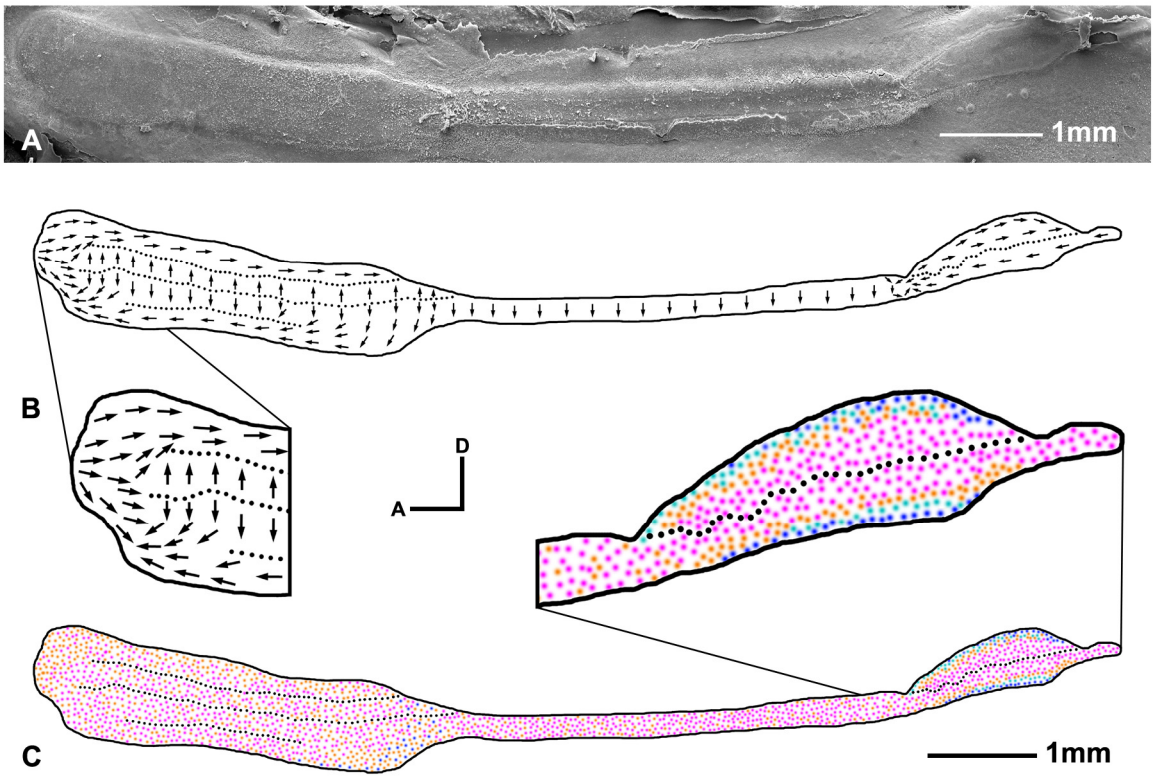



**Figure 2.7.** Ultrastructure of the sensory epithelium of the saccule. **A:** SEM photo of a left saccular macula. **B:** Hair bundle orientation pattern of the saccule. Note that the macula has three distinct segments with eight different bundle orientation groups. **C:** Color coded map of hair bundle types. The bundles on each macula were classified according to the criteria in Table 2.1 and shown in higher magnification in Figure 2.6. Note that the bundle types are more diverse in the caudal segment.

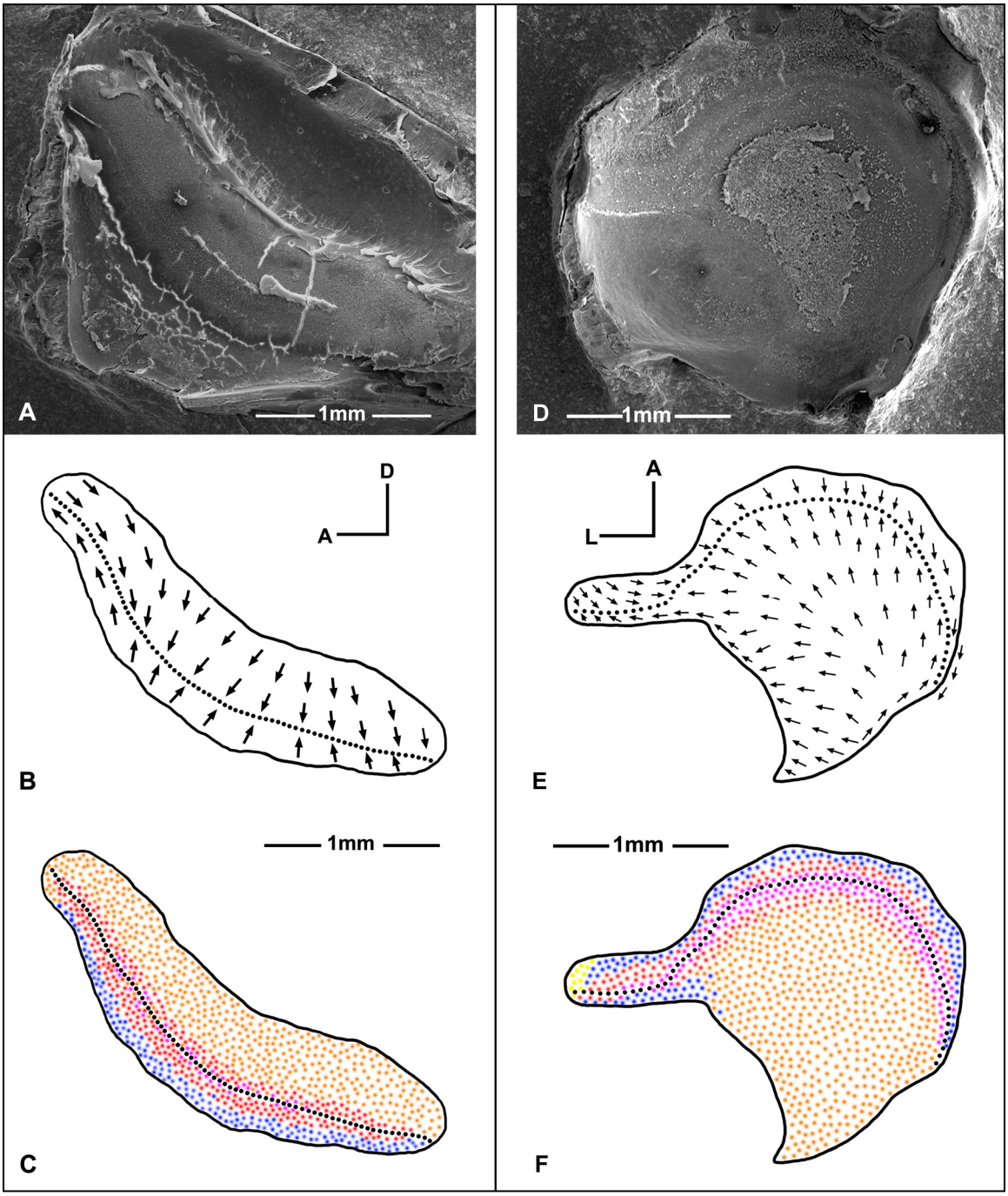



**Figure 2.8.** Structure of the sensory epithelia of the lagena (**A**-**C**) and utricle (**D**-**F**). **A** and **D:** SEM photo of the left lagenar macula and left utricular macula. **B** and **E:** Hair bundle orientation pattern of the lagena and utricle. **C** and **F:** Color coded maps of hair bundle types found in the lagena and utricle. See Table 2.1 and Figure 2.6 for color codes.

# Chapter 3. Interspecific Variations of the Inner Ear in the Deep-sea Fish Family Melamphaidae


## ABSTRACT

Inner ear structures are compared among five species in the family Melamphaidae (bigscales and ridgeheads). Extremely broad interspecific variation is found in the saccular otoliths, including the presence of a long otolithic "stalk" found in the genera *Melamphaes* and *Poromitra*. The variation in saccular otoliths corresponds with a sequential change in the length of the caudal part of the saccular maculae. Most of the sensory hair bundles on the saccular macula are 15–20 μm long with large numbers of stereocilia, including some stereocilia that reach the height of the kinocilium; these bundles may have enhanced sensitivity to bundle displacements. In the utricle, the striolar region separates into two ear-shaped areas that have not been seen in any other vertebrates. The brains in all species have a relatively small olfactory bulb and optic tectum, and a hypertrophied posterior cerebellar region that is likely to be involved in inner ear and lateral line (octavolateral) functions. These findings support the hypothesis that specialized anatomical structures can be found in some deep-sea fishes' ears.


## INTRODUCTION

Fishes are one of the most successful groups of organisms on Earth. They dominate all the aquatic habitats with enormous numbers of individuals and species. They have developed various specializations to enable them to live in all kinds of extreme environments, including the deep-sea.

In the sunlight depleted environment of the deep-sea, many fishes have evolved highly adaptive and sensitive sensory systems for survival, including for vision (Locket, 1977; Douglas et al., 1998; Wagner et al., 1998), olfaction (Marshall, 1980; Herring, 2002), and detection of water motion (via the lateral line system) (Marshall, 1980; Marshall, 1996). On the other hand, sensory organs may regress if they become less useful under certain environment conditions (Marshall, 1980; Herring, 2002). Marshall (1971) suggested that in bottom dwelling species such as benthopelagic and benthic fishes, the olfactory, gustatory, and acoustic and lateral lines organs may compensate for the loss of vision. Previous studies have provided documentation of specializations and adaptations of deep-sea fishes' sensory systems; however, the structure and function of the auditory system in deep-sea fishes have scarcely been explored.

The potential importance of the auditory system in the lives of deep-sea fishes is suggested since hydrodynamic stimulation is continuously presented to fishes, even at great depths; acoustic signals can be detected at much greater ranges from the fishes as compared to light signals, and acoustic signals are far more directional when compared to olfactory signals (Tavolga, 1971; Fay, 1988; Popper et al., 2003). When integrating all of the available hydrodynamic and acoustic information available in their environment, fishes are presented with an "auditory scene" (Bregman, 1990, 2008) that provides an acoustic image of the surrounding world (Fay and Popper, 2000). Thus we hypothesize that some deep-sea fishes have evolved specialized anatomical structures in their hearing organs in order to better perceive, and make use of, the auditory scene.

Fish hearing relies on one or more of three paired otolithic organs: the saccule, utricle, and lagena. Each of the otolithic end organs is a sac with a sensory epithelium –

often called the macula – and an otolith that is coupled to the hair cells on the macula via a gelatinous otolithic membrane (Popper, 1977; Platt and Popper, 1981). In a sound field, the fish body oscillates together with the water, while the far denser otolith moves at a different amplitude and phase. This difference between the motion of the otolith and the rest of the body (including the sensory maculae) results in relative movement between the otolith and the ciliary bundles on the sensory hair cells (Fay, 1984; Popper and Fay, 1999; Popper et al., 2003). The sound signal is then transformed to mechanical stimulation that excites the hair cells (Hudspeth and Corey, 1977).

In most fishes, the saccule is considered to be the hearing end organ and the saccular otolith (called the sagitta) is usually the largest among the three paired otoliths. Besides the often seen flat and oval shape of saccular otoliths, many fish otoliths have sculpted features and even three-dimensional structures (Campana, 2004). Some researchers believe that the shape of otolith may affect its pattern of movement relative to the sensory epithelium (Popper et al., 2005).

Some eco-morphological studies (Aguirre and Lombarte, 1999; Parmentier et al., 2001; Gauldie and Crampton, 2002) have suggested that the difference in saccular otolith shape can be related to the ecological niche or life history of the fishes. For example, in two species of red mullets from the goatfish family (Mullidae), the species with complex shaped saccular otoliths live in muddy bottoms of deeper water, while the species with simpler otolith lives in clearer water and may rely more on eyes in finding food (Aguirre and Lombarte, 1999). Similarly, in orange roughy (*Hoplostethus atlanticus*), which lives at depths of 800-1200m, the saccular otolith is much more complex than that in four other related shallow water species (Gauldie and Crampton, 2002). These studies suggested

that more complex otoliths are associated with greater dependence on hearing for finding food or avoiding predators.

With the consideration of otolith complexity, we have discovered one mesopelagic deep-sea fish family Melamphaidae (bigscales or ridgeheads; Order Stephanoberyciformes) to be particularly interesting for study. Melamphaidae is a typical meso- to bathypelagic family with worldwide distribution in the deep-water and one of the most abundant deep-sea families. With dark brown to black color as camouflaged body colors, these species stay mostly at the depth beyond the influence of sunlight and only migrate to the surface at night. All members of this family have large heads with highly developed cranial lateral line canal organs (Marshall, 1996) and relatively small eyes. Vision may not be important to these fishes and they may rely more on mechanosensory systems such as the lateral line and auditory systems.

This Chapter compares inner ear structures among five different species of the mesopelagic family Melamphaidae. Some structures that have never been reported in any vertebrate are found in the ears from this family. Two unique features are especially intriguing. One is the extremely broad interspecific variations in saccular otoliths, highlighted with a long otolithic “stalk” or a “spur” found in two genera. These correspond with a sequential change in the structure of saccular maculae. The other unique feature is the shape of the utricular maculae. Utricles with this shape have never been reported in any vertebrate.

Extreme environments often reveal the substantial changes in various systems of some organism during evolution. The investigations of the adaptations and specializations in these deep-sea family’s ears will not only provide information of deep-

sea fish's life history, but also provide insights of the evolution and adaptation of the inner ear in fishes.

## MATERIALS AND METHODS

Five species from three different genera (*Melamphaes*, *Poromitra*, and *Scopelogadus*) are used in this study (Table 3.1, Fig. 3.1). The specimens were collected along the Eastern Pacific coast of Central America on a deep-sea research cruise SO 173-2 aboard the FS Sonne during August 8 – September 2, 2003. Two kinds of nets were used during the trawls: A Tucker trawl net with an opening area of 3 m² with a closing cod end controlled by a timer and a rectangular mid-water net with an opening area of 8 m². The trawls were taken at depths of 600–1000 m in water of 2000–5600 m depth. The area of the stations during the cruise covered 10–14°N and 87–93°W.

Fishes were mostly dead after one hour's duration of withdrawing the trawl from the mid water layer to the surface. Fishes were taken onto the deck and collected in trays containing cold sea water. Photographs of fishes were taken before they were handled. Due to the scarce number of specimens from each catch, samples were taken from different body parts by the other investigators on the ship. Afterwards, the selected specimens for this study were measured and then fixed in cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, most with 0.05% $CaCl_2$ and 0.1 M sucrose. Small specimens were fixed whole; while for some of the bigger specimens the fish heads were trimmed and the skull roofs were opened to ensure fast fixative penetration.

The number of specimens used in this study and the size range are shown in Table 3.1. Some fishes were dissected on board the ship immediately after they came to the

surface and the ears and otolith were photographed to obtain the structure of otolith and otolith membrane to eliminate the effect of fixation. Morphological data for species identification were also recorded.

The species were identified according to multiple sources (Carpenter, 2002; Ebeling, 1962, 1975; Ebeling and Weed III, 1963; Kotlyar, 2004, 2008a, b). The identification of species was confirmed by the otolith collection at the Scripps Institution of Oceanography (SIO 64-13, SIO 68-52, SIO 67-52, and SIO 64-12, http://collections.ucsd.edu/mv/fish_collection/otoliths.html) and an otolith atlas (Smale et al., 1995). The geographic distributions of these species at collecting locations were confirmed by the database at Global Biodiversity Information Facility (GBIF, http://data.gbif.org/welcome.htm). After getting to land, the fixative was exchanged with 0.1 M cacodylate buffer and the specimens were stored in a 4°C refrigerator until further analysis.

Fish ears were dissected in 0.1 M cacodylate buffer. If the original fixative contained 0.05% $CaCl_2$, this was also put into the buffer used in subsequent steps. Photographs were taken during the dissection. The ears were then post fixed in 1% $OsO_4$ with 0.1 M cacodylate buffer or PIPES buffer at room temperature for 30 to 60 minutes. After three buffer rinses followed by three double distilled water rinses, the ears were dehydrated for 10 minutes each in 30%, 50%, 75%, 85%, 95% and 3 ×100% ethanol. Critical point drying was done using $CO_2$ as the intermediary fluid. Tissue was then mounted on aluminum stubs using silver paste to preserve the natural curving of lagenae and utricles. The stubs were coated with about 12 nm thickness of Au-Pt on DV 503, and viewed with an AMRAY 1280D scanning electron microscope (SEM).

The relative measurements of hair bundles were taken using SEM Digital Imaging System provided by SEMtech solution INC during the operations on AMRAY 1280D, as well as on SEM images taken during the operations. All measurements are relative because of shrinkage of samples during dehydration and the tilting and bending of hair bundles.

SEM analyses included determination of hair cell bundle orientations and categorization of hair cell bundle types. Mapping of the ciliary bundle orientation was done by scanning up and down through the entire sensory epithelia. Different orientations of bundles were marked onto the overlapping photos of the maculae taken under low magnification.

## RESULTS

### Gross Morphology

The Tucker trawl net equipped with a closing cod end brought up fishes together with cold sea water; thus the specimens were in very good condition. Figure 3.1 shows photographs of freshly caught specimens of representatives of each of the species used in this study. The fish head photos on the left preserve some detailed identification features and the distributions of head lateral line canal pores or neuromasts; the whole fish photos on the right presents the fishes' live color and body characteristics.

The inner ears of these melamphaids species are relatively large as seen in some deep-sea fishes. As a representative example, Figure 3.2 presents the dorsal and ventral view of the brain and both ears from *Melamphaes laeviceps* (bald bigscale). The length of

the ears extended from mid brain to the end of medulla oblongata. The optic tectum is relatively small in this species, and the two hemispheres are pushed aside by the cerebellum and a gigantic fused formation of crista cerebellaris (cerebellar crest) and cerebellar auricle (Fig. 3.2A). The VIIIth nerve to the ear is much larger than the optic tract (Fig. 3.2 B).

Figure 3.3 compares the ears from the three genera, *Melamphaes, Poromitra*, and *Scopelogadus.* There are some variations among the studied species of these three genera.

In *M. laeviceps*, the inferior labyrinth, which consists of the saccule and lagena, is larger than the superior labyrinth, which consists of the semicircular canals and utricle (Figs. 3.3 A,B). The oval-shaped saccular pouch takes up more than half of the inner ear space; inside of which is the large saccular otolith with a "spur" pointing in the ventral posterior direction. The lagena is relatively small and sits on a dorsal posterior position of the saccule. The utricle sits in the junction of anterior and lateral semicircular cannels. The lateral view of the ear (Fig. 3.3 B) shows the eighth nerve innervation to all of the end organs. The overall shape of inner ear in *M. acanthomus* (shoulderspine bigscale) is similar to that of *M. laeviceps*. In both species, the saccule with the elongated sagitta extends anteriorly beyond the superior labyrinth.

In *Poromitra crassiceps* (crested ridgehead) and *P. oscitans* (yawning, or small eyed ridgehead) (Figs. 3.3C,D), the inferior labyrinth occupied about the same space as superior labyrinth. The saccule pouch is also oval shaped and hosts the saccular otolith as seen in *P. crassiceps* (Fig. 3.3C). After removing the lateral side of the saccular pouch and the otolith, the saccular macula can be seen in the center of the medial wall (Fig. 3.3D). The lagena sits dorsal and posterior on the saccule, but it is closer to the caudal

end than in *Melamphaes*. This is due to the deeper profile of the sagitta in this genus, which makes the saccule pouch rounder and deeper than those in Melamphaes.

In *Scopelogadus mizolepis bispinosus* (twospine bigscale) (Figs. 3.3E,F), the ear looks quite different from the other two genera. The inferior labyrinth is smaller than the superior labyrinth. The saccular pouch is relatively small and oval shaped and the caudal end is narrower than the rostral end. The lagena attaches to the saccule at the caudal end of the saccule pouch.

In all ears, the ventral and lateral wall of the saccule pouch is heavily pigmented and brown or dark brown in color (Figs. 3.3A,C,E,F). This part of the pouch is lightly attached to the bubble-like bottom skull, which is soft, thin, and translucent. Figure 3.4 shows the ventral view of the skull in two fresh specimens. One side of the bony capsule was opened to expose the position of saccular otolith. In *M. acanthomus*, the elongated sagitta takes up most of the length of the saccular pouch. A very thin otolithic stalk can be seen pointing ventroposteriorly to the bottom of the skull (Fig. 3.4A). After removing the left sagitta from its sensory epithelium, the fragile otolithic stalk measures only 0.1 mm in diameter, which is as thin as a human hair. The right sagitta is vaguely visible under the translucent cartridge (Fig. 3.4B). In *S. mizolepis bispinosus*, which has a smaller inferior labyrinth, the button-shaped sagitta takes up only 2/5ths of the length of the pouch (Fig. 3.4C),

The size and shape of the sagitta from the two species in Figure 3.4 is apparently different, which reflects the two extreme ends of the interspecific variation in the sagitta of this family.

**Variation in Saccular Otoliths**

The shape of the saccular otoliths in the melamphaids varies considerably among species, from the highly sculpted spear shapes in the genus *Melamphaes* and various gingko-leaf shapes in the genus *Poromitra*, to a non-sculpted button shape in the genus *Scopelogadus*. Figure 3.5 shows otoliths from fishes right after they have been caught. As a result, features of the otoliths that are often lost due to being dissolved by acidic fixatives remain, making it possible to see a number of fine structural features on the otoliths that have never been reported for any members of this family, including these species.

Both *M. acanthomus* and *P.crassiceps* have a long, thin, stalk-like structure protruding from the ventroposterior margin of the saccular otolith (Figs. 3.5A,C). The sagittae of *M. laeviceps and P. oscitans* have a shorter, yet thicker, spur-like structures at the ventroposterior margin (Figs. 3.5B,D). The outline of the sagitta in both genera is wavy, with deep rostral indentations in some species (Figs. 3.5B–D). In contrast, the sagitta of *S. mizolepis bispinosus* is round and smooth and does not have the stalk-like extension (Fig. 3.5E).

Figure 3.6 shows a medial view of the opened otic capsule of two *Melamphaes* species. In *M. acanthomus*, the stalk touches on the ventral wall of the skull (Fig. 3.6A), which is the bottom of the bony labyrinth. Instead of the usual rigid bony wall that is found in most fishes, the bottom part of the bony labyrinth in *M. acanthomu*' is soft and elastics, as shown in bluish color in Figure 3.6A. This kind of contact between otolith and bony capsules has never been described in any studied fishes.

**Variations in Saccular Macula Associated with Sagitta Shapes**

The long and thin otolithic stalk on the sagitta of *Melamphaes* and *Poromitra* is very intriguing. It should be noted that the sensory epithelium of the saccule does not contact the protruding spur. This is shown by fresh dissections of the otic capsule in two species of *Melamphaes* in Figure 3.6. The imprints of the saccular maculae and their original locations are indicated on the sagitta by the otolith membranes remaining in the sulcus. The membrane has a distinct natural pinkish color.

Corresponding to the variation in the saccular otolith, the saccular maculae of the melamphaids fishes also have variations in the shape and bundle orientation groups, and they show a sequential change among different genera (Fig. 3.5F–J). The presence or absence of the otolithic extension appears to be correlated with the shape of the saccular macula. The overall shape of the saccular macula of the two genera that have extensions on their sagitta (*M. acanthomus* and *P. crassiceps* with stalks; *M. laeviceps* and *P. oscitans* with spurs) is shaped like a tadpole, with a tail at the caudal part of the maculae. In contrast, there is no tail part in the saccular macula of *S. mizolepis bispinosus*, which has the button-shaped sagitta.

There is a gradual change in hair cell ciliary bundle orientation patterns corresponding to the tails (Fig. 3.5F–J). All five saccular maculae are similar in their rostral regions but differ caudally. They all have two opposite horizontally oriented bundle groups ventrally and two opposite vertically oriented bundle groups dorsally. The most striking difference is the variations in the caudal "tail" of the maculae (Fig. 3.5F–I). In the genus *Melamphaes*, the caudal "tail" is long and elaborated with vertical and horizontal oriented groups (Fig. 3.5F,G). In the genus *Poromitra*, the macula lacks the

horizontally oriented caudal end (Fig. 3.5H,I). In contrast, there is no tail in the genus *Scopelogadus*, and so its saccular macula actually resembles the enlarged "tadpole head" of the maculae of the other two genera (Fig. 3.5J).

In the saccule of all species, it appears that a separate branch of the nerve goes to each of the separate hair cell orientation groups. Figure 3.7 present the medial view of these innervations in one species from each of the three genera. In *Melamphaes* and *Poromitra*, three distinct nerve branches are associated, respectively, with the rostral vertical group, the rostral horizontal group, and the caudal tail (Figs. 3.7A–B). In *Melamphaes*, a fourth division is separate from the caudal tail branch and assigned to the horizontal group at the caudal end (Fig. 3.7A). *S. mizolepis bispinosus* has no macular tail and thus lacks the third nerve branch (Fig. 3.7C).

**Unique Utricular Macula**

In contrast to the general similarity in utricular structure in fishes, reptiles, and mammals (Lewis et al., 1985), the utricular sensory epithelia in melamphaids have an unusual shape. The outline of the maculae in this family can be described as resembling the head of "Mickey Mouse®." An example would be the utricle of *P. crassiceps* (Fig. 3.8). Other utricular maculae can be described as fox's head in *M. laeviceps* or cat's head in *P. oscitans*. The dominant anterior ear-shaped structures found in the utricle of all species in this family have never been found in any vertebrates. Another feature in these utricles is the exceptionally long and thin lacinia (Latin, *fringe, hem*), which is a narrow part of the sensory epithelium extending laterally from the main macula. (Figs. 3.8, 3.9A–E).

The utricular otoliths in this family are much smaller than the saccular otolith (Fig. 3.6). They are circular or square shaped and form a dome on top of the sensory epithelia, but they do not cover the ear-shaped surface nor the lacinia (Fig. 3.8E), instead, they are covered by the otolithic membrane that extend from the otolith. It should be noted that while the lacinia appears in the images to be on the same plane as the rest of the macula in the opened and flattened SEM samples, it is actually curved and arched over the otolith in an un-opened utricle pouch.

The hair cell ciliary bundle orientation patterns on these maculae are shown in Figure 3.9. The line separating orientation groups is wavy and runs along the edge of the two "ears" and then cuts through the center of the "ears." A number of SEM photos of enlarged portions of epithelium are shown in Figure 3.10. The striola, if present, spreads out over the entire "ear" region.

**Lagena**

The lagenae in this family are very small compared to the saccule (Fig. 3.3). The lagenar otoliths are significantly smaller then the saccular otolith, less than 1/10 of the length and 1/30 of the area of the sagittae (Fig. 3.6B).

The lagenar maculae and their hair bundle orientation in melamphaids are not unusual compared to other fishes (Fig 3.9F–J). The maculae are narrow and positioned with an incline from dorsal-anterior to ventral-posterior. The shape of the macula is relatively consistent within each genus. The *Melamphaes* lagena is shaped like a banana, that in *Poromitra* like a spindle, and that in *Scopelogadus* like a crescent. The dividing line of oppositely oriented hair bundle groups runs along the length of the macula and

divides the macula approximately into half. In *Melamphaes* (Figs. 3.9F,G), the orientations of the hair bundles on either side of the dividing line are pointing towards the line and are mostly perpendicular to it. In *Poromitra* (Figs. 3.9H,I), most bundles are oriented towards the dividing line with the some bundles at the ventral edge shift slightly in the rostral direction. In *Scopelogadus* (Figs. 3.9J), only bundles close to the dividing line are oriented vertically. In contrast, the other bundles shift gradually to caudal or rostral at the dorsal and ventral regions of the macula, respectively.

**Mapping the Distribution of Hair Bundle Types**

The distribution of hair bundles types are mapped on the saccule, utricle and lagena maculae in all five species. Most bundles are categorized according to the criteria described in Table 2.1 of Chapter 2, with the addition of two bundle types. One of these has never before been seen in fish saccules (Figs. 3.11A,B) while the other is a much shorter and smaller version of category 2 bundle in Table 2.1, and is added to reflect the bundle height changes in utricle. One species representing each of the three genera are shown in Figure 3.12. Schematic drawings of each bundle type are featured with the color codes from Chapter 2.

*Saccule*

This unique type of bundles is found on the saccule in the genera *Melamphaes* and *Poromitra*. The bundle length can be as long as 15–20 μm, with a very large elongated rectangular or hexangular cilia base and large number of stereocilia (Figs. 3.11C,D). In a specimen of *M. laeviceps* (standard length 82mm), the average number of

stereocilia at the head region is 98.2 (range 84–114, SD = 10.2) from 10 cells, and 89.1 (range 84–100, SD = 7.6) at the caudal tail from 9 cells. These bundles have a bunch of very long stereocilia that are almost as long as the kinocilium. About half of the stereocilia form a short gradient stub. The schematic drawing of this type of bundle is in Figure 3.11E with the color purple as its mapping color code.

These ultra long thick bundles cover almost the whole surface of the saccule in *Melamphaes* and *Poromitra* (Figs. 3.12). Only a small number of Category 2 bundles adorn the edge of the macula, and a portion of the dividing line, these bundles (coded yellow) have exceptionally long kinocilia and a very short stereocilia staircase with short array length (Fig. 3.11F).

The bundle types on the saccular macula of *Scopelogadus* are very different from the other two genera. Category 3 (coded red) and 6 (coded pink) bundles cover most of the macula with Category 1 (coded blue) bundles on the edge.

*Lagena*

The bundle type distribution on the lagena maculae of these three genera are roughly similar in that they all have Category 3 bundles close to the dividing line and Category 1 bundles around the outer area, the difference are the transitional bundles in between this two (Fig. 3.12).

*Utricle*

In all the utricles from the three genera, the unique ear-shaped areas of the maculae are mostly covered by Category 3 bundles, making the whole “ear” region

resemble the striola region in most other vertebrate utricles (Fig. 3.12). Bundles surrounding these two ear-shaped region are mostly category 1 bundles that have a longer kinocilium but shorter and smaller stereocilia staircase. The long lacinia has small bundles scattered around, with most bundles having very long kinocilia. The bundles distributed over the rest of the sensory epithelia form a gradual gradient in heights. Bundles with a long kinocilia cover the base of the two "ears." Caudal to these bundles, the kinocilia on the bundles becomes shorter, and then the stereocilia gets shorter and shorter towards the medial caudal edge of the macula (Fig. 3.12)

*Supporting Cells*

The supporting cells on the maculae have different surface appearance at different places. Figure 3.13 (A–D) shows a patch of supporting cells along the ventral region of the saccular tails. These microvilli-rich surfaces are organized in a pattern and appear in the same area in all saccules of the genera *Melamphaes* and *Poromitra*. No such cell was found in *Scopelogadus* because there is no tail region in the saccule.

In the ventral region of the saccular head, a clear boundary composed mostly by supporting cells separates the vertical and horizontal bundle groups. This line appears on all the saccules in this family. Pentagonal or hexangular supporting cells are often found at the non-sensory region around the macula in the end organs (Fig. 3.13F).

**The Growth of Saccule**

*Otolith*

The stalk or spur on the ventroposterior edge of the saccular otolith appears only when fish reach half-grown to adult stage. This is seen in fresh otolith samples of *Poromitra crassiceps* from specimens of different sizes. Figure 3.14A shows a stalk-less sagitta from a young *P. crassiceps* (standard length (ST) = 45 mm) inside the outline of an adult (ST = 140 mm) sagitta, with doted lines indicating the growth rings on the adult otolith. The shape of the small otolith matches the growth rings of the bigger otolith. It is clear that the otolithic stalk on *P. crassiceps* only appears after the fish reach a certain size.

Figure 3.14B shows the comparison of a half grown *P. oscitans* (ST = 53 mm) with an adult (ST = 75 mm). On the half grown's sagitta, the base of the otolithic spur just starts to appear, the growth ring on the bigger otolith also indicates the progress of the spur elongation.

*Sensory epithelium*

The sagitta shapes may be different in *P. crassiceps* of different sizes. However the saccular macula in this species retains its characteristic shape from very small size to large adults. Figure 3.15A compares six specimens with a standard length range from 37 mm to 140 mm using the same scale. A schematic drawing of the growth of saccular maculae (Fig. 3.15B) shows that the dimensions of macula seem to expand more horizontally than vertically with the growth of fish. The measurements of the saccular maculae are plotted in Figure 3.16 with the standard length of the fish. The growth of the saccular macula is proportional to the growth of the fish length (Fig. 3.16A) The ratio of

vertical length vs. horizontal length does not show statistic differences with fish's growth (Fig. 3.16B).

## DISCUSSION

Several special features have been found in the inner ears of Melamphaidae in this study: **1)** The shapes of the saccular otoliths varied considerably and ranged from a variety of sculpted shapes in genera *Melamphaes* and *Poromitra* to a non-sculpted button shape in *Scopelogadus*. **2)** Some species in *Melamphaes* and *Poromitra* possess a long, thin, stalk or spur-like structure protruding from the ventroposterior margin of the saccular otolith. **3)** There is significant variation in the sensory epithelia between the different species, and the hair cell bundle orientation patterns are associated with the change in saccular otolith shapes. **4)** A special type of ciliary bundle with very long stereocilia that are almost as long as the kinocilium and very high cilia counts dominate the saccular macula. **5)** The striolar region of the utricular maculae exists as two ear-shaped areas that have not been seen in any other vertebrate. **6)** Beyond the inner ear, gross morphology of the brain reflects a relatively enlarge area involving inner ear and lateral line (octavolateral) functions. These findings evoked many questions regarding function of otolith, dominance in sensory systems, adaptations to deep water living, and evolution.

### Otolith: Does form affect hearing function?

The otolith transfers the vibration of sound to the hair cells because it is denser than the rest of the fish body. Although the shape of otoliths in fish varies among

different families or genera, many of the forms are just variations of an oval to oblong shape. The morphological characteristics of otoliths are often consistent within a family or genus, although many exceptions are known (Campana, 2004). The saccules of melamphaids, like some of the other deep-sea fishes, have very large otolith and have otolith shapes that are seemingly quite different from the shapes found in fishes living in shallower waters.

It has been suggested that otolith size may affect the hearing frequency range and sensitivity, with large saccular otolith responsive to lower frequency sounds than smaller otoliths (Popper and Tavolga, 1981). While not yet tested experimentally, it has been suggested that the increase of sensitivity in fishes with larger otoliths may also be correlated with having a narrower bandwidth of hearing when compared to fishes with smaller otoliths (Lychakov and Rebane, 1993, 2000). On the other hand, the size of otolith relative to the size of the fish body may be correlated with swimming speed in that fast-swimming fishes often have much smaller otoliths than slow-swimming fishes (Popper et al., 2005).

Deep-sea fishes are slow moving animals due to the need to conserve energy in a food-scarce environment. Thus it is possible for these fishes to have larger otoliths. In fact, exceptionally large otoliths are often reported in deep-sea fishes (e.g. Fine et al., 1987; Marshall, 1966; Marshall, 1980; and the *Antimora* and rattail in this study). Since low frequency sound travels longer distances and attenuates more slowly than higher frequency sounds, it may be more useful for deep-sea fishes to have larger otoliths so they are sensitive to a wider acoustic scene (e.g., sounds from greater distances) than shallow-water fishes.

A complex otolith shape may also affect the dynamics of otolith response to vibrations, and may provide richer information for the fish's sense of hearing or balance (Popper et al., 2005). Eco-morphological studies have found that more complex shaped saccular otoliths are found in species that are thought to depend more on hearing than those that do not (Aguirre and Lombarte, 1999; Gauldie and Crampton, 2002; Parmentier et al., 2001).

The otolithic stalk on some species of *Melamphaes* and *Poromitra* (Figs. 4A–D, 5A–B) have not been seen before. The prominent stalk on the otoliths makes them extremely asymmetric, such that these otoliths can no longer be considered as a simple mass point in the movement caused by sound. An asymmetrical otolith may deliver differentiated mechanical stimulations to different areas of the sensory epithelium, which is consistent with the idea that fishes process much of the acoustic information peripherally (Fay, 1988; Fay and Popper, 2000). It is reasonable to suggest that the sculpted otolith structure may also help in enhancing the hearing function. The associated variance in sensory epithelia structure and hair cell bundle orientation patterns also may imply a functional meaning of the otolith structure. Finally, the separate innervation to different saccular regions also leads to the suggestion that the signals from hair cells oriented in different directions may processed separately in the brain. However, without having live species on which to experiment and test hearing capabilities and inner ear mechanics, any ideas must be treated as speculation at this point.

How the otolithic stalk affect otolith movement in the sound field is not known. In *Melamphaes acanthomus*, the otolithic stalk touches the bottom of the skull (Fig. 6A), which is part of the bony labyrinth of the ear. This kind of contact between the otolith and

the fish body has not been found in other fishes. Saccular otoliths are usually suspended on the lateral side of sensory epithelium, and so the movements of the otolith relative to the sensory epithelia are probably under the influence of the direction of the sound field. In contrast to otoliths that are only in contact with the epithelium, it is possible that the presence of the stalk may provide a pivot point for the otolith, limiting its range of motion, thereby "converting" left-right movement into a slight up-down rubbing of the hair bundles.

**Saccule Structure: Similarities among Unrelated Species**

Hair bundle orientation pattern in melamphaids varies sequentially among the three genera (Figs. 4F–J). The different orientation patterns are associated with separate innervations patterns from the eighth nerve (Fig. 7). Thus, the saccular macula has four distinct eighth nerve bundles in *Melamphaes*, three in *Poromitra*, and only two in *Scopelogadus*. The hair bundle orientation patterns in the three genera resemble one another rostrally, but increase in complexity from *Scopelogadus* to *Melamphaes*. The general layout of the bundle orientation pattern does not fit into any pattern described by Popper et al. (2003), and it is more of a combination and modification between "dual" and "opposing" pattern (definition see Fig.1.6 in Introduction) as discussed by Popper and Coombs (1982).

Although the saccular orientation patterns in melamphaids have not been described before, somewhat similar patterns are found in species from unrelated fish groups. *Opsanus tau* (oyster toadfish) from the order Batrachoidiformes (Edds-Walton and Popper, 1995) has a roughly similar layout of the different orientation groups as

melamphaids. They have a rostral area with horizontal groups and middle to caudal region that looks like a transitional form between *Melamphaes* and *Poromitra* (Fig. 3.17A).

The most striking similarity was found in the saccule of another deep-sea fish from the family Myctophidae (lanternfishes), which was studied by Popper (1977). Popper examined the saccules of three species of lanternfishes from three different genera, *Diaphus*, *Ceratoscopelus*, and *Lampanyctus*. All three species had hair cell orientation patterns that resembled the general pattern in *Poromitra* as reported here (Fig. 3.17B). More interestingly, several unusual characters described by Popper in thee lanternfishes are very similar to those in the melamphaids. One is the long hair bundle with several stereocilia that is almost as long as the kinocilium. These bundles also dominate the saccular macula of lanternfishes. The second is a dividing line that separates the vertically and horizontally oriented groups that is devoid of hair cells (Fig. 3.13E). This dividing line also separates the eighth nerve branches to the saccule into two parts, as in melamphaids. Unusual supporting cells were also found just below the ventral-posterior quadrant of the macula in lanternfishes, although the cells look different from the unusual supporting cells in the same area in *Melamphaes* and *Poromitra* (Figs. 3.13A–D).

It is important to note, however, that while there are similarities, as discussed above, the Myctophidae, which are in the taxonomic superorder Scopelomorpha and the Melamphaidae, in the superorder Acanthopterygii, are quite distinct taxonomically (Nelson, 2006). In contrast, the structure of saccular maculae in the squirrelfish (family Holocentridae) of Beryciformes (Popper, 1977), a sister order of the Stephanoberyciformes where Melamphaidae is, are not very similar to those in

Melamphaidae. This suggests that species ancestral to both Melamphaidae and Myctophidae did not have the hair bundle orientation patterns or other ear structures found in the melamphaids.

While there are still few data for fishes that are "between" the Melamphaidae and Myctophidae, we may speculate that the similarities between the two groups represent cases of convergent evolution. It would be of considerable interest, of course, to know the selective pressures that resulted in such similarity between such distantly separated groups of fishes.

The melamphaids and myctophids not only resemble each other in saccule structure, but they also have similar gross morphology in the brain.

**Brain: Special Features in Gross Morphology**

In bony fishes, brain morphologies can reflect specializations in their sensory systems (Wagner, 2001, 2003). The brain of melamphaids looks very different from a typical teleost brain (Fig. 3.2). The optic tectum is rather small, and the cerebellum and its continuous structure push into the space between the two hemispheres of the optic tectum.

In the lanternfish *Diaphus dumerilii*, which is from the same genus as one of the lanternfish *Diaphus brachycephalus*) studied by Popper (1977), the midbrain and hind brain region shows similar features to those in *Poromitra oscitans* (Fig. 3.18). The cerebellum and the gigantic tubuculum acousticum push the optic tectum apart and downwards (Shanklin, 1934). The tubuculum acousticum, later called the crista cerebellaris (cerebellar crest) of the rhombencephalon, is thought to receive inputs from

lateral line and auditory system (Larsell, 1967); the octavolateralis nucleus is under (Butler and Hodos, 2005) or above (Huesa et al., 2003) the cerebellar crest in bony fishes.

This gigantic formation in the posterior cerebellar region may include cerebellar crest, cerebellar auricle, and eminentia granularis, all fused together so that they cannot be distinguished in this preliminary observation. These brain regions in melamphaids may reflect their highly developed head canal lateral line systems, and possibly suggests robust input from the auditory and vestibular system. The optic tectum in *Poromitra oscitans* (Figs. 3.18B,D) is very small and the optic nerves are much thinner than the eighth nerve, which is likely to reflect the fact that *P. oscitans* (yawning, or small-eyed ridgehead) has limited vision. The olfactory bulb in the melamphaids is also very small (Figs. 3.2, 3.18). Similar feature are present in all species in this study. The octavolateral system, which includes auditory, vestibular, and lateral line organs, is evidently the most dominant sense in melamphaids.

**Strange Bundles in Saccule: A specialization?**

One type of hair cell ciliary bundle dominates the saccular maculae of *Melamphaes* and *Poromitra* and has never been reported in fish saccules before. These bundles have a bunch of very long stereocilia that are at almost the same height as the kinocilium. The length of the remaining stereocilia drops dramatically and form a very shallow staircase at the base of the bundle (Fig. 3.11). In the saccule, the whole epithelium is attached to the otolith. It must be noted that bundles of such height have never been found to be associated with an otolith.

The presence of the very long bundles and their homogeneity on the saccule leads to questions, unanswerable here, of the actual physical relationship between the otolith and the sensory epithelium, and the space between the two structures. If we assume that the bundles stand fully upright in vivo, this is likely to mean that there is a considerable distance between the otolith and the base of epithelium. If consider the connection between the hair bundle via otolith membrane to the otolith as a spring system with a dense cap at the top, intuitively, the increased soft substance under the dense otolith will lower the threshold of the system's response to vibrations. It can be suggested that such ears are more sensitive to low frequency sound or head movements.

The architecture of the special type of bundle in saccule may have functional advantages in many aspects. Firstly, bundles with low KS ratio (the ratio between the length of kinocilium and the longest stereocilium) has been suggested to be more sensitive to displacement than bundles with a higher ratio. Baird (1994) compared the response of bullfrog utricular ciliary bundles to bundle displacements and found that type B and C cells (amphibian bundle types with longer kinocilium than the stereocilia, review see Lewis et al., 1985) have lower sensitivity than type F and type E cells (bundle types with high KS ratio). Although Baird (Baird, 1994) concluded that longer bundles are less sensitive to bundle displacements than shorter bundles, the longer bundle in the contexts are the amphibian bundles with longer kinocilium but shorter stereocilia. The saccular bundle in *Melamphaes* and *Poromitra* have very low KS ratio in half of the bundle with the bunch of long stereocilia, thus may be sensitive to displacements.

Secondly, long bundles have a wider operation range if the displacement force is applied at the tip of the hair bundle (Baird, 1994). For the saccular bundles in

*Melamphaes* and *Poromitra*, displacement comes from the otolith at the top. Their long kinocilium, together with long stereocilia, may increase the bundles' operation range without compromising sensitivity.

Thirdly, the short stereocilia staircase near the base of these long bundles may also enhance sensitivity because at this level, small displacements cause large angular rotations (Baird, 1994). And larger numbers of stereocilia means more transduction canals are available to produce currents. The stereocilia number in these bundles may be the highest among all fish ears that have been studied so far (Platt et al., 2004).

With the above considerations, the saccular bundle with a long kinocilium and two high and low compartments of stereocilia staircases and high number of stereocilia may have multiple ways to increase sensitivity and operation range. This may be a specialization in the inner ear structure with enhanced sensitivity to bundle displacements, though it needs further investigation to determine if it is for hearing or vestibular function.

**Utricle: Unique Structure That Brings the Family Together**

The highly diverse saccular otolith and macula structure in melamphaids makes one wonder how these species could be included in the same family or even the same genus if just looking at the ears. However, the peculiarly unique structure of the utricle that has never been discovered in any other vertebrates does bring these family members together (Fig. 3.8).

In the middle of the ear-shaped region, a reversal line of opposite oriented bundle groups separates the two areas resembling "ears" into approximately two halves. The reversal line on either side of the two ear-shapes and in the middle of two ear-shapes are

so close to the edge of the macula that there is almost no room for opposing bundles (Fig. 3.9A–E).

The hair bundles on the melamphaids utricle are very heterogeneous in bundle types. Six different categories of bundles are mapped on the macula, and the height of bundle varies gradually at some areas. The majority of bundles in the ear-shaped region are classified as category 3 bundles as described in Chapter 2. These bundles are generally bigger, with a longer stereocilia array and steeper slope (Fig. 3.10). These characters fits into the description of type I striola bundles in the striola of turtle (*Trachemys scripta elegans*) utricles (Xue and Peterson, 2006). However, type I hair cells, the type with cell body in a calyces (Wersäll, 1956), have not been confirmed in fish end organs, except for a TEM study of a cichlid fish *Astronotus ocellatus* (oscar) that established a type I-like cells in the striola region of utricle (Chang et al., 1992). It is possible that the cells in the melamphaids are also type-I like but further analysis of the cell body is needed to confirm that.

The hair bundles surround the ear-shaped area (category 1) resemble the type II bundles (cell body contacted by boutons) in the turtle utricle near the striola region. The very short bundles in the medial caudal region of melamphaids utricle, however, do not resemble those in the turtle utricle.

Hair bundle types have been studied in great detail in the utricles of some vertebrates (Li et al., 2008; Moravec and Peterson, 2004; Xue and Peterson, 2006). A series of parameters are used to characterize hair bundle types (Xue and Peterson, 2006), such as bundle height, stereocilia array length and slope, KS ratio, etc. Together with the analysis of cell body types the profile of hair bundle type distribution are established for

the utricle. Although it is difficult to do precise quantitative analysis of bundle types on many deep-sea fishes due to the scarce number of specimens, the richness of bundle types on melamphaids utricle is worth investigating with quantitative methods should new specimens be available.

Why does the striola region in melamphaids take on a two-ear-shaped other than a continuous crescent shape like other vertebrate utricles? It seems these utricle maculae may have expanded to fill the entire striola region anteriorly, but are restricted by limited space at the junction with anterior ampullae of the semicircular canals. An expanded striola region may be beneficial because it contains the type I-like hair bundles with low K/S ratios that possibly increase sensitivity (Baird, 1994; Xue and Peterson, 2006). And, the higher number of stereocilia numbers may provide stronger transduction currents (Moravec and Peterson, 2004).

Another speculation is based on the orientation of striola bundles. The expanded striola region retains bundle groups that are mostly polarized at two axes on the horizontal plane: anteriolateral-posteriomedial and anteriomedial-posteriolateral. This leaves the anterior-posterior axis empty. Displacements at the anterior-posterior axis can be encoded by the horizontal oriented bundles in saccule, thus reduce the redundancy of sensory cells for this direction - an energy conservation strategy.

**Tradeoff among Sensory Systems**

All species studied in this family have large inner ears. In the genera *Melamphaes* and *Poromitra*, the inferior labyrinth is larger than the superior labyrinth, which is opposite to what N. B. Marshall concluded in the 1970s. After comparing inner ears of

fishes from different depths, Marshall found that mesopelagic and bathypelagic fishes have large utricles and small saccules, whereas most benthopelagic fishes (grenadiers and rattails, deep-sea cods, and brotulids) tend to have very large saccules and saccular otoliths (Bierbaum, 1914; Marshall, 1980). The current findings of very large saccules in the mesopelagic family suggest that it is not possible to make a general conclusion about deep-sea fishes base on habitats. Bierbaum's (Bierbaum, 1914) collection of ears also included one melamphaids species, *Melamphaes* (now *Poromitra*) *megalops*. The ear of this species looks very similar with the other two *Poromitra* in this study. Among benthopelagic fishes (Buran et al., 2005), very large and very small saccules relative to the super labyrinth in Elopomorph fishes (eels, etc.) have also been found.

The species suggested by Marshall as representations of underdeveloped saccules in meso- and bathypelagic fishes are *Chauliodus sloani*, a viperfish that prays on lantern fishes, and *Sternoptyx diaphana,* a hatchet fish with large eyes and light organs. These fishes are likely to rely heavily on vision. On the other hand, big saccules are found in benthopelagic fauna include rattails, deep-sea cod, and brotulids, all of which are families with members that are thought to be sound producing (Marshall, 1966). For bottom living fishes, no sunlight is available at the depth and bioluminescence is less abundant than in the mesopelagic layer. A large saccule otolith in these fishes could be an indication of dominant role of hearing in their lives.

It would be ideal for a fish to have good vision, olfaction, and mechanosensory systems altogether. However, specializations in any sensory system require large brain areas to accommodate them, and the volume inside a head is somewhat limited. Secondly, a remarkable constraint of deep-sea living is the scarce source of food energy (Gartner et

al., 1997). So there is simply not enough space and energy for an animal to develop specialization in all sensory systems. Tradeoffs in representations of sensory system in the brain have been confirmed in vision and olfaction (Wagner, 2001, 2002, 2003). Overrepresentation in one sensory system often comes with a reduction in less important ones.

The melamphaid fishes do not have light organs and their eyes are medium sized or small. This implies that vision is not dominant in their sensory system. Instead they have very elaborate head lateral line organs (Fig. 3.1), although the canals may be reduced on the body. The large ear, very large cerebellar area involved in octavolateral input, and robust eighth nerve innervation (Figs. 3.2, 3.7), suggest that inner ear function is well developed for these fishes. Anatomical evidence from the melamphaids' inner ear structure and brain gross morphology does not oppose the hypothesis to be applied to the investigated species, especially for *Melamphaes* and *Poromitra*.

**Phylogenetic and Evolutionary Considerations**

The structural variation in the saccular otolith and macula may be correlated with the family's phylogenetic history. A phylogenetic tree of this family will be needed to test this hypothesis. Many deep-sea fish family's taxonomy is under revision and the genetic relationships between groups have yet to be established. This includes the family Melamphaids and its order Stephanoberyciformes (Colgan et al., 2000). Many current studies are still at the family level, so no existent trees at the genus or species level have been found for this family.

A tentative attempt to establish a tree from the available DNA sequence was performed by using DNA sequences for the bar-coding gene (mitochondrial cytochrome c oxidase subunit I). These sequences were obtained from GenBank and BOLD (Barcodes of Life Data) System for *Poromitra crassiceps, P. oscitans, P. capito, P. megalops, Melamphaes lugubris, Scopelogadus mizolepis,* and *S. bispinosus.* The outgroup was chosen from the sister family Stephanoberycidae. Unfortunately, the constructed gene tree has poor resolution at the genus level. This may be due to inadequate samples. Thus, it is not possible to establish the phylogenetic relationship for the studied species.

However, given more available data in the future, a phylogenetic tree will be available for analysis of the evolutionary pathway of these variations in the ears of melamphaids.

At the morphological level, Ebeling (1962) tried to establish the relationships of species within the genus *Melamphaes* using 28 characters to evaluate the specialization in each species. Within these characters, for example, the degree of ossification in bones reflects the degree of adaptation for deep-water living, as is the increase of head size accommodating more canal organs. From this somewhat subjective analysis, *Melamphaes acanthomus* is in a group slightly more specialized than *M. laeviceps*, this does agree with the longer otolithic stalk in *M. acanothomus* and shorter spur in *M. laeviceps*, assuming the otolithic stalk is a derived character. In the most "primitive" group, otolith images are available for *M. lugubris* (SIO 67-101 from Scripps) and *M. polylepis* (PPP3055 from NMITA: Neogene Marine Biota of Tropical America) and both otolith have the "normal" oval shape with only small papilla at the same spot as the

otolithic stalk. However, another species that was evaluated as more specialized for deeper living, *M. simus*, has a similar saccular otolith (Smale et al., 1995) as the two "primitive" species. It is also pointed out that different species may have different set of specialized or unchanged characters.

The genus *Scopelogadus*, on the other hand, was described as a relatively "young" genus, and is more completely adapted to bathypelagic environment with more specialized characters like low density bones and completely regressed swim bladder (Ebeling and Weed III, 1963). They live in deeper and more sterile water. The reduced form in saccular otolith and macula structure may well be a derived character from adaptations for its life style.

In order to understand the evolutionary relationship between these different ears, ears from the other two genera in this family, *Scopeloberyx* and *Sio*, need to be studied. And more species within the same genus should be included, especially for the most diverse genus *Melamphaes*.

The similarity in saccular macula and in brain morphology in myctophids and melamphaids is also very interesting. They are very distant species from different taxonomic superorders. However, they live in the same meso- to bathypelagic water layers. During the cruise that captured the specimens used here, myctophids and melamphaids were the two most abundant fish groups in all catches. One of the major difference between lanternfishes and bigscales is that lanternfishes have well developed light organs, which suggests that they are likely to rely on vision, at least for near-by objects and to find mates. Nevertheless, the highly similar saccular macular structure and

some similar features in the brain's morphology may imply that sharing of similar environmental niches may a induce convergent evolution in some of the sensory systems.

**ACKNOLEDGMENTS**

Thanks to Dr. Hans-Joachim Wagner for inviting me to join the deep-sea research cruise in the project Sensory Mechanisms in Mesopelagic Fish. The research cruise was funded to H-J. Wagner by the German Ministry of Education and Research (BMBF) under project No. 03G0173B. Thanks to the Master and crew of the FS Sonne. Thanks to Tim Maugel for his expert advise on SEM work. Also thanks to H.J. Walker and Cindy Klepadlo from Scripps Institute of Oceanography for providing melamphaids otolith collection photos to confirm some of the species identification. And thanks to Gang Chen for helping in phylogenetic analysis. Portions of the microscopy work were supported by P-30 grant 2 P30 DC004664 from the National Institute of Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health.

## TABLES

**Table 3.1.** Species name, depth and standard length (ST) of melamphaid fishes examined in this study

| Species name | Common Name | Depth Range | Maximal ST | ST Range | Number of Specimens |
|---|---|---|---|---|---|
| *Melamphaes acanthomus* Ebeling, 1962 | Shoulderspine bigscale | 250 - 3500 m | 110 mm | 85-110mm | 7 |
| *Melamphaes laeviceps* Ebeling, 1962 | Bald bigscale | 400 - 1109 m | 134 mm | 82-93 mm | 4 |
| *Poromitra crassiceps* Günther, 1878 | Crested ridgehead | 0 - 3400 m | 180 mm | 37-140 mm | 7 |
| *Poromitra oscitans* Ebeling, 1975 | Yawning | 800 - 5320 m | 82 mm | 53-75mm | 3 |
| *Scopelogadus mizolepis bispinosus* Gilbert, 1915 | Twospine bigscale | 300 - 3385 m | 94 mm | 50-71 mm | 8 |

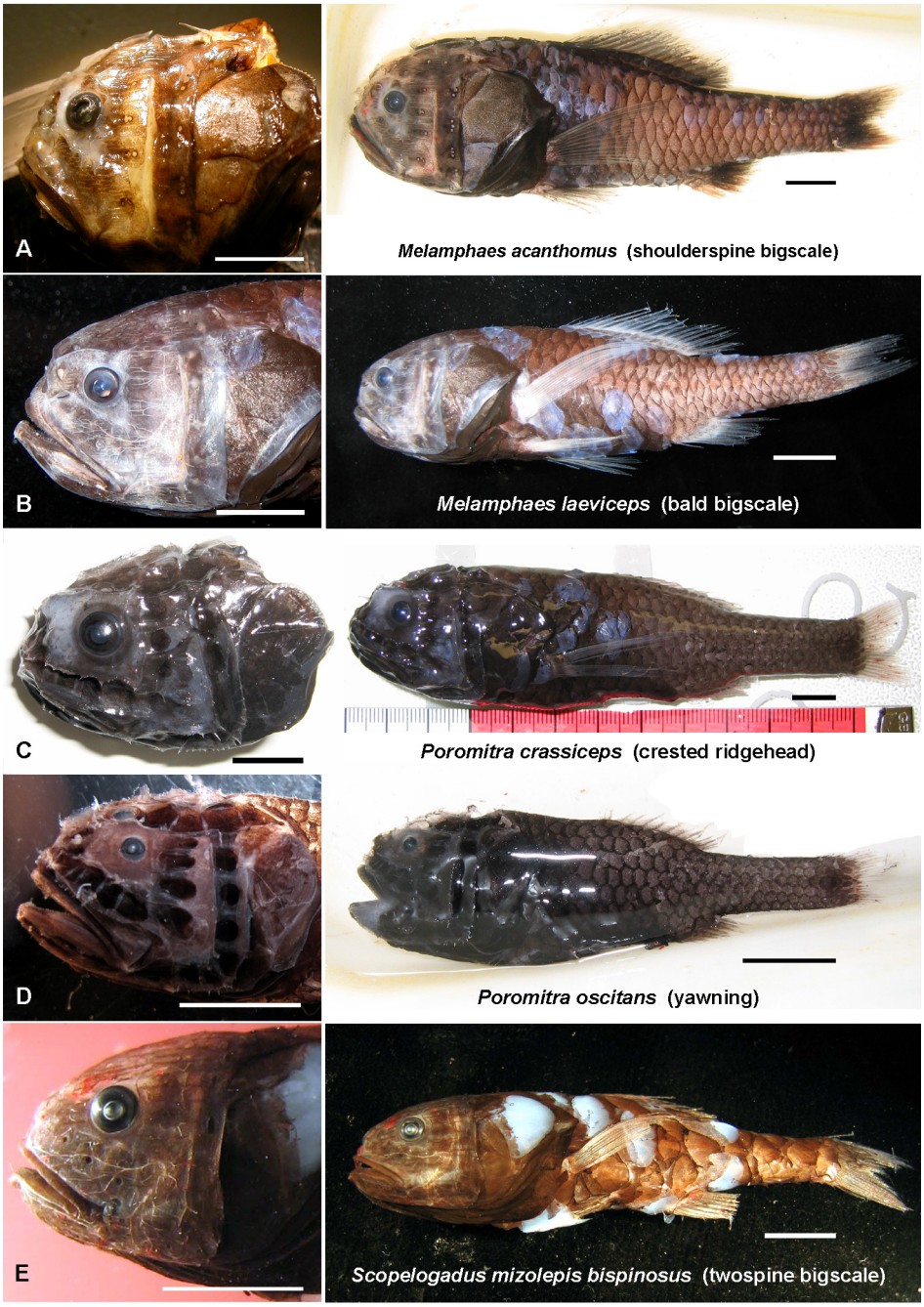



**Figure 3.1.** Photographs of the actual specimens used in this study. Each fish head on the left and the fish body on the right belong to the same specimen. Pictures of whole fish were taken on fresh specimens without fixation to preserve the fishes' live color, body characteristics, and some scales. The heads in **B**, **C**, and **E** were unfixed; whereas **A** and **D** were photographed after fixation. The fish heads show the distributions of head lateral line canal pores, or neuromasts if the head skin were removed. Scale bar = 10 mm.

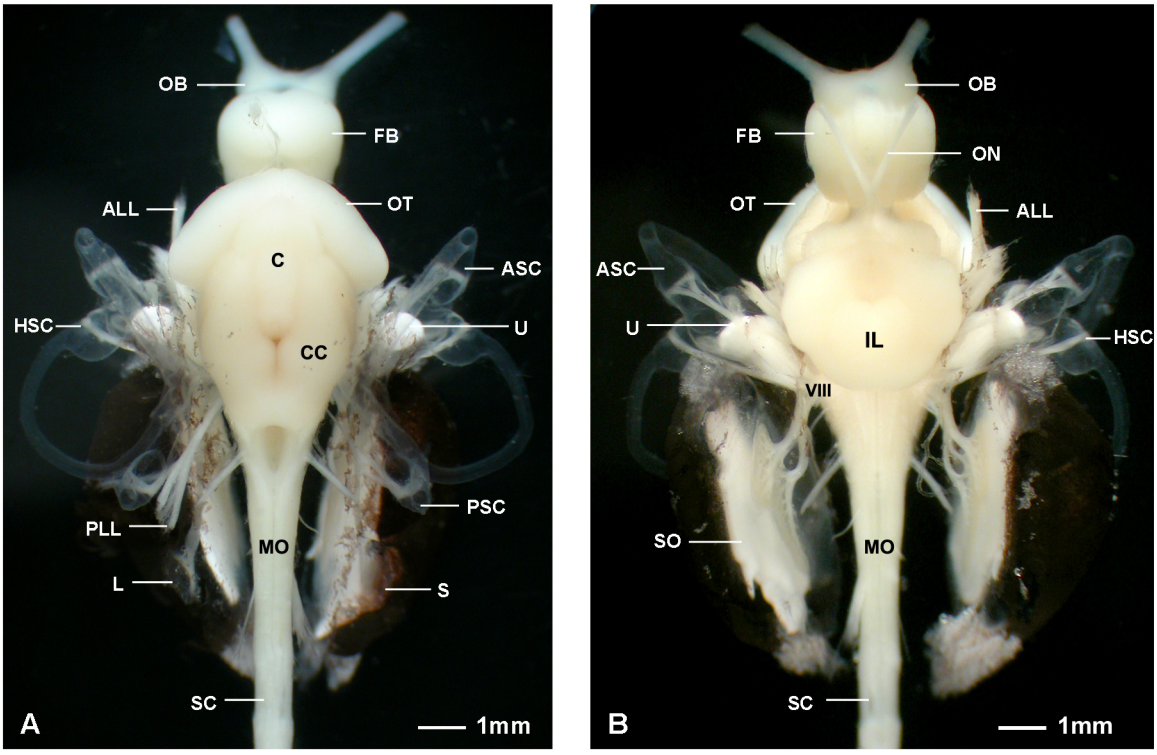



**Figure 3.2.** Brain and ears of *Melamphaes laeviceps*. **A:** Dorsal view. **B:** Ventral view. The ears extent from optic tectum to the end of medulla oblongata. Note the gigantic crista cellubellaris that pushes over the relatively small optic tectum (**A**). The VIII cranial nerve to the ear is much larger than the optic nerve (**B**).

ALL, anterior lateral line nerve; ASC, anterior semicircular canal; C, cerebellum; CC, crista cerebellaris (tubuculum acousticum in older literature); FB, forebrain; HSC, Horizontal semicircular canal; IL, inferior lobe; L, lagena; MO, medulla oblongata, OB, olfactory bulb; ON, optic nerve; OT, optic tectum; PLL, posterior lateral line nerve; PSC, posterior semicircular canal; S, saccule; SC, spinal cord; SO, saccular otolith; U, utricle; VIII, the eighth cranial nerve.

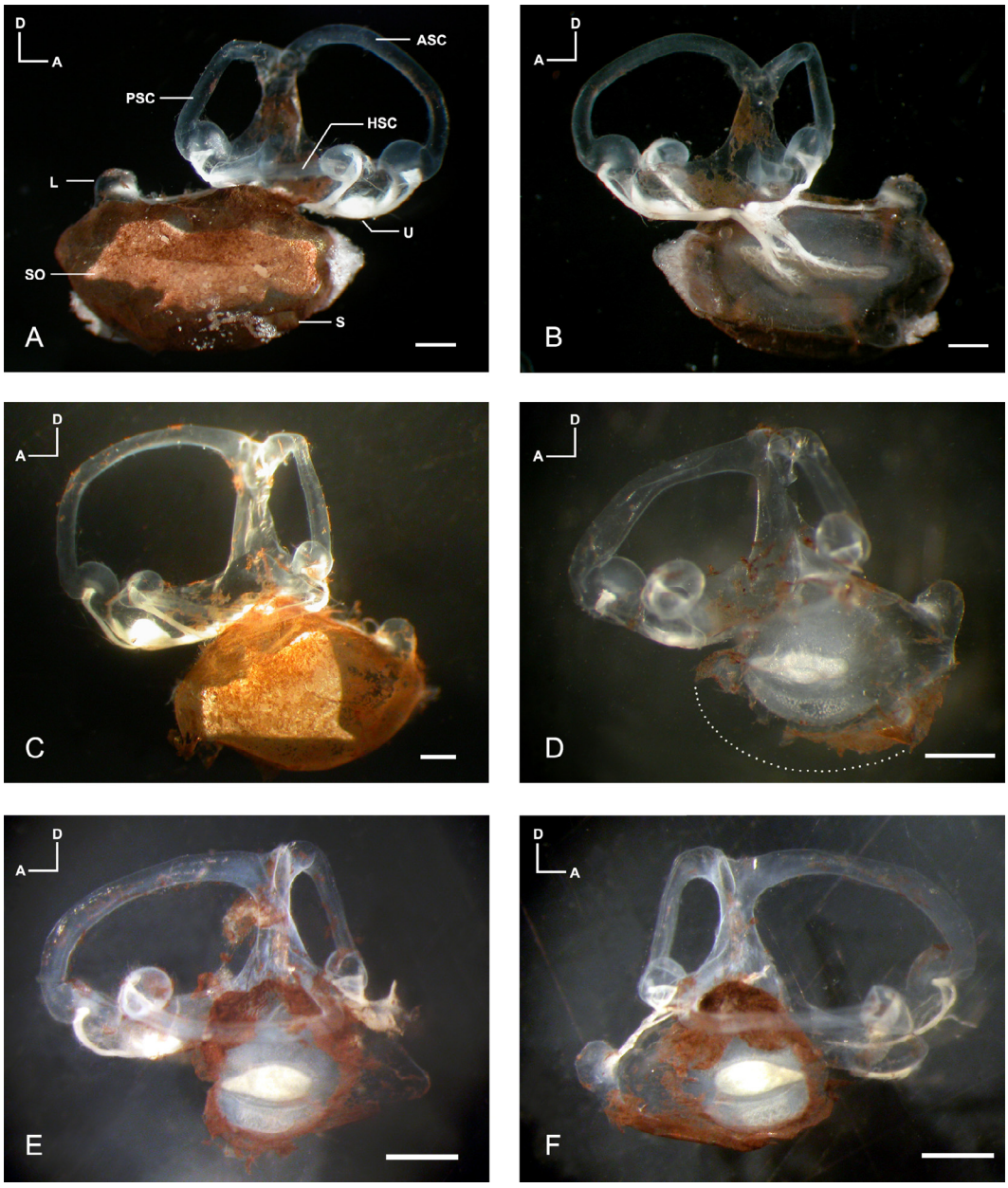



**Figure 3.3.** Ears of melamphaid fishes. **A:** lateral view of a right ear of *Melamphaes acanthomus*, the otolith is inside saccular pouch. **B:** lateral view of a right ear of *M. acanthomus* showing the VIII cranial nerve innervation to all of the end organs. **C:** lateral view of a left ear of *Poromitra crassiceps*. **D:** lateral view of a left ear of *P. oscitans*, the saccular otolith was removed to show the macula. The dotted line shows the outline of the saccular pouch that was broken when removing the otolith. **E, F:** lateral view of a left (**E**) and a right (**F**) ear of *Scopelogadus mizolepis bispinosus* with the saccular otolith removed. ASC, HSC, PSC, anterior, horizontal and posterior semicircular canal, respectively; L, lagena; S, Saccule, U, utricle. Scale bar = 1mm.

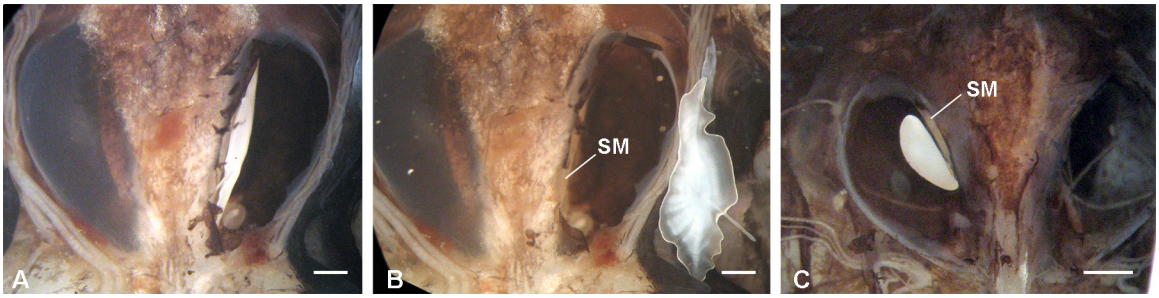



**Figure 3.4.** Ventral view of the bony capsule in *Melamphaes acanthomus* (**A, B**) and *Scopelogadus mizolepis bispinosus* (**C**). **A:** The position of saccular otolith while still attached to the macula. The otolithic stalk can be seen pointing to the ventroposterior direction. **B:** The saccular otolith was removed from the macula. The otolithic stalk is 0.1 mm in diameter. **C:** The button shaped saccular otolith of *S. m. bispinosus* is still attached to the macula. Scale bar = 1 mm.

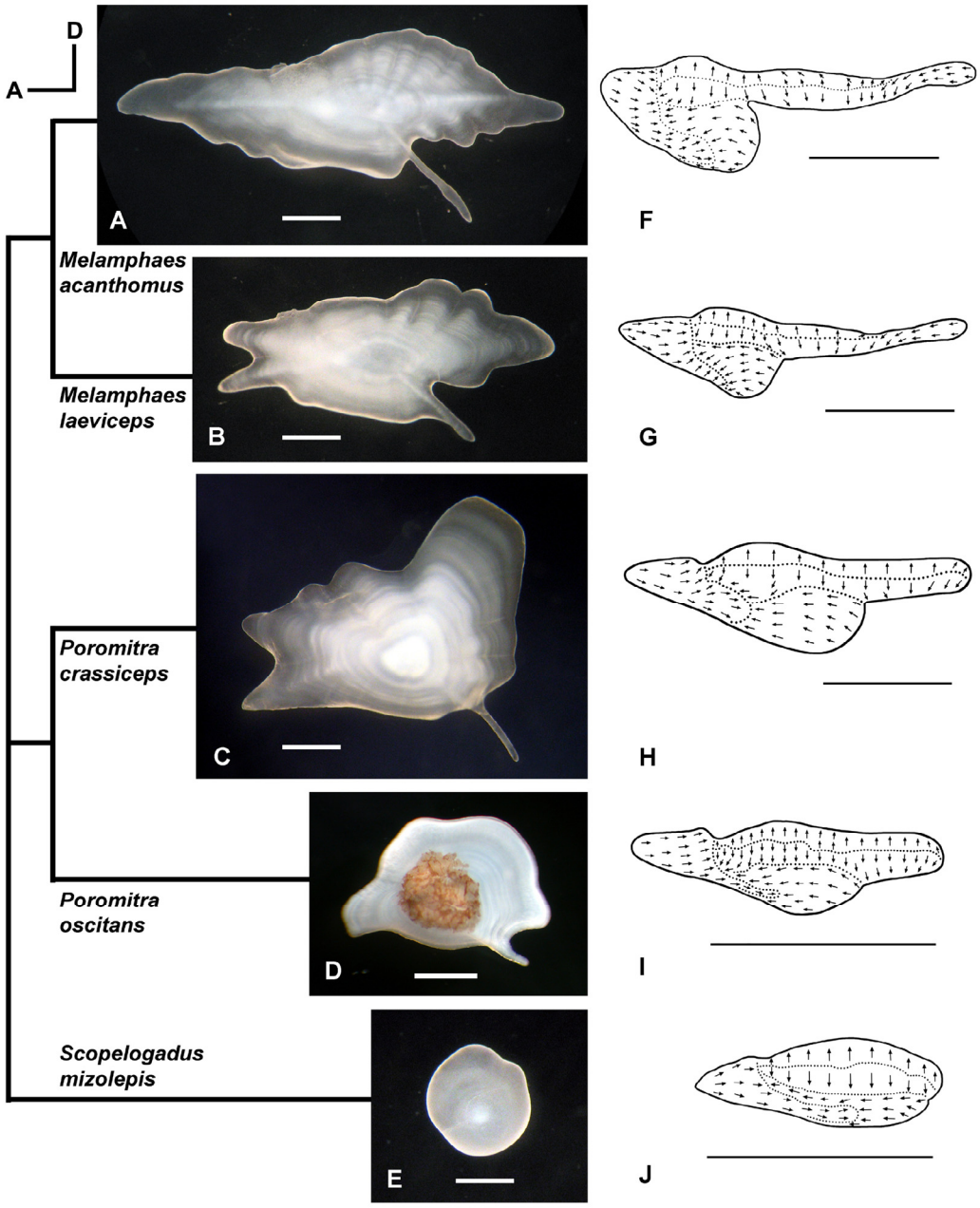



**Figure 3.5.** Saccular otoliths and maculae of melamphaid fishes. **A**, **B** and **E** are media view of right otoliths, **C** and **D** are lateral view of left otoliths. The long otolith stalk is conspicuous in the saccular otolith of *Melamphaes acanthomus* (**A**) and *Poromitra crassiceps* (**C**). Shorter spurs can be found in *Melamphaes laeviceps* (**B**) and *Poromitra oscitans* (**D**). In *Scopelogadus mizolepis bispinosus* (**E**), the otolith is round and smooth. **F-J:** Hair cell bundle orientation patterns on the saccular maculae of each species. In *Melamphaes* (**F, G**), the caudal "tails" of the macula is long and elaborated; *Poromitra* (**H, I**) lack the horizontally oriented end; whereas there is no tail in *Scopelogadus* (**J**). Scale bar = 1 mm.

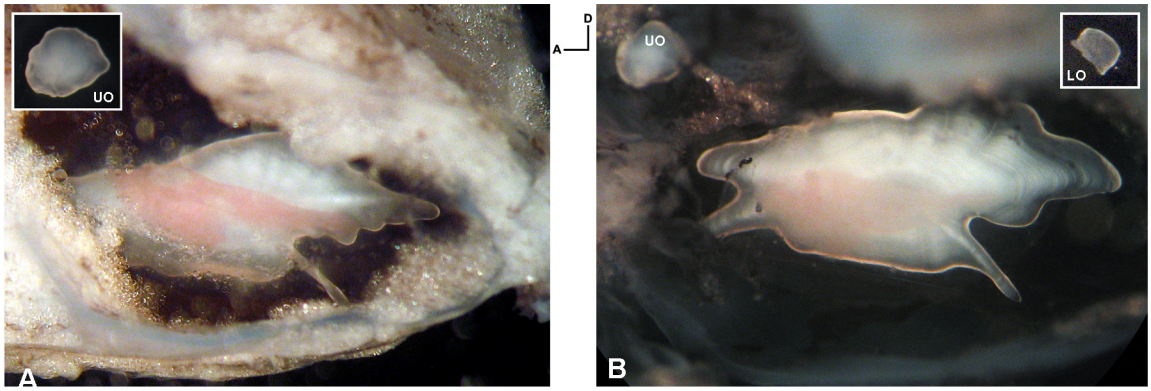



**Figure 3.6.** Medial view of the saccular otolith and otolithic membrane in two *Melamphaes*. **A:** An imprint of the saccular macula is shown by the natural pinkish color of the otolith membrane on the otolith of *Melamphaes acanthomus*. The stalk on the saccular otolith touches the bony labyrinth, which is cartilaginous and indicated by the translucent blue color. The sensory epithelium has no contact with the stalk. Insert is the utricular otolith of the same ear. **B:** The imprint of saccular macula on the otolith of *M. laeviceps*. The shorter spur on the otolith does not touch the bony labyrinth. The utricle otolith (UO) is in its original position. Insert is the lagenar otolith (LO) from the same ear. Scale bar = 1 mm.

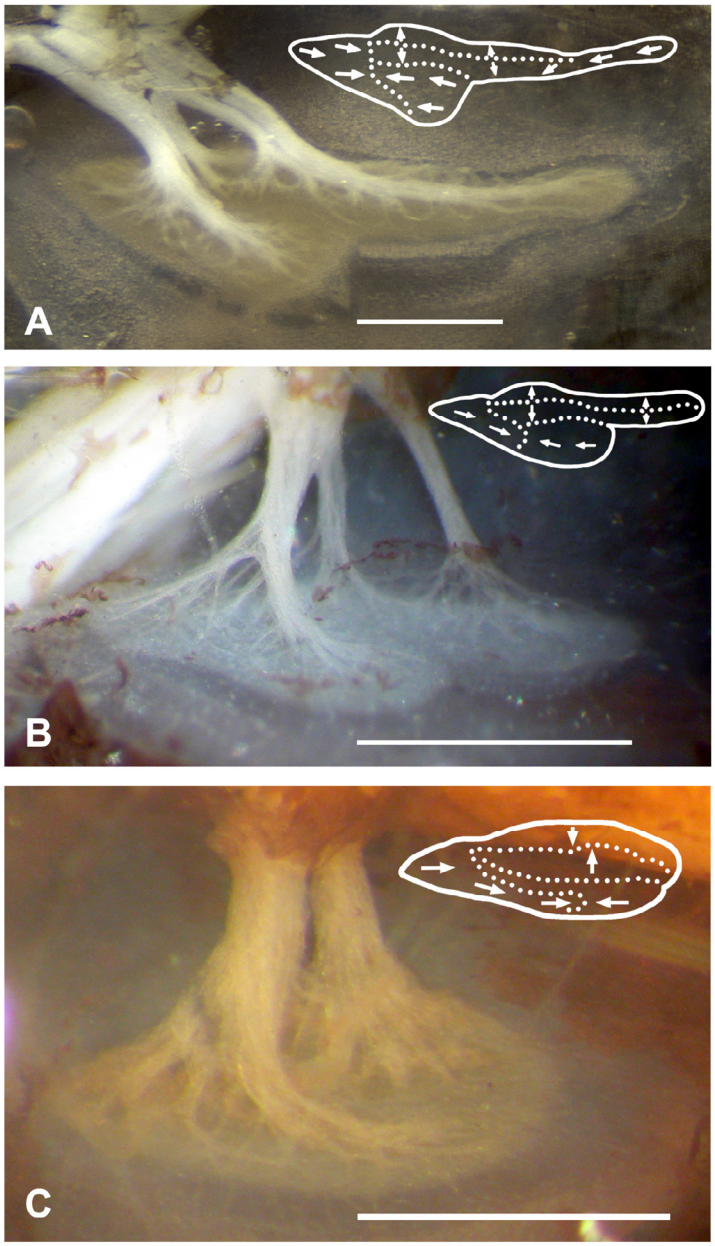



**Figure 3.7.** Innervation of the saccular maculae of melamphaid fishes. Different hair bundle groups are innervated by separate nerve branches. In *Melamphaes laeviceps* (**A**), four nerve bunches can be assigned to the rostral vertical and rostral horizontal groups and the vertical and horizontal groups on the caudal tail. In *Poromitra crassiceps* (**B**), three distinct nerve branches innervate different orientation groups. In *Scopelogadus mizolepis bispinosus* (**C**), the macula has no caudal tail and lacks the third nerve branch. A simplified bundle orientation pattern is shown for each epithelium. Scale bar = 1 mm.

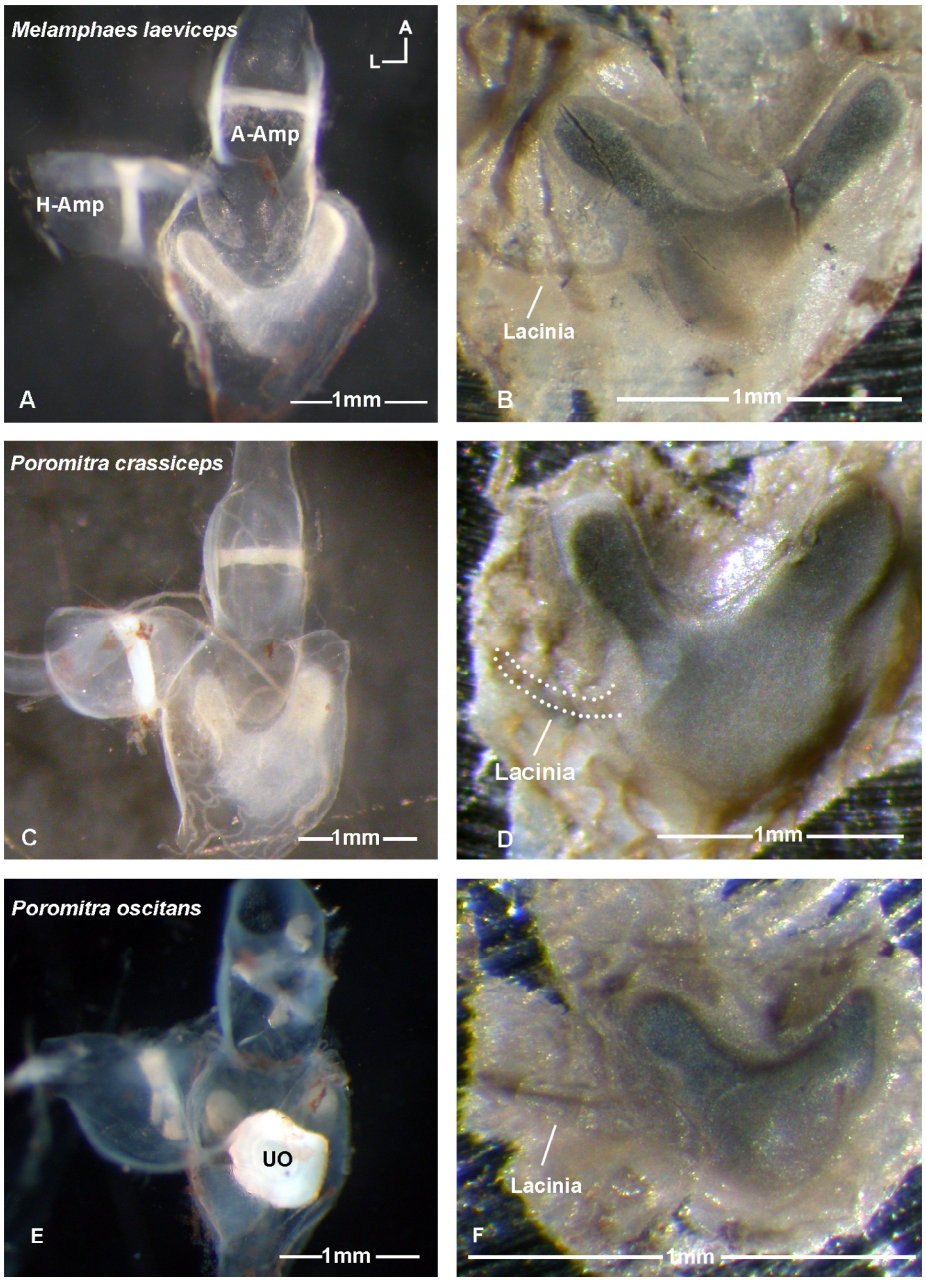



**Figure 3.8.** Left utricles of three melamphaid species. Images on the right are utricles with the ampulla from anterior and horizontal semicircular canals. **A** and **C** have the otolith removed; **E** demonstrates that the otolith does not cover the ear-shaped region of the macula. Images on the left are flattened maculae after osmium staining and show the entire surface of the macula. The ear-shaped region and the lacinia are prominent on the macula.

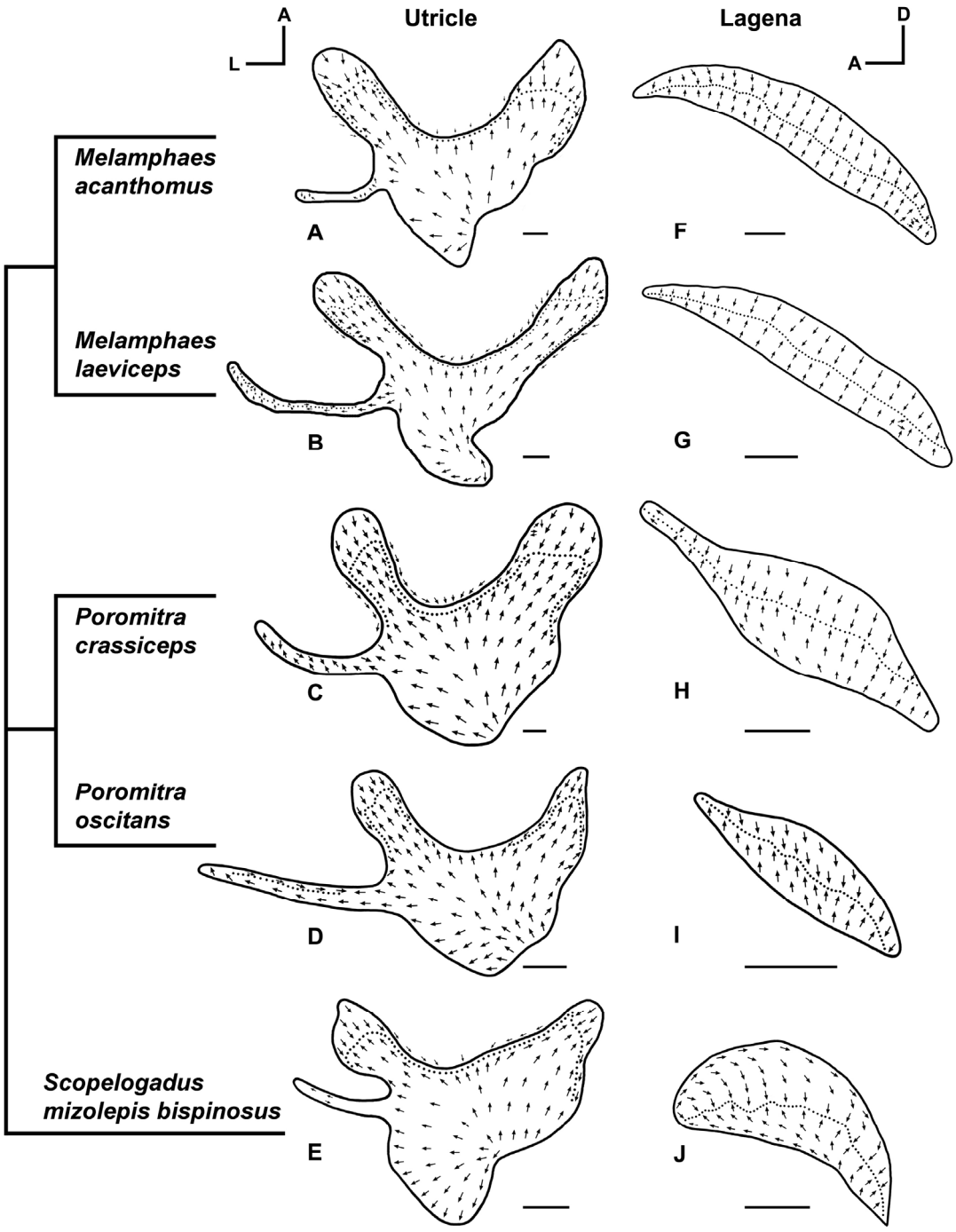



**Figure 3.9.** Hair bundle orientation patterns in utricles and lagenae of melamphaid fishes. In the utricle, the bundle orientation patterns are very similar in all species. In the lagena, the orientation patterns are consistent within genera, but vary slightly among different genera. Scale bar = 100 μm. (All other information is on the figure itself.)

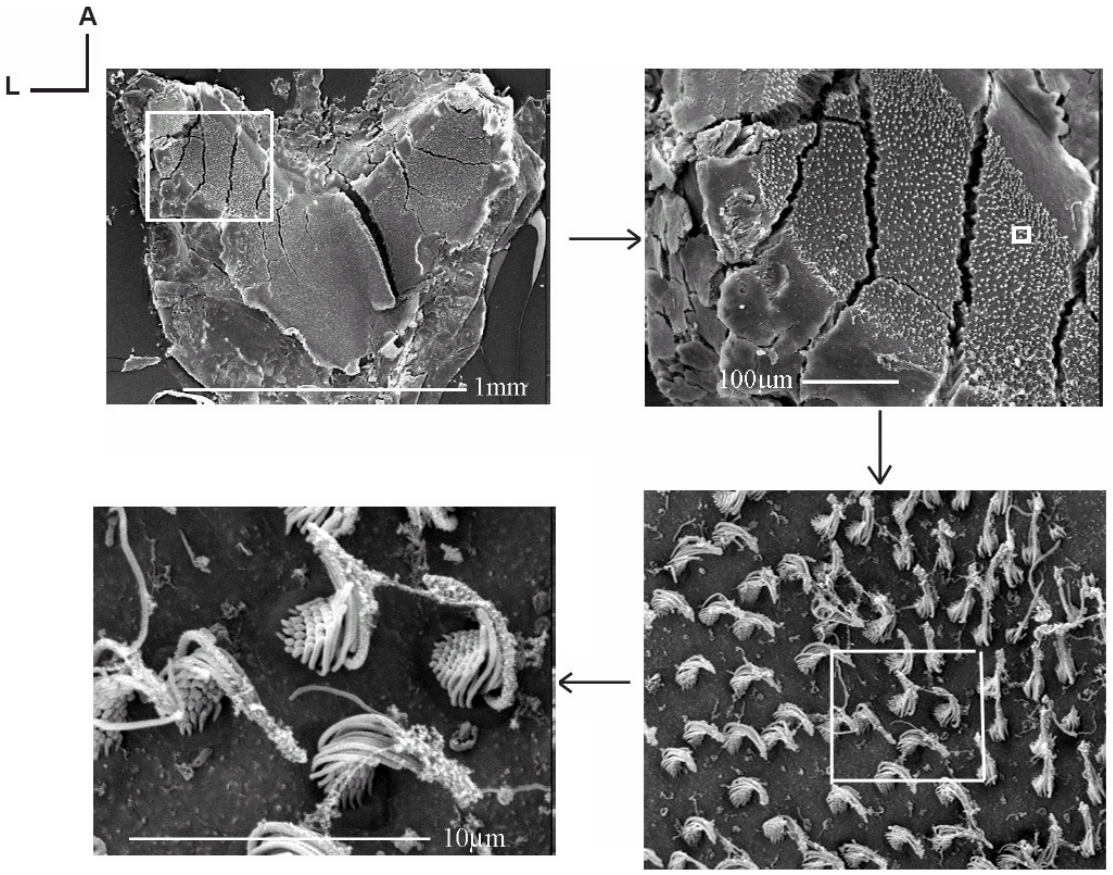



**Figure 3.10.** SEM photo of a left utricular macula in *Melamphaids acanthomus*. Serial enlargements show details along an orientation dividing line on the macula and hair bundles that are orientated to opposite directions.

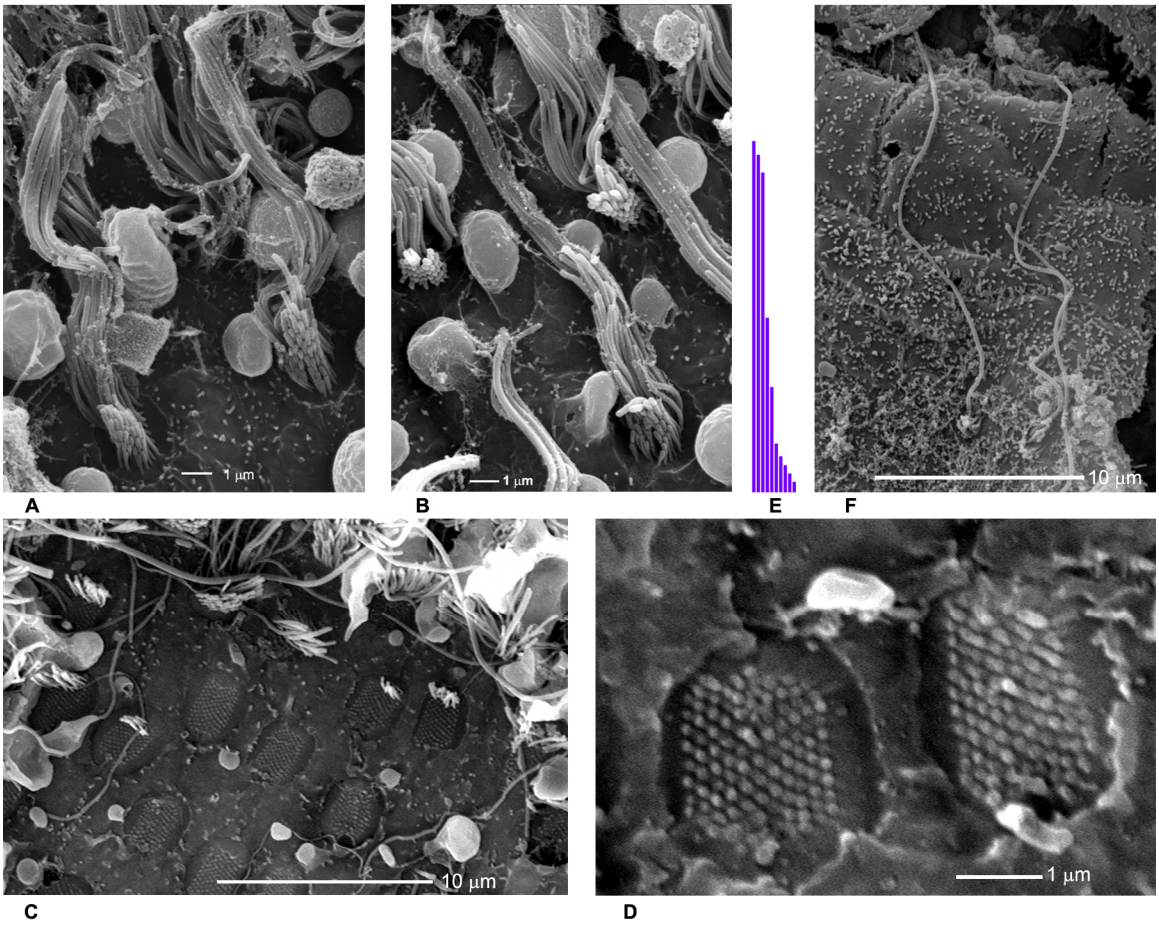



**Figure 3.11.** Exceptionally long hair bundles are found on the saccular maculae of *Melamphaes* and *Poromitra*. **A-D:** Hair bundles as long as 15-20 µm comprise more than 80% of all bundles on the saccule. These bundles have a bunch of stereocilia that are as long as the kinocilium (**A, B**) and a large rectangular or hexangular stereocilia base, with average stereocilia counts of 98.2 ± 10.2 (**C, D**). **E:** A schematic drawing of this long bundle with color purple as its mapping code. **F:** Bundles with a very long kinocilium and a small stereocilia stub are found on the edge of the macula.

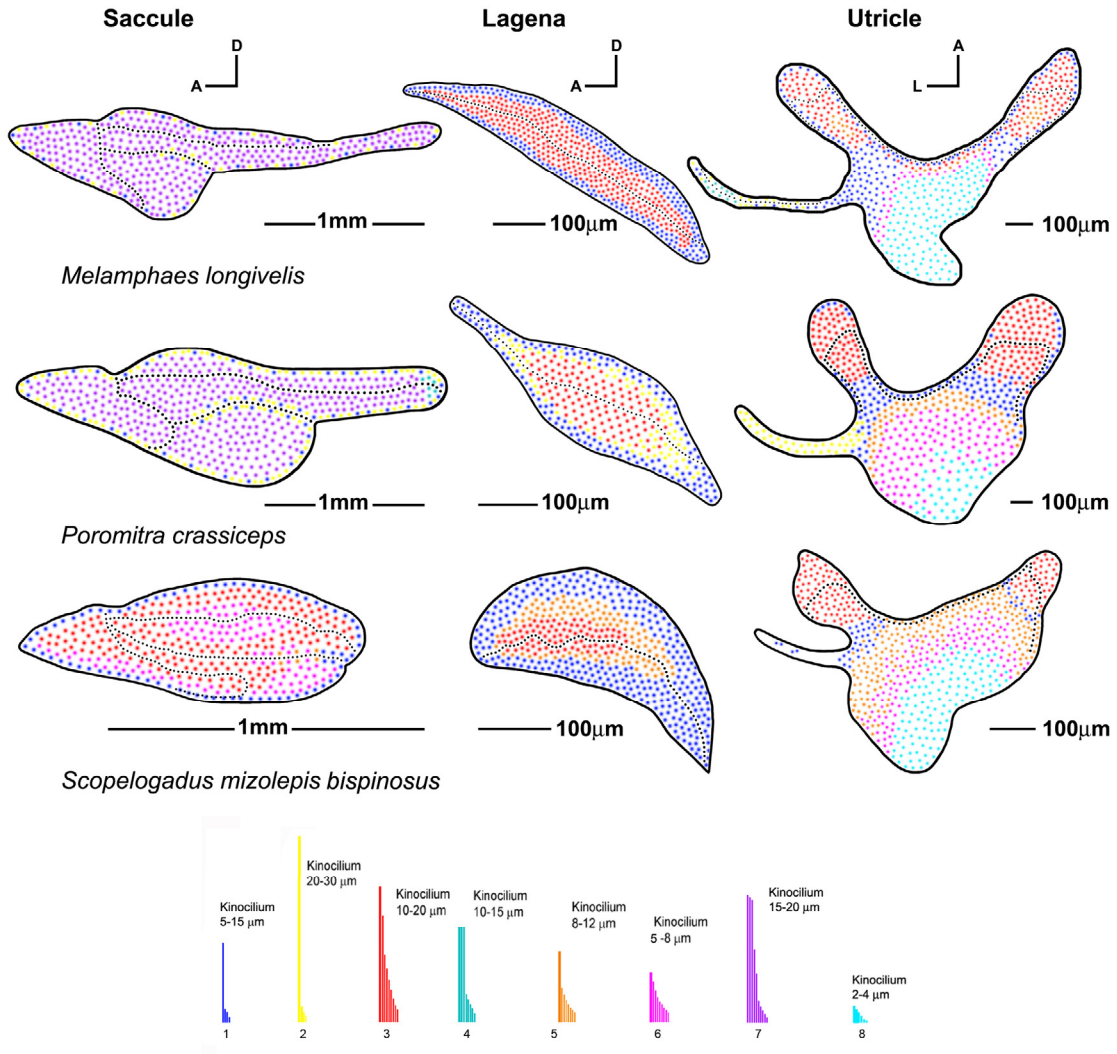



**Figure 3.12.** Color coded map of hair bundle types on the saccule, lagena and utricle in melamphaids. The bundle type distributions are consistent within genus in *Melamphaes* and *Poromitra*. **Saccule:** in *Melamphaes* and *Poromitra*, the big bundle with 15-20 µm long kinocilium and stereocilia, as described in Figure 3.11, dominant the whole macula. *Scopelogadus* has different types of bundles. **Lagena:** bundle type distributions do not vary much among the three genera. **Utricle:** distribution map are similar among genera.

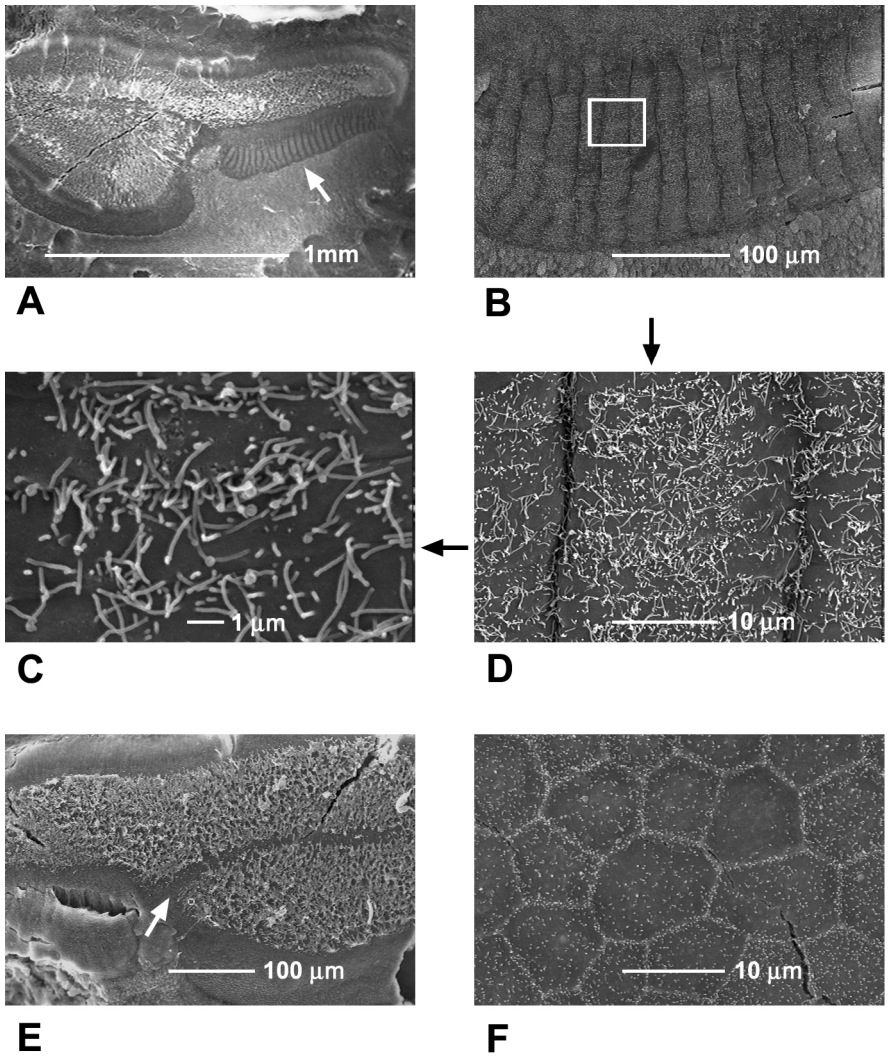



**Figure. 3.13.** Supporting cells on the macula. **A-D:** A patch of supporting cells along the ventral region of the saccular tails appears in all investigated species of *Melamphaes* and *Poromitra*. They are microvilli-rich and are organized in a pattern. **E:** A clear boundary comprise mostly by supporting cells separates the vertical and horizontal bundle groups. **F:** Microvilli-lined supporting cells along the lacinia of the utricle.

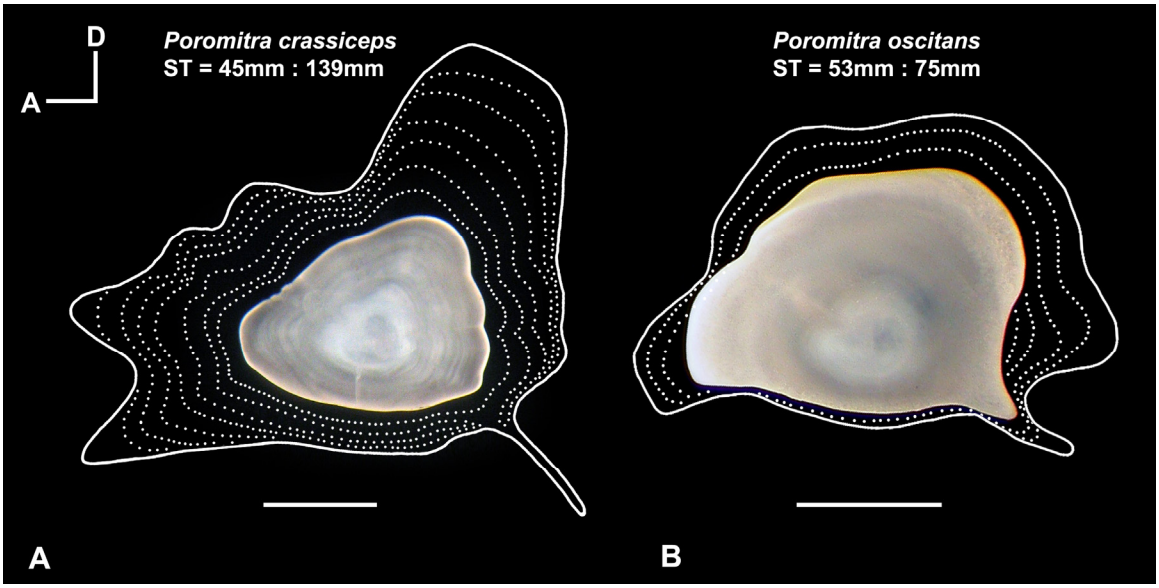



**Figure 3.14.** The growth of saccular otolith in the genus *Poromitra*. The saccular otolith from a fish of smaller standard length (ST) is shown inside the outline of a larger otolith, with dotted lines indicating the growth rings on the larger otolith.

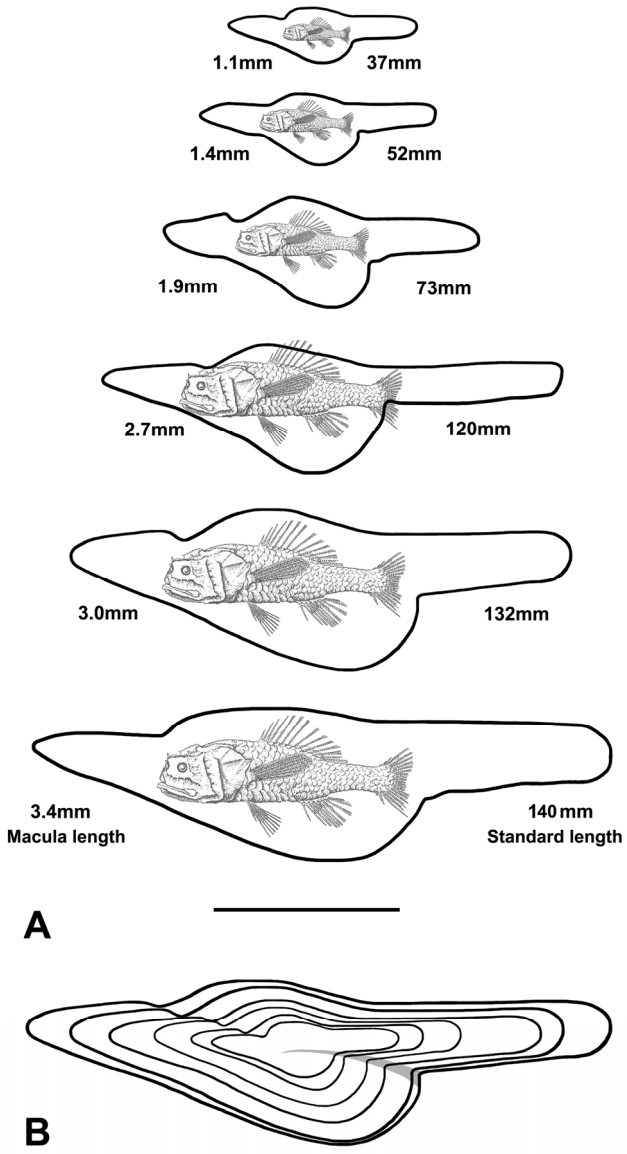
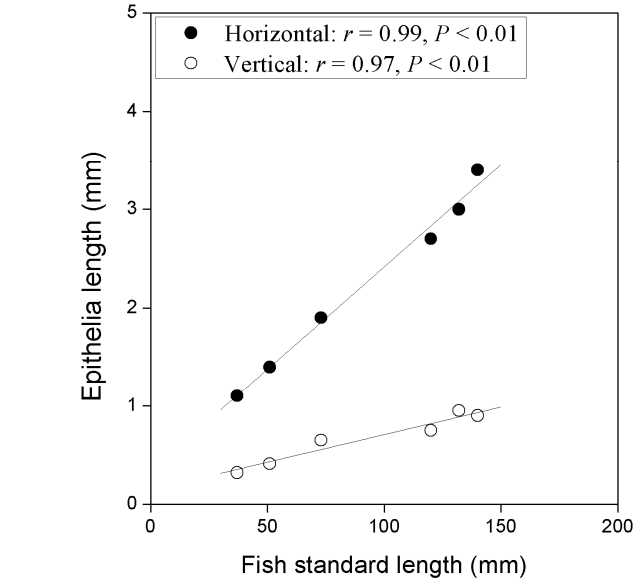
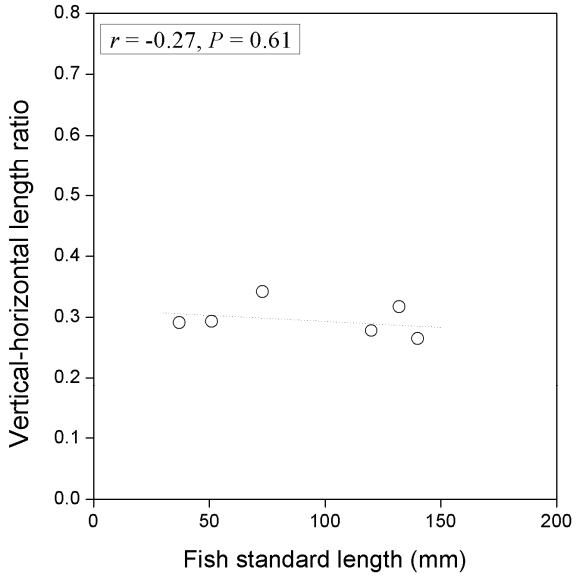



**Figure 3.15.** The growth of saccular macula in *Poromitra crassiceps*. **A:** Outline of the macula in six different specimens. Number on the right is the horizontal length of macula; number on the left is the standard length of each fish. Scale bar equates 1mm for macula and 100 mm for fish. **B:** Schematic drawing of the growth of saccular maculae. The dimension of macula expands more horizontally than vertically with the growth of the fish. The shaded area represents a portion of the bundle orientation shift line on which hair bundles are much less dense than in the surrounding area. This line may be the growing edge of the expanding macula.




A




B

**Figure 3.16.** The length of saccular macula vs. standard length of fish in *Poromitra crassiceps*. The growth of saccular macula is in a linear relationship with the growth of fish. The ratio of vertical length vs. horizontal length does not show statistic differences with fish's growth.

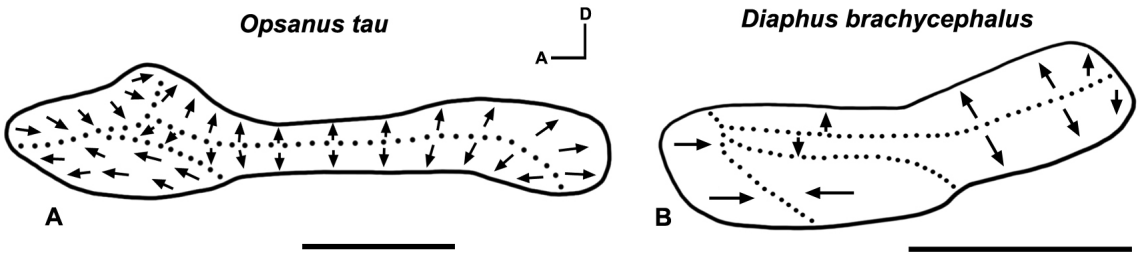



**Figure 3.17.** Saccular hair bundle orientation patterns similar with melamphaids. **A:** *Diaphus brachycephalus* (short-headed lantern fish), redrawn from Popper, 1977. **B:** *Opsanus tau* (oyster toadfish), integrated drawing of six maculae from Edds-Walton and Popper, 1995. Both species are from a different super order than melamphaids.

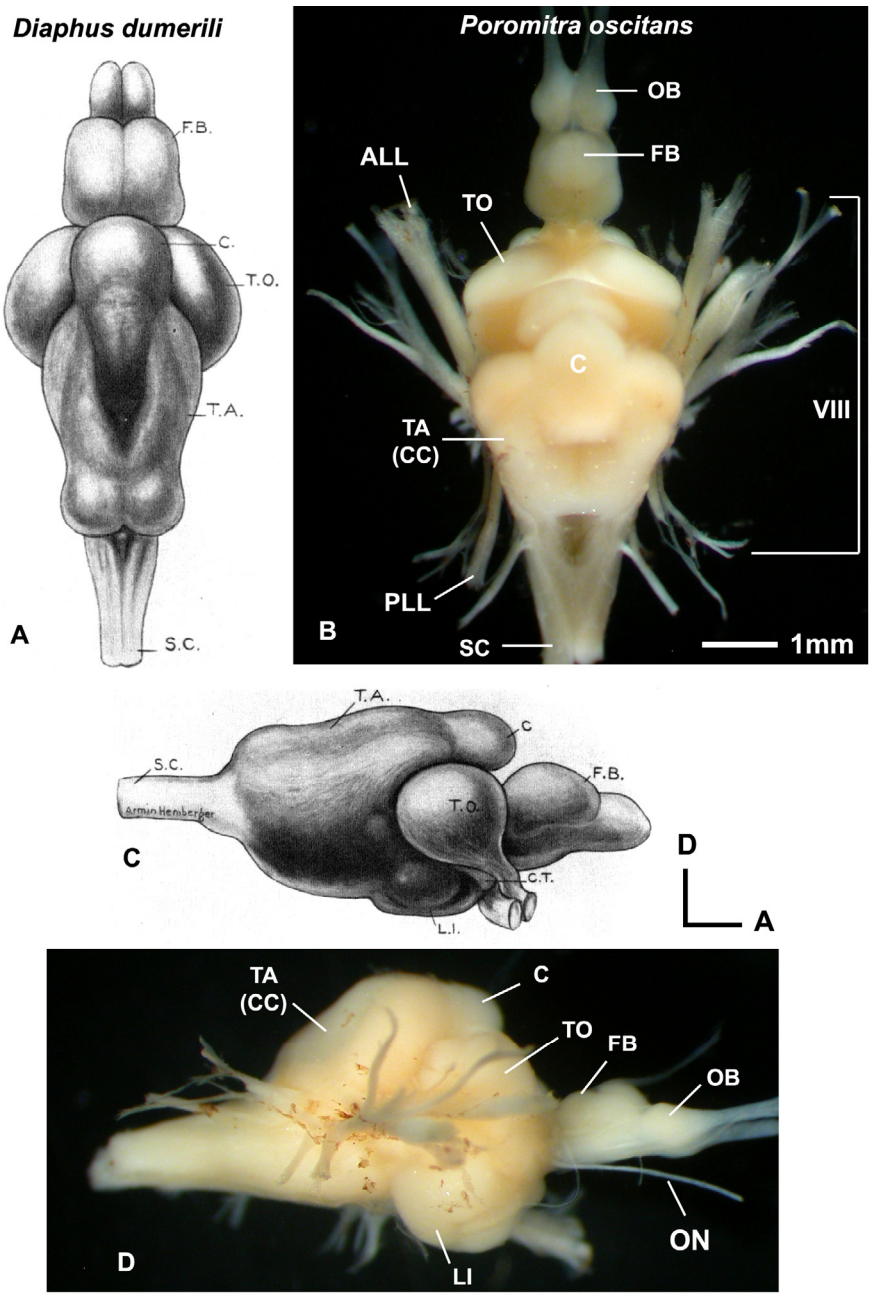



**Figure 3.18.** Comparison of brains from a lanternfish *Diaphus dumerili* (Shanklin, 1934) and *Poromitra oscitans*. **A, B:** Dorsal view of the brain, anterior is to the top. **C, D:** Lateral view of the brains. The brains from this two distant species look very similar except that the optic tectum and optic nerve in *P. oscitans* are much smaller than those in *D. dumerili*. ALL, anterior lateral line nerve; C, cerebellum; CT, commissura transversa; FB, forebrain; LI, Lobi inferiors; OB, olfactory bulb; SC, spinal cord; TA, tubuculum acousticum (CC, crista cerebellaris); TO, tectum opticum.

# Chapter 4: Comparison of the Saccules and Lagenae in Six Macrourid Fishes from Different Deep-Sea Habitats


## ABSTRACT

Ears were compared between six species of Macrouridae (grenadiers and rattails) that live at different depths. The Differences found in the inner ear anatomy of these species reflect the sensory advantages of different habitats that are related to the benefits and constraints at different depth; and it also reflect the fish's particular life style and the trade-off among different sensory systems. From this study, the most obvious trade-off among sensory systems is found between vision and hearing.


## INTRODUCTION

Macrourid fishes are one of the most dominant slope dwellers in the deep-sea. They are distributed world-wide in large numbers and high biomass. Macrourids are adapted to a variety of habitats in the deep ocean (Marshall and Iwamoto, 1973).

The ears of macrourid fishes are of particular interest since, as slope dwellers and potential sound producers (Marshall, 1980), they may have developed specializations in their hearing organs. Many macrourid species are known to have drumming muscles on their swim bladder and have very large saccules and saccular otoliths (Marshall, 1971). The ears of macrourid fishes have not been explored in detail except for the drawing of an ear of *Hymenocephalus* by Bierbaum (1914), which showed a very large saccule. The saccular otoliths of the genus *Coelorinchus* have been studied in great detail regarding species identification (Lombarte and Moralesnin, 1995).

Depth-related changes in saccular otolith have also been investigated in species in the genera *Nezumia* and *Coryphaenoides* (Wilson, 1985).

The macrourid fishes are popular subjects in deep-sea biological and ecological investigations due to their world-wide distribution and abundance. Many studies have been conducted on their distribution, feeding habits (Drazen et al., 2001)), diets (Carrasson and Matallanas, 2002), etc. *In situ* studies using bait cameras have extensively investigated the macrourid fishes' locomotion, metabolism, development, and feeding behavior (Bailey et al., 2002; Collins et al., 2005; Jamieson et al., 2006; Collins et al., 1999; Priede and Merrett, 1996). Comparison studies on their sensory brain morphology have been done in many species from the genus Coryphaenoides (Wagner, 2001, 2002, 2003). These studies provide a rich collection of data on many aspects of macrourid fishes and are particularly useful to understand their life history.

This study used six species of macrourids that represent different depth and habitats. Most of the species are from the benthopelagic fauna; one species is from the mesopelagic fauna. The gross morphology and ultrastructure of the inner ear were investigated, with a goal of looking for potential variations that may be associated with different depths or habitats. The findings reveal the trade-off between this species' hearing organ and the other sensory systems, some of which may be related to constraints in some particular habitats, and some of which may be related to inherent differences in the life styles of different species. Specializations in the inner ear are evident in one of the species with potential sound producing abilities.

## MATERIAL AND METHODS

Six species from the family Macrouridae were used in this study. Five of the species were collected using semi-balloon-otter-trawls from the Porcupine Seabight in the northeast Atlantic Ocean during Discovery cruises D252 in April 2001, D255 in August 2001, and D260 in March 2002. One mesopelagic species was collected along the Eastern Pacific coast of Central America on a deep-sea research cruise SO 173-2 aboard the FS Sonne during August 8 - September 2, 2003. A list of the species used in this study is shown in Table 4.1.

The sampling procedure is the same as described in Chapter 2 and 3. Gross morphology and SEM studies were done on fixed samples obtained during the cruises. During Discovery cruise D260, many fresh fish specimens were dissected on board the ship to investigate the morphology of fresh otolith and confirmed that the dentate features on the lagena otoliths described below were not a result of fixation artifact. Some fish specimens were also dissected to observe the structure of internal organs, especially the swim bladder. These specimens were not reflected in the numbers presented in Table 4.1.

The size of macrourid fishes were usually recorded as head length or pre-anal total length. This is because their long and fragile tail (from which the family gets its common name, “rattail,” is often lost during collecting. Head lengths, which measured from the tip of the snout to the end of the most posterior point of the opercula, were used in this study to describe the size of specimens since they are more reliable and reflect the relationship between sensory organs better.

The protocol for SEM analysis is the same as described in Chapter 2. For comparing the size of saccular and lagenar maculae, areas of the maculae were calculated by using the outlines of maculae traced from SEM photos. The number of pixels inside each macula outline were obtained from the Histogram in Photoshop 7.0 and then divided by the number of pixels inside a square made by the macula's scale bar. The results are the actual area of each macula in $mm^2$. The calculated area reflects the relative size of each macula after dehydration and critical-point drying, which usually causes 20-30% shrinkage in length from fresh samples.

## RESULTS

### Gross Morphology

The macrourid fishes in this study all have very large ears relative to the size of their brain. Figure 4.1 shows the brain and ears from two species from two different habitats. *Coryphaenoides armatus* is a scavenger that lives close to the abyssal bottom as adults. It has a large forebrain and a relatively small optic tectum (Figs. 4.1A, C). The relative size of the saccular otolith is much larger than the otolith from *Nezumia parini* (Figs. 4.1B, D), which is a mesopelagic species that live in mid-water and which has very large eyes. The relative size of the optic tectum in *Nezumia* is larger than that of *C. armatus* (Figs. 4.1A, B).

Figure 4.2 presents whole ear photos of four species taken under a stereoscope. The dimensions of the saccular pouch are as large as the upper labyrinth in all species except for *Nezumia parini* (Fig. 4.2A). This species has a smaller lower labyrinth (*pars inferior*) than upper (*pars superior*). The saccular macula is positioned on the vertical

plan and its length is parallel to the horizon (Figs. 4.2 B, D, F). The lagenar macula is also on the vertical plan, but its longitude length rises anteriorly with a small angle from the horizon (Figs. 4.2C-F).

The saccular otoliths are large in this family (Fig. 4.3). When normalized by head size, *Nezumia aequalis*, the only one in the six studied species with drumming muscle on its swim bladder, has the largest saccular otolith (Fig. 4.3A). *Nezumia parini*, the only mesopelagic fish studied in this family, has the smallest otolith (Fig. 4.3B).

**Sensory Epithelium of Saccule**

The sensory epithelium of the saccule has a very consistent shape among the six species (Fig. 4.4). The saccular maculae are all shaped like two wings connected by a narrow band. The orientation of hair cell ciliary bundle resembles the "dual" pattern (Fig.1.6 in Introduction) summarized by Popper and Coombs (1982). The bundles have a bi-directional vertical pattern in the middle and a gradual shift to bi-directional horizontal patterns at both ends (Fig. 4.4). More interestingly, the dividing line that separates the opposite orientated bundles has a very similar path on all the maculae. The starting and ending points, and the winding pattern, are all comparable among the six saccules; the locations where the bundles' axes shift between horizontal and vertical directions are also consistent on the maculae.

The compositions of hair bundle types are similar among the six species with a slight variation on the overall length of the bundles between difference species. The majority of bundles on the saccular maculae are category 5 or 6 (Chapter 2) bundles. Figure 4.5 shows some examples from *C. mediterraneus*. Bundles in the middle of

macula are mostly category 6 (Fig. 4.5B), while bundles at the periphery area are mostly category 5 (Fig. 4.5E). Bundles at the edge are slightly longer, and some bundles at the dorsal edge have longer kinocilia.

**Structure of Lagena**

The lagenae in the macrourid family are beautiful in many ways. The shape of the lagena is like an upside-down flask connecting to the saccule via its narrow bottle neck (Figs. 4.6A, C). This is a typical appearance of this end organ, and the name of the organ is from the Latin word lagena, meaning "flask." The lagenar otoliths in some species have ornate dentations along the edges (Figs. 4.6A-F). The transparent edges and lines on the otolith are vaterite, one of the crystal structures of calcium carbonate, as opposed to the non-transparent aragonite crystal structure in most otoliths.

The lagenar otolith does not cover the whole surface of the macula and there is considerable variation in the amount of coverage among different species. Figure 4.7 presents the outline of lagenar maculae and the overlaying otoliths on top in three species. The coverage varies from 20% in *C. guentheri*, to 60% in *C. rupestris*, and 80% in *N. aequalis*. The uncovered area is usually the anterior narrow tip of the macula, except in *C. guentheri*, which has part of the anterior and the majority of the middle to posterior surface of the macula exposed from the otolith (Fig, 4.7A).

The shape of lagenar maculae and their hair bundle orientation patterns are similar among the six species except for *C. guentheri* (Fig. 4.8). Most species have a group of anterior-dorsally oriented bundles at the anterior tip of the maculae. The rest of the bundles gradually shift to two vertical bi-directional groups on the bulk of the

maculae. The bundles are either perpendicular to the dividing line (*C. mediterraneus* and *C. armatus*, Fig 4.8C, D), or at an angle with the dividing line, but are perpendicular to the horizon (*C. guentheri* and *C. rupestris*, Figs. 4.8A, B). The lagena macula of *C. guentheri* is shaped very differently from the other species, and it has different bundle orientations groups at the anterior and posterior areas of the macula.

In contrast to the saccules in this family, hair cell ciliary bundles on the lagenae of macrourid fishes have extraordinary diversity in bundle shapes and heights (Fig 4.9). Six categories of bundles were found on the maculae of the six species studied here. Five of the categories were described in Table 2.1 of Chapter 2. One new type of bundles was added for this family. These bundles are 15-20 μm long and have several stereocilia that are as long as the kinocilium, these bundles lack the short staircase of stereocilia as compared with the category 4 (Chapter 2) and 7 (Chapter 3) bundles.

Figure 4.9 shows the various types of bundles and their actual locations on serial enlarged SEM photos of the lagena from *C. rupestris*. Same types of bundles are often aggregated in certain areas on the maculae. Category 2 bundles with very long kinocilia are found in regions of the maculae that are not covered by the otolith. A striola-like strip appears along the ventral region of the macula between the dorsally and ventrally oriented bundle groups (Fig. 4.9). Hair bundles are less dense within the strip and have striola-like category 3 bundles.

Figure 4.10 presents a color coded map of the ciliary bundle types on the lagenae. There are similarities as well as differences in the distribution of hair bundle types among different species. Some of the bundle types appear on similar locations on different species, and more similarities are found within the genus. The size of the

regions in which each kind of bundle is located also varies between species. There are more bundles of longer types (2, 3, and 4) in *Nezumia* than in *Coryphaenoides* species in this study.

**Comparison of saccular and lagenar macula size associated with different habitats**

Previous sections have presented the differences in the size of saccular otolith and the differences in lagena structure among these species. While the saccular maculae of the six species have very similar shapes and hair bundle orientation patterns, the maculae vary considerably in their size relative to the head length. In order to compare the size of the maculae between species with different maximal body sizes, adult specimens from each species were chosen and the apical surface area of saccular and lagenar maculae were calculated. The area data were then normalized to the average head length (54.8 mm) of the selected specimens (head length range from 38 to 75 mm) and plotted in Figures 4.11 and 4.12.

Figure 4.11 presents the apical surface area of the saccular maculae from six adult specimens. The shaded outlines of the saccular and lagenar maculae for each species are also normalized to the head length and presented with a corresponding area bar. Each species dominant sense, as described by Hubbs and Iwamoto (1977) and Wagner (2001), are indicated inside the bar. *Nezumia aequalis* is indicated as a species with potential auditory dominance by its drumming muscle on the swim bladder. Two species live in shallower depths and have vision as their dominant sense (*N. parini* and *C. rupestris*) and these species have below average size (for all of the six species) of saccular maculae. The potential sound producing species, *N. aequalis*, has the biggest

saccular macula and it is much larger than the rest of the group. *Coryphaenoides mediterraneus* were identified with two dominant sensory areas in their brain (Wagner, 2001) including areas related to the eighth cranial nerve; this is reflected by the above-average size in its saccular macula. *Coryphaenoides armatus* has olfaction as its dominant sense and its saccule is larger than the shallower non-sound-producing species. *Coryphaenoides guentheri* does not have a dominant sense and the size of its saccular macula is median among the group.

Figure 4.12 analyzes the size of lagenar macula and its relationship to the saccular macula. Except for *C. guentheri* which has a lagenar macula that is almost as big as its saccular macula, the relative size of lagenar maculae in the other five species does not vary much (Fig 4.12A).

The area ratio of lagenar vs. saccular maculae varies considerably among all the six species (Fig 4.12B). It is the variation in the size of saccular maculae that causes the area ratio of lagena/saccule to differ so much between species. The shallower and vision dominant species have higher lagena/saccule ratios than the deeper or sound producing species. *Coryphaenoides guentheri* is an outlier with its extra large lagenar macula, and it is hard to compare its lagena/ saccule ratio to the other species.

## DISCUSSION

### The Size and Shapes of Otoliths

The size of the saccular otolith varies considerably in the six species from this family (Fig. 4.3). This difference is marked by an extra large otolith relative to fish head length in *Nezumia aequalis* and the very small otolith in a mesopelagic species *N. parini*.

It is suggested that the size of saccular otolith is positively related to the ability of sound production in sciaenids (Paxton, 2000; Cruz and Lombarte, 2004), and macrourids (Marshall, 1980). This is supported by the findings in this study.

Previous studies on the depth-related changes in saccular otoliths in macrourid fishes revealed a decrease in sagitta length vs. head length with increased depth (Wilson, 1985). It was also suggested that the carbonate saturation level as well as genetic conditions regulate the size of the otolith in some gadiform deep-sea fishes (Lombarte and Lleonart, 1993). The findings in this study, however, do not necessarily support the idea that deeper species have small otoliths relative to the head length. Instead, the observations here suggest that vision dominant, relatively shallow water species have smaller saccular otoliths than the deeper species. The conclusion from this observation is that the size of the saccular otoliths is not only controlled by the habitats, but also by the shift in the sensory modality. Thus, otolith size is not necessarily related to depth.

The ornate teeth-like structures in the lagenar otoliths are very intriguing. This kind of indentation appears more often in deep-sea fishes than in shallow water fishes. For example, the orange roughy, American angler, and Mediterranean slimehead all have indentations, concavities, or holes in their saccular otolith (Campana, 2004). The indentation structure could potentially affect the otolith movement related to ciliary bundles, but there is a total lack of experimental evidence to support this idea or any other hypothesis regarding the shape of these otoliths. On the other hand, deep-sea fishes have lower density body tissue than shallow water fishes and so it is possible that the teeth-like structure decrease the density of otoliths, and this may be a co-adaption to compensate for the loss of density in the fish body.

**The Sensory Epithelia**

The hair cell ciliary bundle orientation patterns in the macrourid saccular maculae (Fig. 4.4) are very similar to those found in gadiform fishes to date. Existing data on *Gadus morhua* (Atlantic cod) (Dale, 1976), *Bregmaceros* (Popper, 1980), *Merlccius capenss*, and *M. oaradoxus* (Lombarte and Fortuno, 1992) all show a dual pattern on their saccular maculate that is similar to that reported here for the macrourids. In the saccule of *Gadus morhua*, the height of the ciliary bundles is greater on the epithelial edge than in the middle of the macula (Dale, 1976). The saccular hair bundle heights in some of the macrourid fishes studied here do not vary as much between the edge and the middle area (Fig.4.5).

The hair bundle orientation patterns in the lagenar maculae in the macrourids studied here are relatively similar within the family except for *C. guentheri*, which has an eccentric epithelium shape and more complicated bundle orientation pattern at the rostral and caudal portions of the macula (Fig. 4.8). The bundles in the middle section of the lagenae in *C. mediterraneus* and C. *armatus* are oriented at a small angle from the vertical, which is slightly different from the vertically oriented bundles on the same area in *C. rupestris* and *C. guentheri*. This is interesting because *C. armatus* is a scavenger and *C. mediterraneus* use its gustatory sense on barbel to search for food. Both species swim with their heads pointing downwards most of the time (Bailey et al., 2007). As a consequence, the slightly angled orienting bundles on the lagenae in these species may actually have their sensitive axes aligned into a vertical direction while swimming.

The bundle types in the lagenae of macrourids are highly diverse (Figs. 4.9, 4.10). Six different types of bundles are found on the epithelia. Bundles with long kinocilia are distributed at the edge, and thicker, shorter bundles are in the middle. The largest bundles are along the dividing line between hair cell orientation groups, just as happens in the goldfish (*Carassius auratus*) where thicker, oppositely oriented bundles are found along the dividing line on the center of the macula (Platt, 1977). However, some shorter bundles in the center of the macrourid maculae are also very thick with a large number of stereocilia.

The lagena macula of *Gadus morhua* also has a striola region similar to that found in macrourids with longer stereocilia (Dale, 1976). However, the longest stereocilia in macrourids are not found in the striola region, but near the base of the anterior tip of the macula, with a special type of bundle that has not been found in the lagena or saccule in other fish species. These bundles have several very long stereocilia and without the short stereocilia staircase. These very long bundles as well as many category 2 long bundles are located in an epithelial region that is not covered by the otoliths. Similar unloaded areas are also found in the anterior portion of goldfish lagena (Platt, 1977), but the bundles are not as long as those in macrourids. These unloaded bundles may be under the influence of endolymph flow more than the otolith, and provide different response patterns to the fish than the other bundles. It is very possible the lagena in this family is a vestibular organ because the saccule is very likely the hearing organ due to its positive correlation in size with fish's sound producing structures.

**The Size of Saccular Maculae and the Trade-Off between Different Sensory Systems**

One of the most interesting findings in this study is the diversity in saccular macula size relative to fish head length (Fig. 4.11). Using earlier data on the structure of sensory regions of the brain in macrourid species (Wagner, 2001, 2002), the variation in the size of saccule and its relationship to the fish habitats and life styles are accounted for in this study.

The two shallowest species, *N. parini* and *C. rupestris*, have saccular maculae that are below-average size for this family. *Nezumia Parini* is a mesopelagic species with huge eyes that are one third to two fifths of the head length. This species also has a reduced swim bladder and lateral line (Hubbs and Iwamoto, 1977). Vision is very possibly the most important sense for this species. *Coryphaenoides rupestris* lives close to the continental slope and has only one specialized sense – vision – as described by Wagner (2001, 2002) from the comparison of the volume of different sensory areas in the brain. These two species live in very different habitats, but both have the advantage of the twilight and the bioluminescence. It may be hypothesized that the substantial use of vision may be correlated with the much below-average sizes seen in the saccular sensory epithelia.

Between the two deepest dwelling species in this study, *C. mediterraneus* is thought to have an octavolateral system (ear and lateral line) as two of its dominant sensory modalities (Wagner, 2001). This is confirmed by an above-average sized saccular macula within the macrourids. *Coryphaenoides armatus* spends its adult life in very deep water and mostly feeds on carrion. Baited camera studies confirm that that

this species can locate carrion very quickly (Armstrong et al., 1992), probably using olfaction. Thus, olfaction is a very important sense for this species. Studies on the relative size of the sensory brain area also confirm that olfaction is the most specialized sense for this fish (Wagner, 2001). Hearing is potentially useful in the deep where there is no sunlight and where bioluminescence is less abundant than the mid water. Findings in this study reveal that *C. armatus* has a reasonably large saccular macula as compared with the shallower fishes in the same family. It is possible that hearing could be a useful sense to compensate for the degradation of vision in that habitat.

*Nezumia aequalis* lives at a similar depth as *C. rupestris*, but the relative size of its saccular macula is several times larger than that of *C. rupestris*, even without head length normalization. In fact, the saccular macula on a 42 cm head length specimen is larger than that in a 75 cm specimen of *C. rupestris*. The most parsimonious explanation for this difference is the presence of the drumming muscle on the swim bladder of *N. aequalis*. As a species that probably produces sound, hearing is the most important sense and this is reflected by an over-sized saccular.

*Coryphaenoides guentheri* is identified as a generalist (fishes that do not have any dominant senses) and is average in a few senses (Wagner, 2001). The size of saccular macula is below-average for macrourids, but the size of the lagena is very much above average. The size ratio between lagenar and saccular sensory area reach reaches 0.9. This kind of size ratio has only been found in Ostariophysan fishes such as the goldfish (Popper and Platt, 1983).

Except for *C. guentheri*, all other macrourid species have similar size lagenar maculae (Fig. 12). The variation in lagenar/saccular macula area ratio is mostly caused

by the variation of saccular macula sizes. It appears that although different species have different sized saccular maculae depending on the trade-offs between sensory systems, the size of lagena macula stays relatively constant. This phenomenon suggests that lagenae in these species may not participate in audition, but may have vestibular function that does not change with the shift between other sensory modalities. *C. guentheri*, on the other hand, has a lagena that may have more complicated functions than the other lagenae in the studied species.

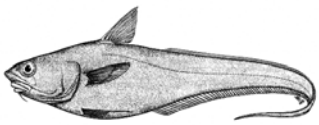
**Conclusion**

The goal of this study is to search for possible trends in inner ear structure that may be correlated with depth in macrourid species. The findings in the six species' inner ear reflect the characteristics of habitats at different depth to some extent. However, no consistent trend can be established regarding depths. Instead, the differences found in the inner ear anatomy are dictated not only by the sensory advantages of different habitats that are related to the benefits and constraints at different depth, but also by the fish's particular life style and the trade-off among different sensory systems. From this study, the most obvious trade-off among sensory systems is found between vision and hearing, and these may be the two most energy consuming senses for deep-sea fishes.

The macrourid fishes seem to be very flexible in terms of shifting among different sensory modalities. Vision, olfaction, or hearing specialized species are found to be highly adapted to different habitats. This could be one of the reasons why macrourids are such a successful family and dominate the deep ocean.

## TABLES

**Table 4.1.** Macrourid species used in this study.

| Species | | Common name | Depth Range | Max Size (Total length) | No. of ears used |
|---|---|---|---|---|---|
| *Nezumia parini* |  | Parin's grenadier | 0-1350 m | 230 mm | 4 |
| *Nezumia aequalis* |  | Common Atlantic grenadier | 200-2320 m | 360 mm | 2 |
| *Coryphaenoides rupestris* |  | Roundnose grenadier | 180-2200 m | 1100 mm | 2 |
| *Coryphaenoides guentheri* |  | Günther's grenadier | 831-2830 m | 500 mm | 3 |
| *Coryphaenoides mediterraneus* |  | Mediterranean grenadier | 1200-3000 m | 730 mm | 4 |
| *Coryphaenoides armatus* |  | Abyssal grenadier | 1830-4700 m | 1020 mm | 2 |

## FIGURES

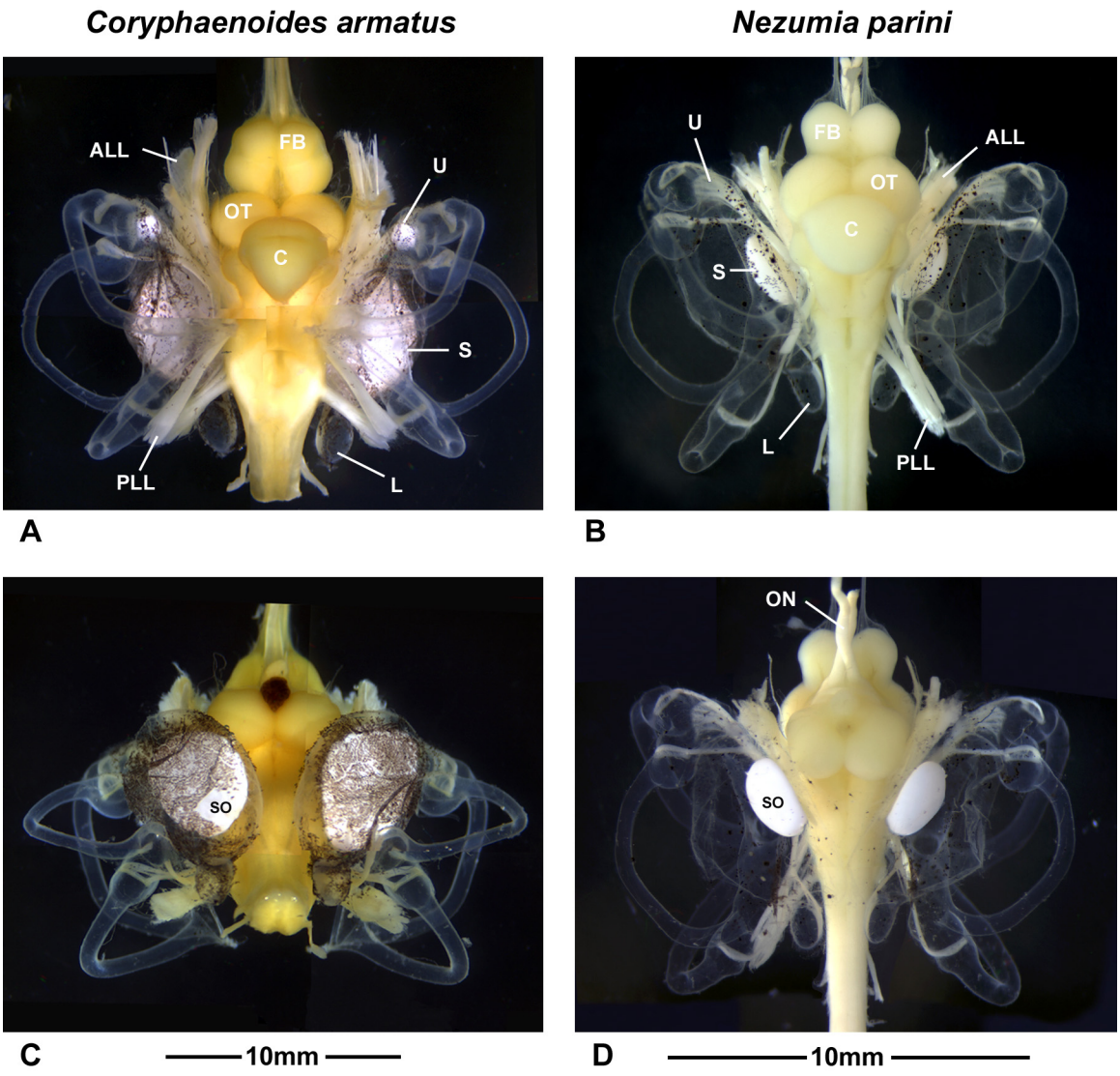



**Figure 4.1.** Brain and ears of macrourid fishes from two different habitats. **A** and **B** are dorsal views; **C** and **D** are ventral views. Anterior is to the top. *Coryphaenoides armatus* is a demersal bottom dweller which lives up to 5000 m deep. *Nezumia parini* is a mesopelagic species that lives near the twilight zone and has huge eyes. The gross morphology of the brain and ears in these two species differs in the relative size of saccular otolith, optic tectum, and forebrain. ALL and PLL, anterior and posterior lateral line nerve; C, cerebellum; FB, forebrain; L, lagena; ON, optic nerve; OT, optic tectum; S, saccule; SO, saccular otolith; U, utricle

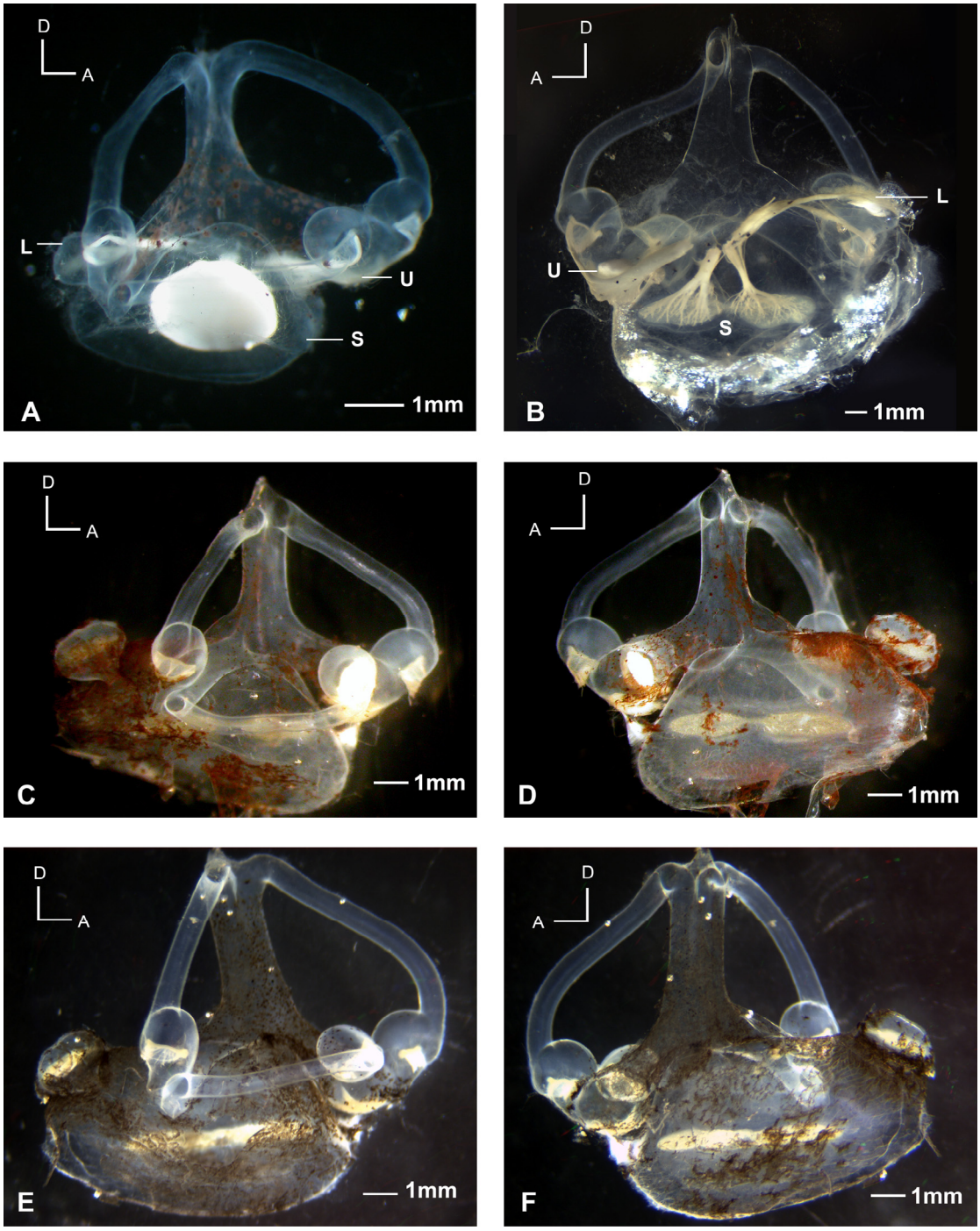



**Figure 4.2.** Ears in macrourid fishes. All are right ears. Pictures on the left are a lateral view while those on the right are a medial view. All ears have a relatively large saccular pouch and a flask shaped lagena connecting to the saccule via a narrow bottle neck. **A:** *Nezumia parini,* the otolith is still inside the saccule. **B:** *Coryphaenoides rupestris*, medial view showing the innervation to the end organs. **C** and **D:** *C. mediterraneus*, the saccular macula can be seen in the lateral view. **E** and **F:** *C. armatus*, saccular and lagenar maculae are seen in the medial view. L, lagena; S, saccule; U, utricle.

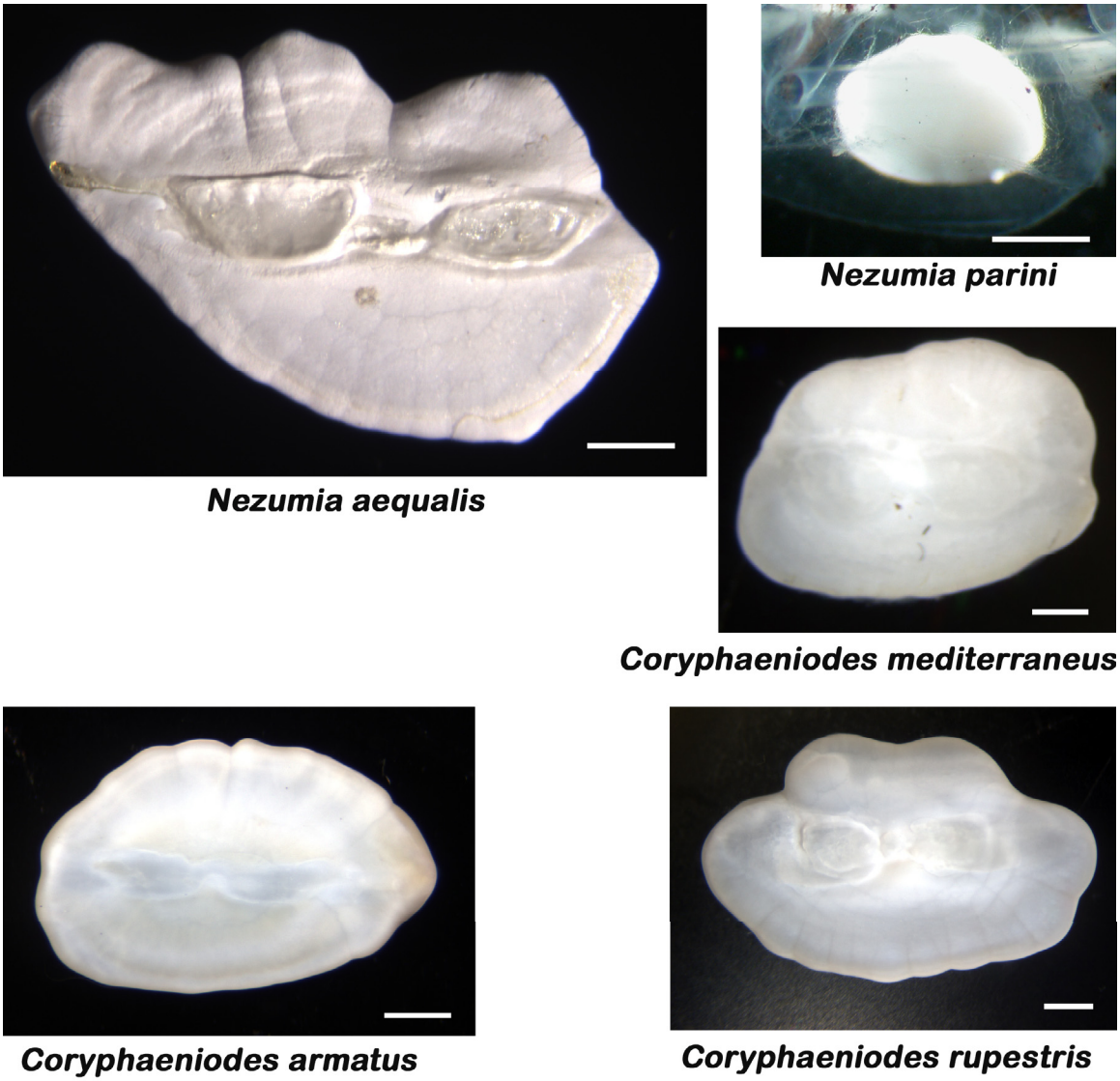



**Figure 4.3.** Saccular otoliths in macrourid fishes. All are medial view except *Nezumia parini*. Some images were flipped horizontally (using Photoshop) to have the same orientation. The sound producing *N. aequalis* has the biggest sagitta relative to its head length, and its actual size is also bigger then some of the larger species. *N. parini* has the smallest sagitta in both normalized size and actual size. Scale bars = 1 mm.

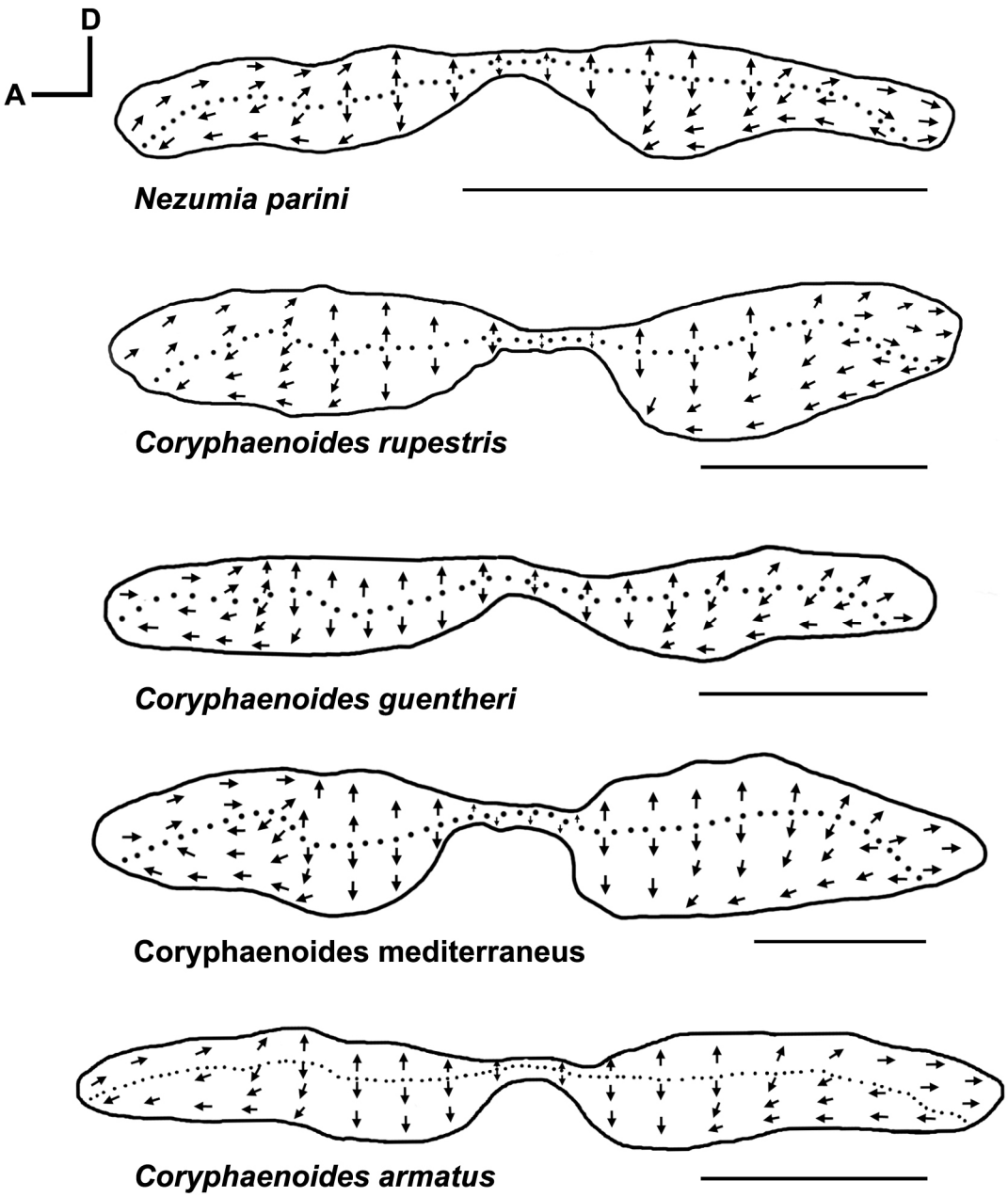



**Figure 4.4.** Hair cell ciliary bundle orientation patterns on the saccule. All saccular maculae are shaped like two wings connected by a narrow band. The orientation of the hair cell ciliary bundles are similar in all species and have a bi-directional vertical pattern in the middle and a gradual shift to a bi-directional horizontal pattern at both ends. The dividing line that separates the opposing bundles runs a very similar path on all the maculae. Scale bars = 1mm.

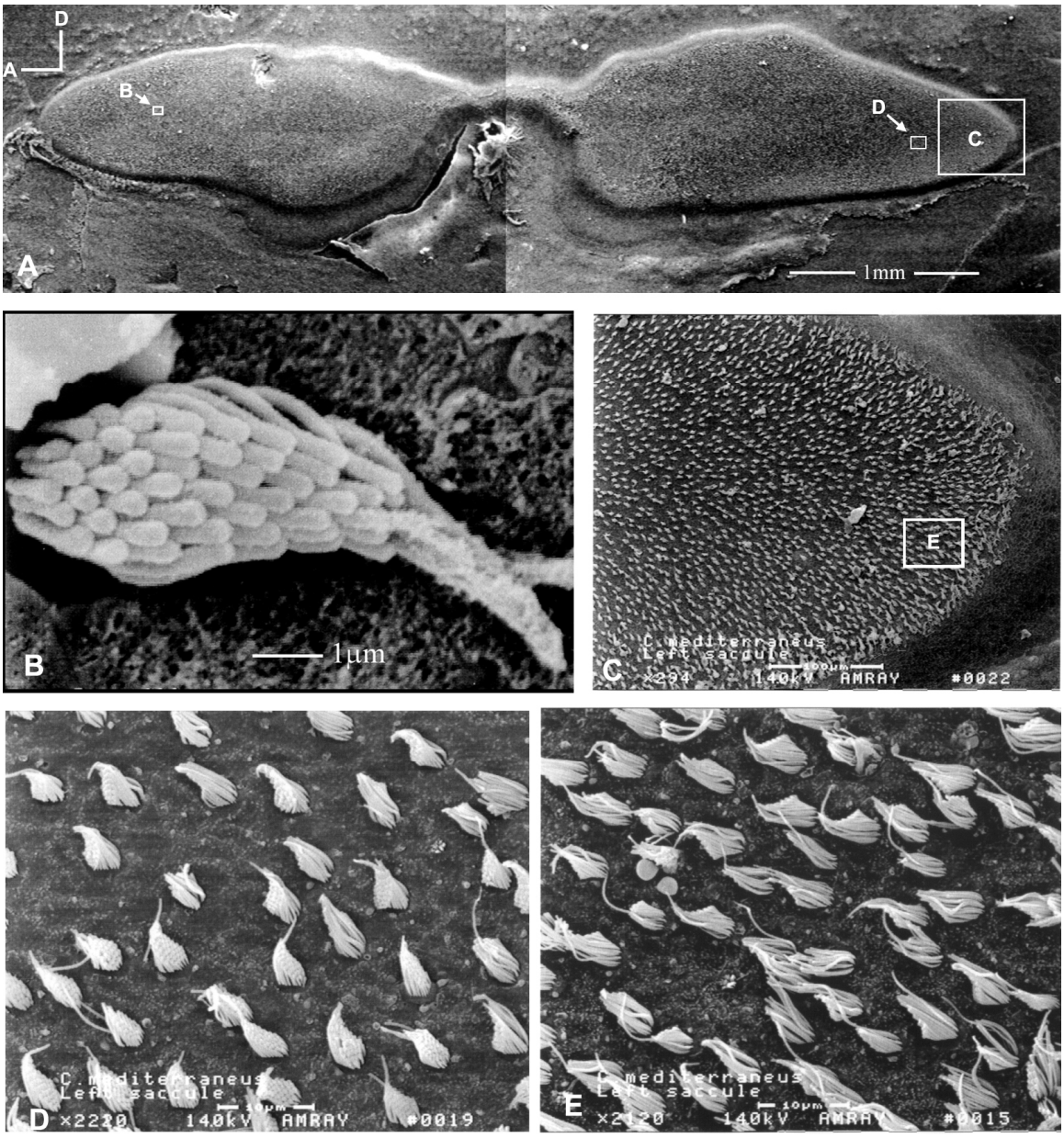



**Figure 4.5.** Hair cell ciliary bundles on the saccular macular of *Coryphaenoides mediterraneus*. Most bundles in the saccule are category 5 or 6 (Chapter 2). **A:** SEM photo of the saccular macula. **B:** One category 6 bundle from the middle of anterior segment. **C:** Posterior areas in A, all bundles are evenly disturbed. **D:** Opposite oriented bundles from posterior segment of **A**. **E:** Bundles near the edge of the macula (from **C**) are slightly longer than those in the center.

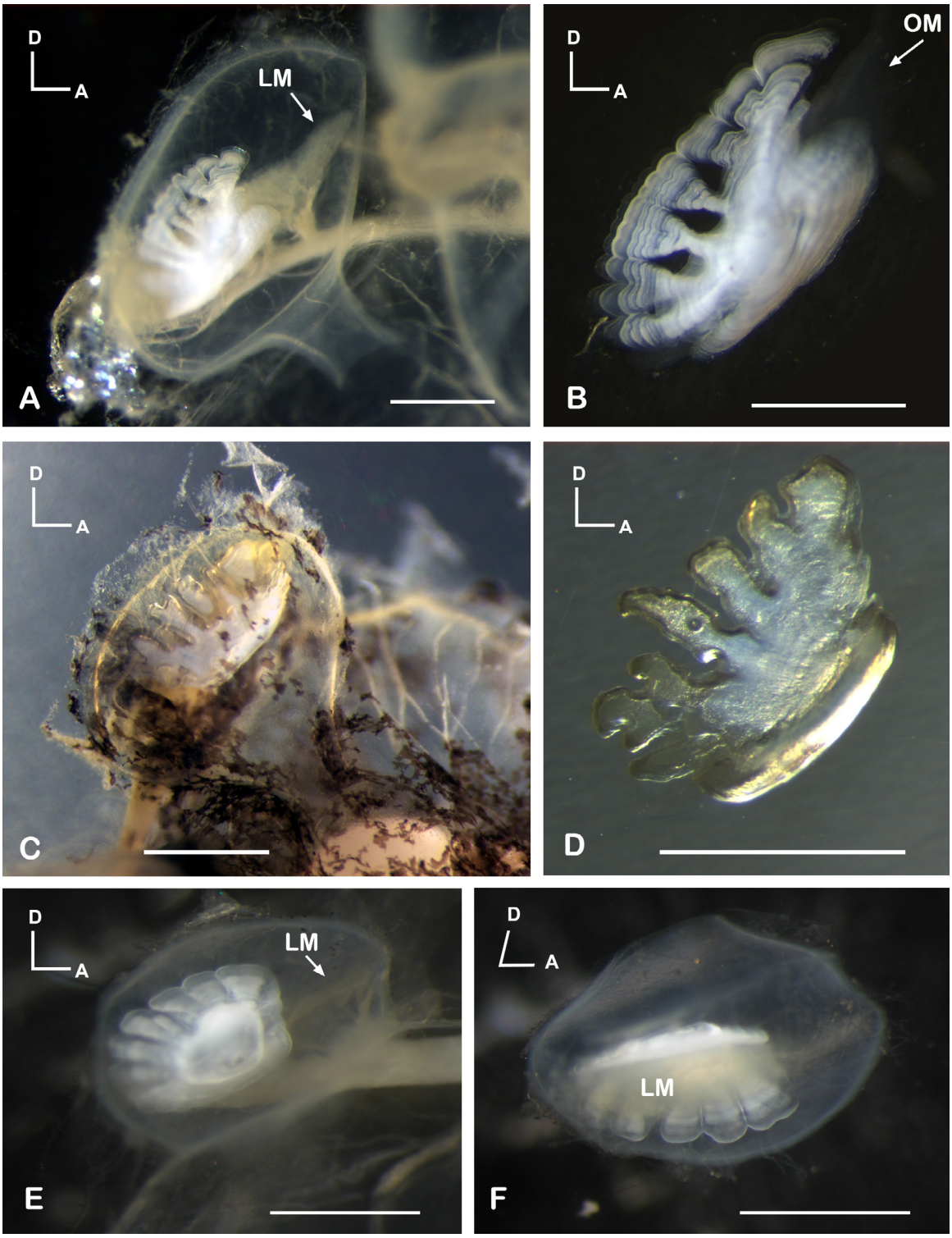



**Figure 4.6.** Lagena and otolith of macrourid fishes. **A:** Lateral view of a right lagena in *Coryphaenoides rupestris*, part of the lagenar macula (LM) is not covered by the otolith.

**B:** Lateral view of a left lagenar otolith (flipped horizontally using Photoshop). The otolithic membrane (OM) is seen at the anterior indentation of the otolith. **C:** Lateral view of right lagena in *C. mediterraneus*. **D:** Medial view of a left otolith, the brown color is from osmium stain. **E:** Lateral view of a right lagena in *Nezumia aequalis*. **F:** The same lagena in **E** looking from the ventral medial side. The otolith is seen arching on top of the lagenar macula. Scale bars = 1mm.

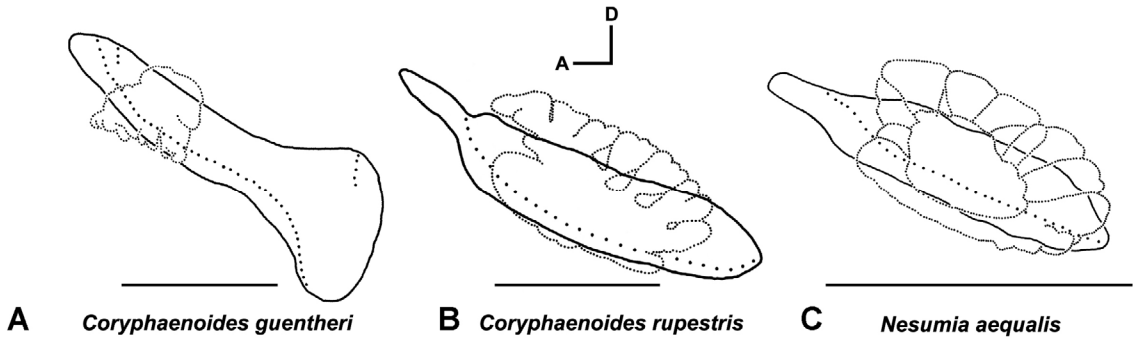



**Figure 4.7.** Variation in coverage of lagenar otolith on the macula. The otoliths are shown with the fine doted lines over the surface of the maculae. The thick doted lines are the dividing lines of the oppositely oriented bundles. The coverage varies from as less as 20% of the macula in *C. guentheri*, 60% in *C. rupestris*, to 80% in *N. aequalis*. Scale bars = 1 mm

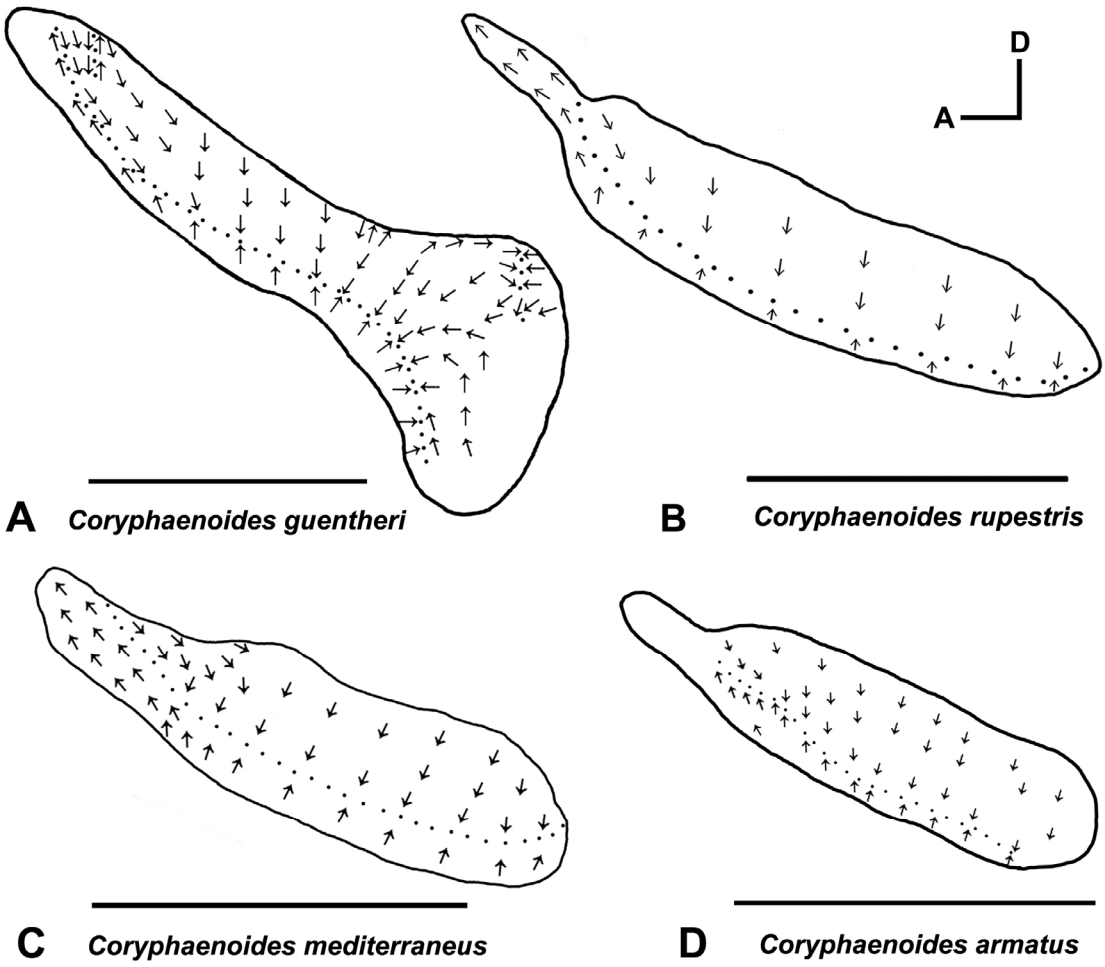



**Figure 4.8.** Hair cell ciliary bundle orientation patterns on the lagena. Bundles on the anterior tip of the macula orient to the anterior-dorsal direction, and then gradually shift to a vertical bi-directional pattern on the bulk of the macula. Scale bars = 1 mm.

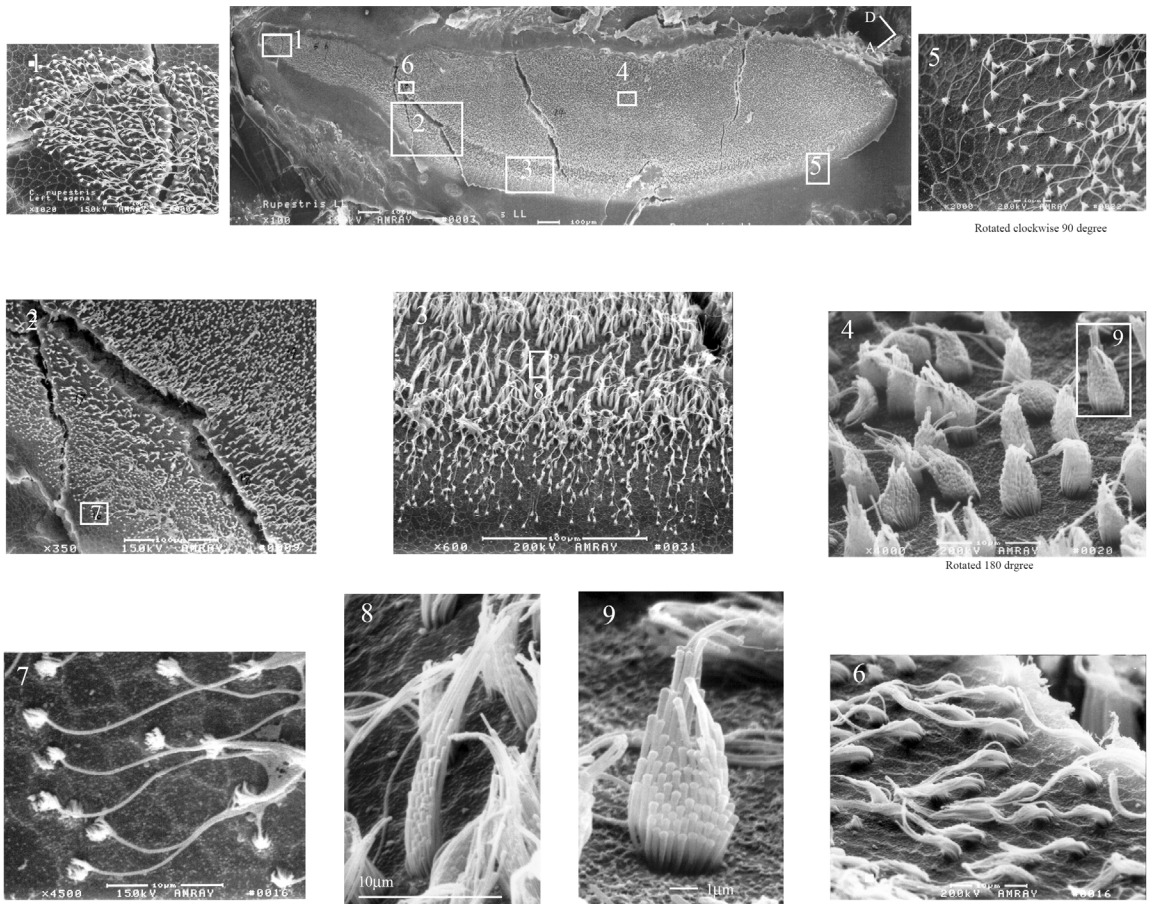



**Figure 4.9.** Hair cell ciliary bundles on the lagenar macula of *Coryphaenoides rupestris*. Bundles are diverse with different lengths and shapes. Top center picture is an SEM photo of a lagenar macula. Different areas are numbered and enlarged in subsequent SEM photos. **1, 5, 7:** Category 2 bundles on the anterior tip or ventral edge of the macula. **2, 3:** Striola-like region along the dividing line. **4, 9:** Category 6 bundles from the center of the macula. **6:** Category 4 bundles from the anterior center of macula. **8:** Category 3 bundles near the dividing line.

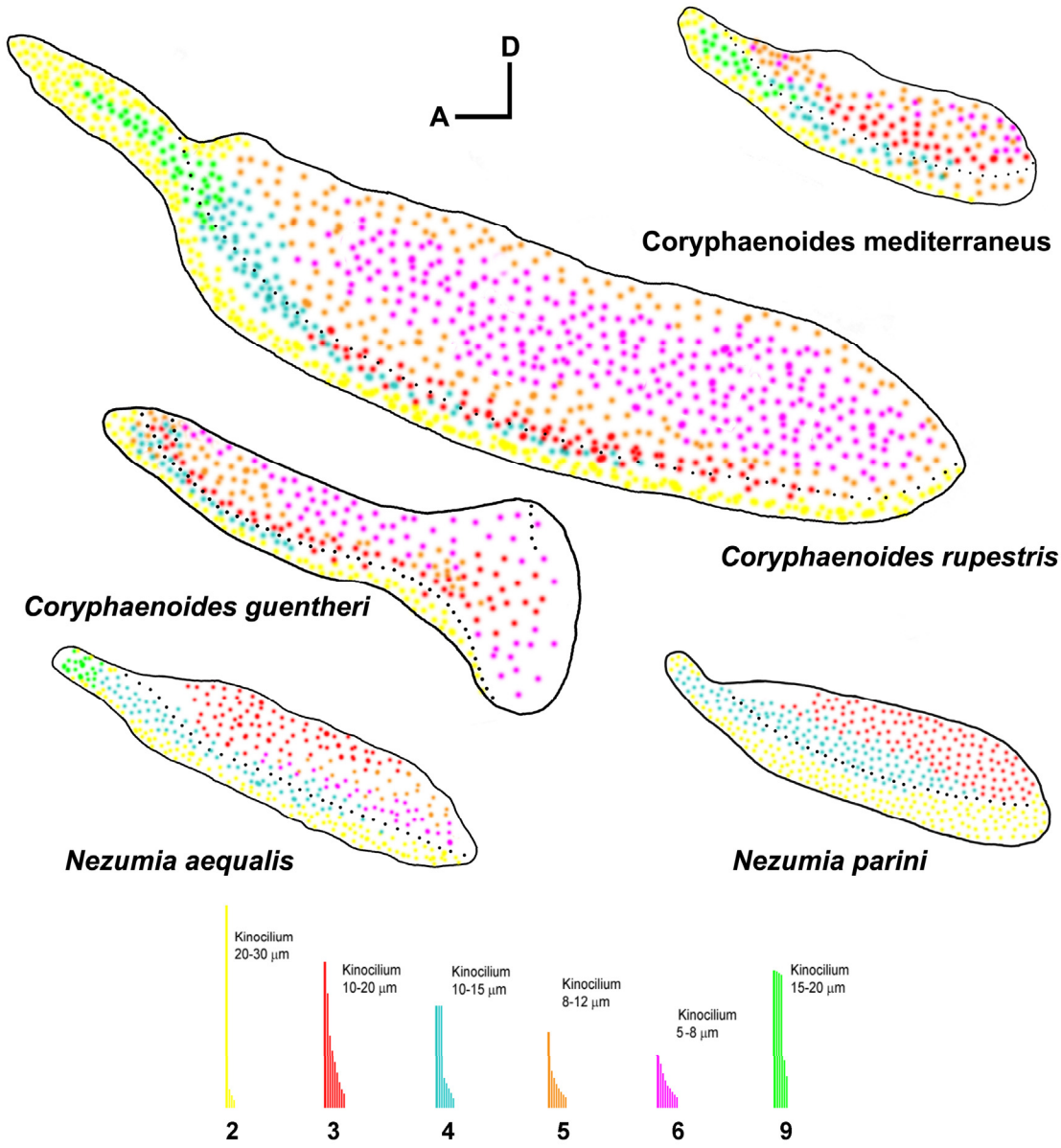



**Figure 4.10.** Color coded map of the distribution of different bundle types on lagenar maculae. Different types of bundles aggregate in similar areas on the maculae of different species. Maps were not drawn to scale. The larger map of *Coryphaenoides rupestris*'s lagena refers back to Figure 4.9. Color codes of different categories are the same as Table 2.1 in Chapter 2. A new category (**9**) is added for the bundles with a bunch of long stereocilia and no short stereocilia staircase at the base.

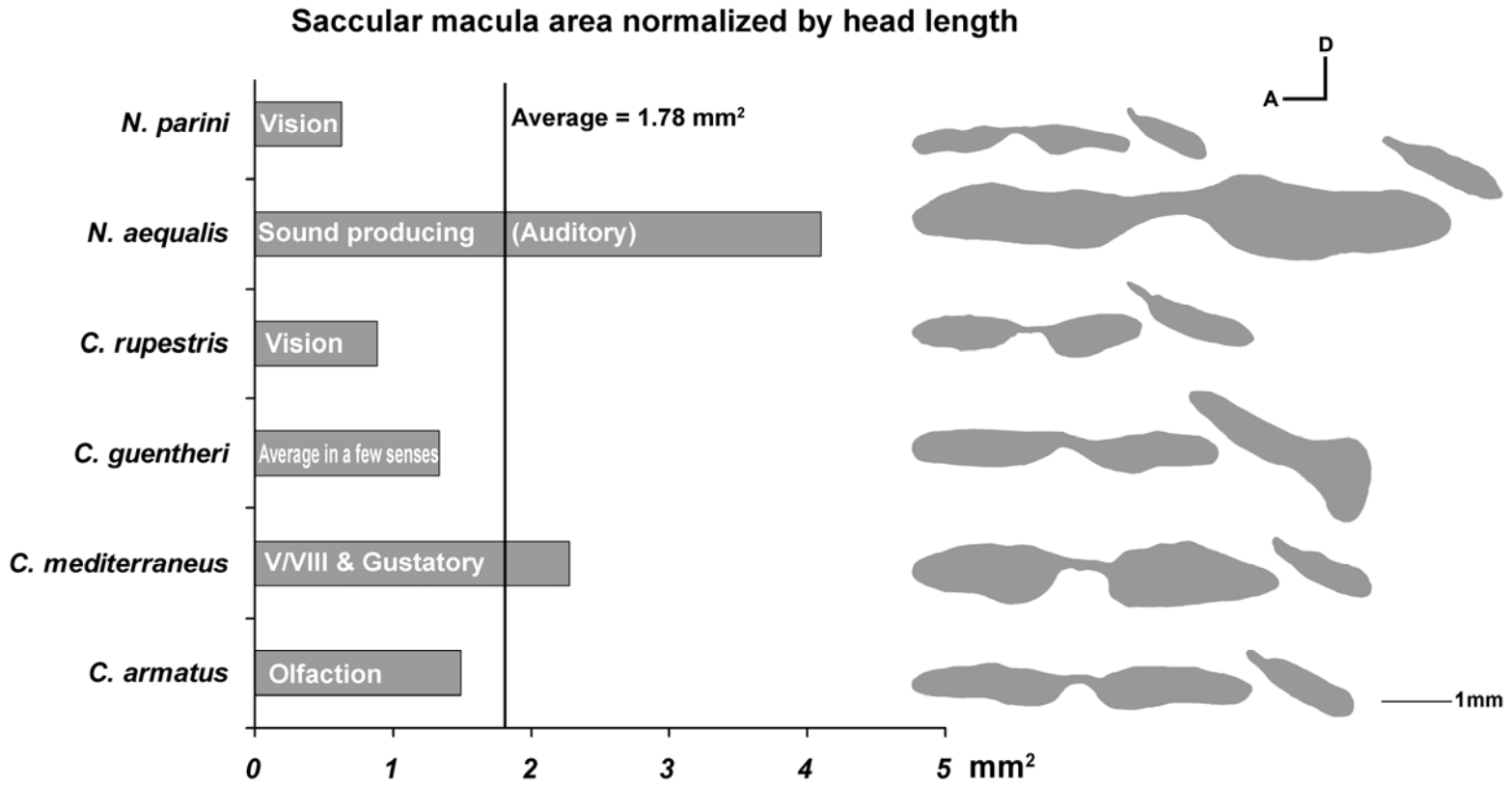



**Figure 4.11.** Calculated areas of the saccular maculae of macrourid fishes from different habitats. The bars indicate the area of macula surface normalized to an average head length of 54.8 mm (the head lengths range from 38 to 75 mm). The outlines of the normalized saccular maculae are on the right. Each species' dominant sense (Hubbs & Iwamoto, 1977; Wagner 2001) is indicated inside the bar. Vision or olfaction specialists have below-average saccular maculae; the sound producing *Nezumia aequalis* has the largest saccular macula.

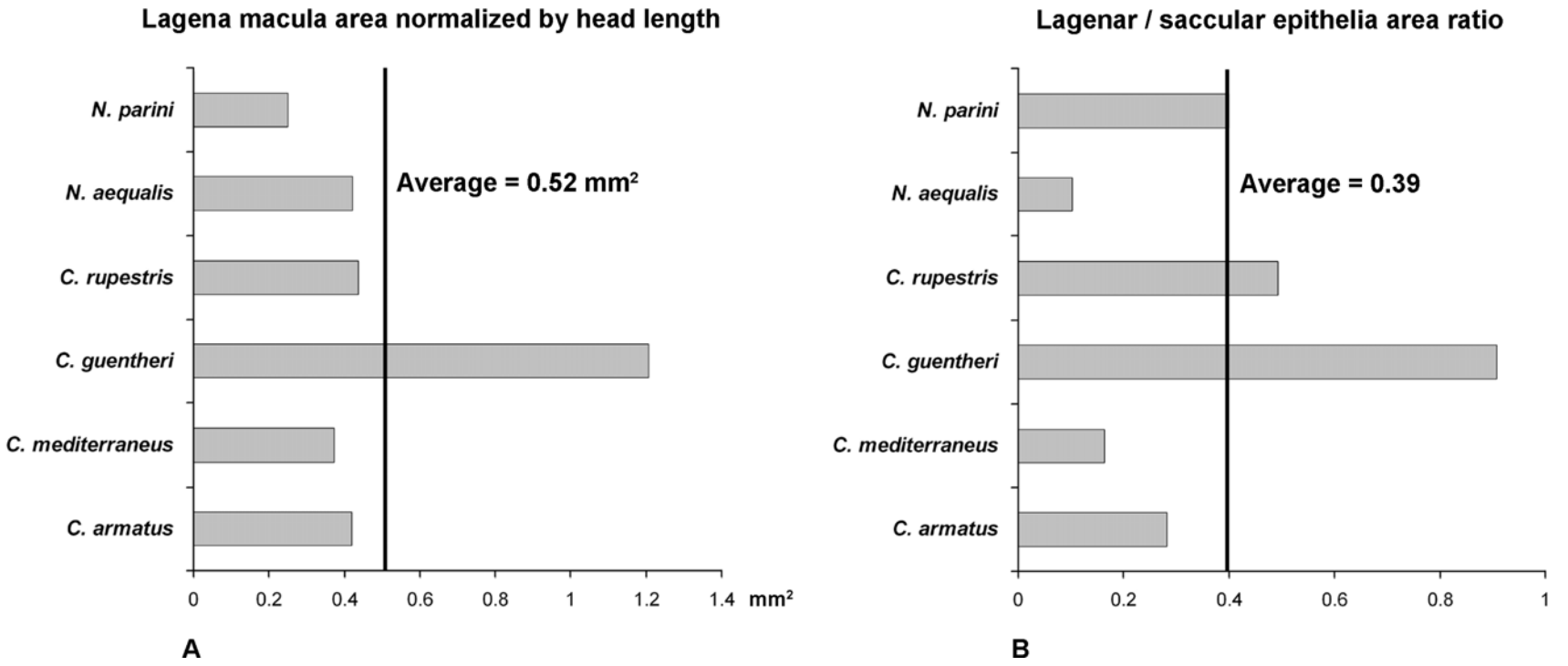



**Figure 4.12.** Lagenar macula area in macrourid fishes from different habitats. **A:** Area of macula surface normalized to a head length of 54.8 mm. **B:** Ratio of lagenar / saccular maculae areas. Deeper species (*Coryphaenoides mediterraneus* and *C. armatus*) and the sound producing *Nezumia aequalis* have a low lagenar / saccular area ratio. *C. guentheri* has an exceptionally large lagena macula and a 0.90 ratio in lagenar / saccular macula area.

# Chapter 5: General Discussion

## Overview of Relationship among Fish Groups

A cladogram of teleost fishes showing the relationship of different fish groups is presented in Figure 5.1. This is updated from Figure 1.2 in Chapter 1 with a list of the families mentioned in this study added to the cladogram. The common names of species or species groups were included for these families to provide an overview of their locations in the teleost taxonomy. The sequence of super orders and orders is based on Nelson (2006) and Helfman et al. (1997).

It is interesting to note that similar or comparable inner ear structures are found in very distantly related fish groups. For example (Chapter 2), the connection between swim bladder and the saccular region of the ear and the corresponded enlarged rostral part of the saccule with elaborated hair bundle orientation patterns are found in fishes from distant superorders. *Antimora rostrata* is a gadiform fish, the pinecone soldierfish is a beryciform, and the clown knifefish is from the much older (in terms of evolution) Order Osteoglossoformes. The special features in these species are very different from the other members in their orders without a swim bladder connection to the inner ear (e.g. squirrel fish in Beryciformes, and Atlantic cod and European hake in Gadiformes). The other example (Chapter 3) is the striking similarity in saccular hair bundle orientation pattern between melamphaids from the Stephanoberyciformes and the lanternfishes from the Myctophiformes. These findings provide examples of possible convergent evolution in the inner ears among phylogenetically very distant groups.

**Specialized Structures Found in Ears of Deep-Sea Fishes**

Based on the extensive evidence of adaptation and specialization in the sensory systems of deep-sea, including vision, olfaction, and lateral line system, it was hypothesized that the auditory system may have also evolved specialized inner ear structures to improve hearing capabilities. This was hypothesized since acoustic information may be very useful to deep-sea fishes when there is very limited light. Enhanced hearing can help fishes perceive the "auditory scene" and for interspecific communication if sound production has evolved in the species. In addition, maintaining an upright posture in the dark may also require specializations in the vestibular function of the inner ear when visual cues are limited.

The findings in this study support this hypothesis in various ways in the different fish groups studied.

In *Antimora rostrata,* a member of the morid family (Chapter 2), the inner ear is rigid in some parts and attached tightly to the bony capsule in other parts. This structure, along with its intimate connection of the ear to the swim bladder, may help the ear to follow the sound oscillation from the swim bladder with better precision than would occur in a softer ear. The proportion of the ear that consists of the saccule is larger in *Antimora* than has ever been seen among known fishes. The elaborate structure of the saccular macula is comparable in some way with the saccules of two shallow water hearing specialists that do not have Weberian ossicles connecting the swim bladder and the inner ear. This observation suggests that enhanced sensitivity may be inherent in the auditory system, and particularly the ear, of *Antimora*.

In the melamphaid fishes (Chapter 3), long and thin otolithic "stalks" or shorter "spurs" have been found on the saccular otoliths of two genera. Although the function of these otolithic extensions is unknown, they cannot be regarded as a developmental irregularity in the otolith because these formations are very consistent within specimens of the same species. These structures may change the kinetics of the otolith's response to sound or head movements, and thus affect the saccule's function in some way. The increased distance between the otolith and the base of epithelium may also contribute to this dynamic. The cilia making up the predominant type of hair bundles on the saccule of *Melamphaes* and *Poromitra*'s are exceptionally long, and the ciliary bundle consists of a large number of stereocilia arranged in two different layers. It is hypothesized that some characteristics of in the structure of these bundles may help to increase sensitivity to displacement according to physiologic findings from bullfrog utricular bundles (Baird, 1994). Bundles of this length were only previously encountered in vestibular end organs, and only in areas without the load of otolith. The slow locomotion in deep-sea fishes may allow, or even require, that the bundles and otolith to be arranged in this way. Thus, it is possible that the saccule in these fishes may be used in vestibular function or for detection of low frequency sound. Although it is not possible to test its function yet, the anatomical evidence suggests that *Melamphaes* and *Poromitra* may have very sensitive ears.

The "Mickey Mouse®" shaped utricular macula found in the melamphaids has never been reported in any other vertebrates (Chapter 3). These ear-shaped maculae are ubiquitous in this family and may be a way to increase the size of striola region, for reasons that would need to be explored experimentally. The anterior concave regions that

gives these maculae their characteristic two-eared shape may be a result from lack of space or cells at the junction between the utricle and ampullae, or an energy conservation strategy by omitting a large portion of anterior-posterior oriented striola bundles, which can be compensated by cells with the same axis in the saccule. The ear-shaped striola, which is also shaped as two ears, is only covered by the otolithic membrane. This may provide a special geometric surface for the endolymph flow.

In macrourid fishes (Chapter 4), the positive relationship between saccular otolith size and sound production is confirmed in this family. *Nezumia aequalis*, the only species that has drumming muscles on its swim bladder among the six species in this study, has by far the biggest saccular otolith and macula when normalized to head size. The lagenae in this family have many different bundle types. Indeed, bundles with different shapes and lengths are found at different location on the macula, with one bundle type often aggregating in one area. The teeth-shaped edge of the lagena otolith and the absence of the otolith covering in some areas of the macula may provide differentiated stimulation to different types of bundles. In the genus *Coryphaenoides*, the two species that live at shallower depths have a larger lagena/saccular area ratio then the two deeper species. The ratio is almost 1:1 in *C. güntheri*. This ratio has only been reported to date in the ostariophysan fishes (Popper and Platt, 1983). The above-average size of the saccular or lagenar macula in different species may indicate specializations in different aspects of the inner ear function. In the case of saccule reported here, the size may possibly be related to the sound production.

**Adaption to Deep-Sea Environment**

The previous section summarized data and suggested that some structures found in this study may be regarded as inner ear specializations in deep-sea fishes. However, these are only suggestions of adaptations in deep-sea fishes for enhanced hearing or vestibular function, and they need confirmation from physiological or behavioral study. However, such confirmation is unlikely due the difficulties of getting, and keeping alive, these species when they are brought up from great depths.

Specialization is only part of the story when talking about adaptations in the sensory system of deep-sea fishes. Specialization is a narrower concept than adaption. This is suggested since specialization is often referred to enhancing in function in a certain organ. Specializations may result in above-average ability, such as the enhanced hearing ability in goldfish via Weberian ossicles, or maintaining function under extreme conditions, such as the tubular lens for gathering light or the multiple layers of rods for increasing the number of receptors in the eyes of some deep-sea fishes to cope with low light (reviewed in Chapter 1).

Adaption, however, is a broader term. Changes in an organism's structure and function may go different ways during evolution. Some of the changes happened only because of selective constraints by a specific environmental condition. For example, the reduction of muscle protein and the presence of low density skeletons in deep-sea fishes probably evolved due to selection pressure imposed by low nutrition supplies. Additional selection pressure may have resulted in reduction of body density as a result of potential difficulty in maintaining a large amount of gas in the swim bladder under the high

pressure. Some of the changes may seem disadvantages if looked upon alone, but they may serve an important function when looking at the fish and its environment as a whole.

When a species possesses an integration of various adaptations for deep water living, we then regard this species as being "specialized" to the deep-sea.

Many aspects in the deep-sea may affect the evolution of the fish ear. Water pressure has a major effect on swim bladder structure and this may affect the properties of sound that is transmitted into the inner ear when such a mechanism exists. The higher density gas and rigid swim bladder wall give the swim bladder less room for vibration, and this may reduce the amplification effect for sound by the swim bladder. The rigid ear and rigid connection to the swim bladder in *Antimora rostrata* may help to receive as much vibrations from the swim bladder as possible (Chapter 2). Pressure does not seem to have an observable effect on the inner ear's structure per se, because the liquid filled membranous labyrinth is less compressible than structures that include gas. Although the biochemical events inside the hair cells and neurons will be affected by pressure and temperature, this is a different scope of investigation than this study.

The issue of darkness is addressed in my hypothesis, which may lead to enhanced hearing to help the fish to perceive the "auditory scene." This will help the fish deal with difficulties in finding prey and mates.

Some structures in the ear of deep-sea fishes that are less frequently found in shallow water fishes may not be related to hearing, but instead serve as adaptations to other aspects of deep-sea life. The teeth-like vaterite formation on the lagena otolith in macrourid fishes (Chapter 4), as well as the various teeth, holes, and indentations found in the saccular otoliths of many other deep-sea fishes, may have functional effects on

stimulation of the hair bundles, or they may simply be a co-adaption to compensate for the lost of density in the fish body. The reduced size of the saccular otolith and the resultant reduction in the size of the saccular macula in the genus *Scopelogadus* from the melamphaid family (Chapter 3) may also be adapted to its very low density bones.

The heavy pigmentation on the inner ear is seen in two deep-sea families in this study, especially on the ventral and lateral wall of saccule sac in melamphaids (Chapter 3, whole ear pictures) may serve as camouflage in a few species. This is suggested since the large saccular otolith in these species is likely to reflect lights and these fishes' body is small and semi translucent in some body regions. Thus it is possible that the pigment prevents light reflection and helps conceal this white target from potential predators.

**Trade-off among Sensory Systems**

Adaption to extreme environment, such as the deep-sea, requires specializations in various organs and structures. In the sensory system, fishes that do not have specialization in any sense are less common because some senses may be useless without special structures to overcome the limitations of the extreme conditions; whereas some senses may be especially useful for certain environments or particular life styles. Specialization in a sensory organ usually result in hypertrophy in the brain area dedicated to that sense (Wagner, 2001), thus consuming a lot of energy. The energy efficient way of life in the deep ocean cannot afford to have hypertrophy in all senses; and the space in the cranial cavity is also limited. As a consequence, one or two senses often became dominant during evolution and the rest are suppressed to various degrees (e.g. the ocular degeneration in some deep-sea fishes (Munk, 1964). This kind of trade-off among

sensory system happens very often in the sensory system of deep-sea fishes, and this is also observed in this study.

In melamphaid fishes, the inner ear and lateral line organs have robust outputs to the brain (Chapter 3). The size of saccule in this family is relatively large among mesopelagic fishes. Unlike many mesopelagic species that have light organs and highly specialized eyes, the melamphaids lack these specializations. Gross morphology in the brain indicates hypertrophy in the octavolateral area of the cerebellum, and reduction of size in vision and olfactory centers. The head lateral line organs and inner ear are probably the two dominant sensory systems for this family.

In the macrourid fishes, it is known that the family have some members with sound producing ability and the saccular otolith is larger in those sound producing members (Marshall, 1966). This is also confirmed by the one sound producing species in this study (Chapter 4). Thus we can assume that the saccule is the hearing organ in this family and the size of saccule relative to the head length reflects the degree of their dependence on hearing.

The difference in dwelling depth of the studied species is roughly reflected in the inner ear anatomy, though there are exceptions. The factors that affect the anatomy of the inner ear in this family are a combination of different sensory advantages from different habitats and the fish's particular life style.

The two shallowest species have a below-average size of saccular maculae; this is because they are all specialized in vision to take advantage of the twilight and the bioluminescence at the depths at which they live. In the two deepest species that live below the twilight zone where bioluminescence is very limited, vision is suppressed and

other senses apparently “take over,” so these species have relatively larger saccules than the two shallowest species. The deepest species among the two have a smaller saccule because, as a scavenger fish, olfaction is by far the most dominant sense so hearing is less important. In the two species that live at mid-depths, one has many senses in average and is the only generalist among the six (Wagner, 2001). The size of saccular macula is not impressive, but the size ratio between lagenar and saccular sensory area reach an intriguing 1:1 ratio. The other one is a sound producing species as mentioned before, and it has the most above-average size in saccular macula among the six species.

The findings in this study provide evidence from the inner ear to the studies of trade-off among sensory systems in deep-sea fishes.

**The Size of Saccule vs. Water Depth**

From the observations in this study, differences in inner ear structures may reflect the dwelling depth of the fishes to a certain extent; however, they also reflect the life styles and the trade-off among sensory systems. In order to get an overview of the relationship between the inner ear structure and depth among the studied deep-sea species, the size of the saccular macula (represented by the apical surface area of saccular macula) and the area ratio between lagenar and saccular maculae were chosen to plot against the maximum depth of each species (Fig. 5.2).

Figure 5.2A plots the size of saccule vs. depth. The areas of the saccular maculae of selected adult individuals were normalized to an average head length of 50mm. The scatter plot indicates that the sizes of saccular maculae of deeper living species (below 2500m) are closer among different families (gathered between 0.8-1.7mm$^2$), whereas the

data are more scattered in the shallower species. The extreme point is from *Nezumia aequalis,* which has exceptionally large saccules and is the only one in the 12 species that has drumming muscles on its swim bladder.

Figure 5.2B plots the lagenar/saccular maculae area ratio vs. depth. The deeper species (below 3000m) have relatively small lagenar maculae (ratio below 0.3). Larger lagenar maculae relatively to the saccule are mostly found in shallower species. The largest is found in *Coryphaenoides guentheri*.

The scatter plots provide a limited overview of the relationship between sensory epithelia size and depth and they suggest that deep-sea fishes live in shallower water layers may have more variations in the ear. As has been discussed in the previous section, this may reflect the difference in dominance of particular sensory systems. Shallower layers of the deep-sea provide residual sunlight and richer bioluminescence than the deeper layers. The importance of vision to a fish should be taken into account when discussing the relationship between inner ear size and depth.

Figure 2A and 2B only show two dimensional diagrams between two variables. In order to include more variables to explore the clusters of the collected data, a cluster analysis (Ward, 1963) was conducted for the 12 species of deep-sea fishes. The three variables used in this analysis are the normalized saccular macula size, the lagenar/saccular area ratio, and the orbit diameter/head length ratio. The last variable may roughly reflects the importance of vision to a fish. The number of cases (12 species) is within a reasonable range for the number of variables (k) used, given that the minimal sample size should be no less than $2^k$ cases (Dolnicar, 2002). The original data with assigned clusters are listed in Table 5.1.

The resulting dendrogram is shown in Figure 5.3. Three clusters were decided for the 12 species of fishes based on the values of semi-partial R-square, which measures the loss of homogeneity by merging neighboring clusters (Program: SAS Proc Cluster, SAS9.1). The resulting clusters separate three relatively shallow and vision oriented species (cluster 2, green) from the rest of the group (yellow); fishes in the later group (yellow) have relatively larger saccules. *Nezumia aequalis* (cluster 3, red) was separated from the remaining eight species, which reflects its exceptionally large saccule. This cluster (3) separates the only fish with potential sound production ability from the rest of the fishes. Cluster 1 (cyan) contains mostly deeper species with large saccules. The two subdivisions within cluster 1 reflect differences in taxonomy as well as habitats, one subdivision contains five mesopelagic melamphaid species (purple), and the other one contains three demersal slope dwellers from gadiform fishes (blue).

The clusters analysis confirms that the difference of saccule size found in these fishes is related to the difference in depth and habitat and the importance of vision or hearing to fishes.

**Diversity in Fish Inner Ears**

The findings in this study revealed many structures in the inner ear that have never been reported in other fishes. Among them are the "Mickey Mouse®" shaped utricular macula and the long and thin otolithic stalks on the saccular otolith in melamphaids, and the exceptionally large saccule and the rigid thick membranous labyrinth in *Antimora rostrata*. Some structures are not common among shallow water fishes, like the teeth-like indentations on the lagena otolith of macrourids, the elaborate

bundle orientation pattern in *Antimora*'s saccule, and the exceptionally long hair bundles found in some of the species.

These special structures may reflect the adaption in deep-sea fishes's ears to the extreme environment; they may also reflect the fact that ears in general are very diverse. The number of species among all fishes that have been studied is so limited, and fishes have more number of species than any other vertebrates, what we have seen may be just the "tip of the iceberg" with regard to inner ear and auditory system diversity among all fishes. More adapted features will be discovered in fish ears when we broaden the known species list, and these adaptations may not be constrained to deep-sea species.

## Perspective of Future Work

### *Neuroanatomy*

In *Antimora rostrata* (Chapter 2), the array of innervation of the eighth cranial nerve along the length of hind brain is very interesting and suggests that input to the brain from the ear is very extensive and possibly spread out. However, it will require neuroanatomical study using anterograde neural tracer to help to identify the brain area associated with this robust innervation,

The enlarged octavolateral area in the cerebellum of some melamphaids is only a rough observation in gross morphology (Chapter 3). Further neuroanatomical investigation is needed to understand the inner structure of this formation, and to identify and separate areas that are devoted to the lateral line organs and the different inner ear end organs. In particular, it would be interesting to compare these brain structures with those from shallow water hearing specialists, and perhaps other deep-sea fishes that are

likely to have hearing specializations. One of many interesting questions concerns whether there is any convergence in the way that the octavolateral regions of the brain are organized in fishes that are only very distantly related to one another such as the melamphaids and myctophids (Chapter 3).

***Extended to Related Species***

In order to understand the phylogenic relationship and evolution of the special structures found in the families involved in this study, extending the investigation to other species in the same family, or close related families, are necessary.

Regarding *Antimora rostrata*, it is known that all members in this deep-sea cod family possess a connection between the inner ear and the swim bladder (Paulin, 1988). The saccular otoliths in other members of the morid family have similar features to the ones in *Antimora* base on existing otolith atlas (Campana, 2004). It would be interesting to find out if similar inner ear structures can be found in these related species, or if they are only special features in *Antimora*. The comparison may also help us to understand the inner ear function in this group.

In the melamphaid family, it is important to find out the structure of saccular otoliths and maculae in species from the other genera, *Scopeloberyx* and *Sio*. This will further reveal the trend between the shapes of the saccular otolith and the underlying maculae. In the most diverse genus *Melamphaes*, some members do not have the otolithic stalk on their sagitta and it would be interesting to find out if there is any difference between the saccular macula from stalked or non-stalked species. This may help us to understand if there is any functional significance of the otolithic stalk. Molecular studies

on the phylogenetic relationship in this family will also help us to understand the evolutionary pathway of these otolithic extensions and the various saccular maculae in this family.

### *Baited Camera with Acoustic Study*

Except for some species for which there is anatomical evidence for sound production and the inner ear specializations that are presumed to be involved with hearing, there is no direct evidence, which could only come from physiological or behavioral studies, to indicate whether deep-sea fishes utilize, or respond to, sound. Baited camera on free dropping vehicles or controlled landers has been used to study deep-sea fishes' distribution, metabolism or behavior (e.g. Collins et al., 1999; Jones et al., 2003). These landers can potentially be equipped with acoustic devices to play or record sounds in the deep-sea. However, such experiments depend on development of acoustic devices that can work under the very high pressure of the deep ocean, and the availability of chances to use these devices on deep-sea research cruises.

### *Quantitative Analysis of Hair Bundle Types*

The investigations on the bundle type distributions in this study is only on a qualitative level due to the large number of species involved and the limited number of specimens of each species. Quantitative analysis of the bundle types is necessary to document the range of bundle length, the K/S ratio, the number of stereocilia, the arrangement of stereocilia arrays, and the bundle density on different areas, etc. Suitable candidates for this study include a number of species. For example, the saccule of the

sound producing *Nezumia aequalis* is a good candidate since the ear is probably specialized for hearing. The lagenae in *Coryphaenoides rupestris* and *C. guentheri* would be of interest because of the richness in bundle types on the macula, and the higher than usual lagena/saccule area ratio. The "Mickey Mouse®" shaped utricular macula in melamphaids would be of particular interest because of the unique striola structure and richness in bundle types.

To achieve a better classification of the different hair bundle types, cluster analysis may be used to group the different types of bundle based on variables like cilia length, number of cilia, size of bundle, ratio between kinocilia and stereocilia length, the slop of stereocilia, etc.

**Summary**

The findings from this study revealed many interesting structures in the inner ear of deep-sea fishes and the trade-off in relationship between the ear and other sensory systems in some families. The data in this dissertation supports the hypothesis that some deep-sea fishes have evolved specialized inner ear structures that may help to improve hearing or vestibular capabilities.

From the three very different and taxonomically unrelated deep-sea families involved in this study, many structures are found in the inner ear that are potentially specializations for hearing or vestibular sense. In contrast, most of the epipelagic oceanic species for which we have data on the inner ear and auditory system structure (e.g., salmonids, perch, and tuna), do not have specializations in the ear or auditory system.

Thus, this observation further supports the hypothesis that deep-sea fishes, in general, have more specialization in their ears than shallow water fishes.

Of course, while it is interesting to hypothesize that a greater proportion of deep-sea fishes have hearing specializations, further investigations, such as those described above, and the far more difficult studies of testing hearing and inner ear physiology, are needed to fully test these hypotheses.

## TABLES

**Table 5.1** Maximum Depth, Sensory Epithelia Area and Ratios, Head Length, and Orbit/Head Length Ratios of 12 Species of Deep-Sea Fishes

| Family | Species Name | Cluster* | Max Depth (m) | Saccular Area ($mm^2$) | Lagenar Area ($mm^2$) | L/S Area Ratio | Head Length (mm) | Normalized Saccular Area** | Orbit Diameter/ Head Length Ratio |
|---|---|---|---|---|---|---|---|---|---|
| **Morids** | ***A. rostrata*** | 1 | 3000 | 5.14 | 1.565 | 0.30 | 90 | 1.586 | 0.22 |
| **Melamphaids** | ***M. acanthomus*** | 1 | 3500 | 1.201 | 0.103 | 0.09 | 44 | 1.551 | 0.10 |
| **Melamphaids** | ***M. laeviceps*** | 1 | 1109 | 0.850 | 0.060 | 0.07 | 35 | 1.735 | 0.11 |
| **Melamphaids** | ***P. crassiceps*** | 1 | 3400 | 1.309 | 0.053 | 0.04 | 54 | 1.122 | 0.12 |
| **Melamphaids** | ***P. oscitans*** | 1 | 5320 | 0.360 | 0.015 | 0.04 | 26 | 1.331 | 0.07 |
| **Melamphaids** | ***S. mizolepis bispinosus*** | 1 | 3385 | 0.306 | 0.046 | 0.15 | 31 | 0.796 | 0.12 |
| **Macrourids** | ***C. armatus*** | 1 | 4700 | 1.489 | 0.437 | 0.29 | 55 | 1.231 | 0.21 |
| **Macrourids** | ***C. mediterraneus*** | 1 | 3000 | 2.895 | 0.523 | 0.18 | 65 | 1.713 | 0.22 |
| **Macrourids** | ***C. guentheri*** | 2 | 2830 | 1.306 | 1.234 | 0.94 | 54 | 1.120 | 0.25 |
| **Macrourids** | ***C. rupestris*** | 2 | 2200 | 1.647 | 0.824 | 0.50 | 75 | 0.732 | 0.26 |
| **Macrourids** | ***N. aequalis*** | 3 | 2320 | 2.483 | 0.254 | 0.10 | 42 | 3.519 | 0.32 |
| **Macrourids** | ***N. parini*** | 2 | 1350 | 0.297 | 0.119 | 0.40 | 38 | 0.514 | 0.35 |

* See **Figure 5.2**.

** The saccular surface areas were normalized to an average fish head length of 50mm.

## FIGURES

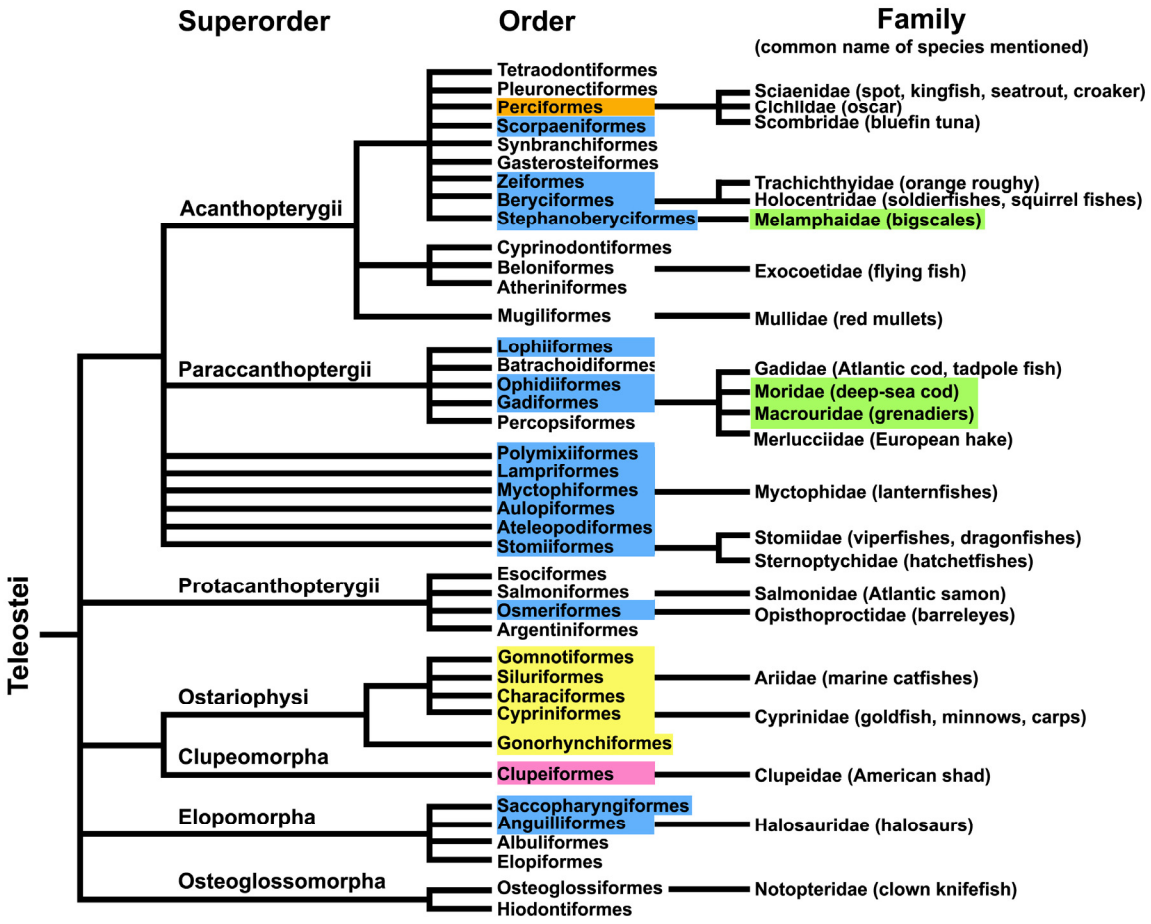



**Figure 5.1.** Cladogram of teleost fishes showing the relationship of different fish groups. The sequence of orders is based on Nelson (2006). The representations of commonly seen fish names listed in **Figure 1.2** were removed and a list of mentioned families in this dissertation was added. The common names of species or species groups discussed in this study were listed for these families. The orders containing deep-water living fishes are highlighted in blue. The three deep-sea fish families used in this study are in green. The lengths of branches do not represent time.

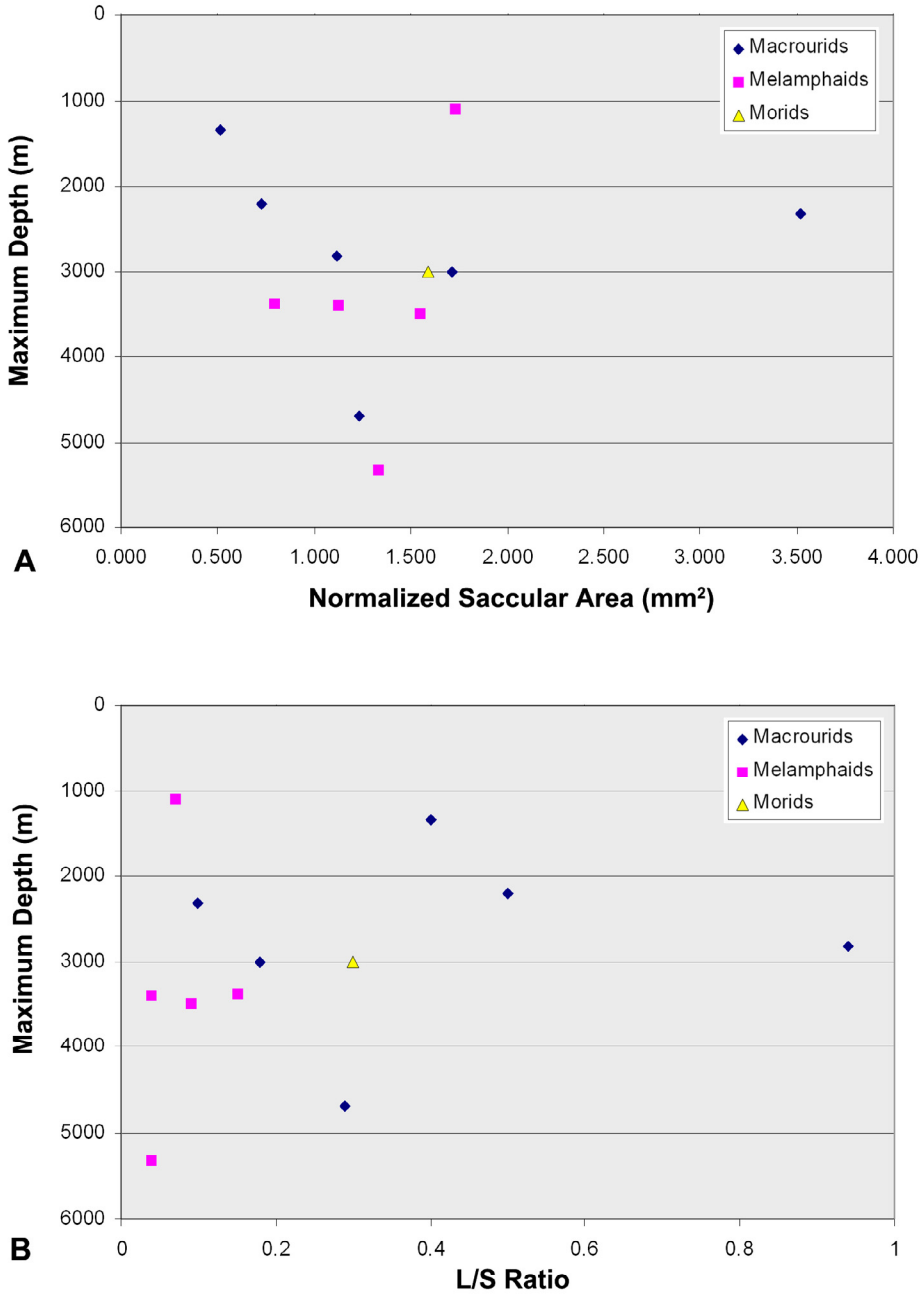



**Figure 5.2.** Scatter plot of sensory epithelia area vs. maximum depth of 12 species of deep-sea fishes. **A:** Saccular surface areas vs. maximum depth of each species. The saccular areas were normalized to an average head length of 50mm. **B:** Lagenar/saccular area ratio vs. maximum depth. Fish species and values are listed in **Table 5.1**.

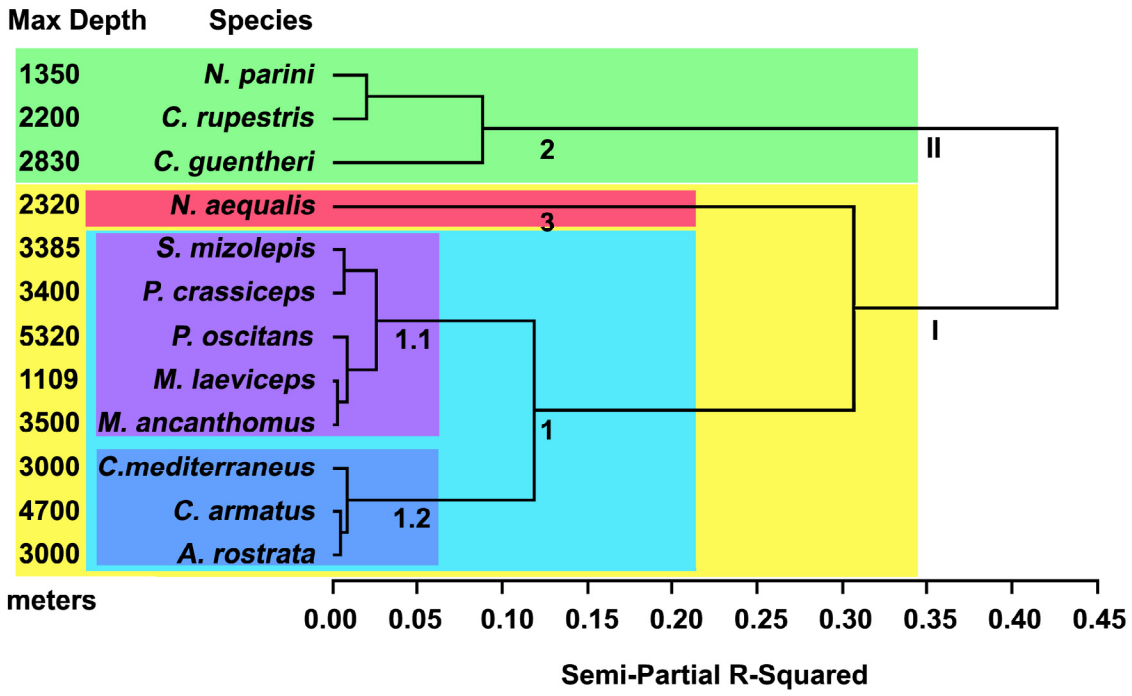



**Figure 5.3.** Dendrogram of a cluster analysis of 12 species of deep-sea fishes using three variables (normalized saccular area, lagenar/saccular area ratio, and orbit/head length ratio). The hierarchical tree system was based on Ward's method (Ward, 1963). Semi-Partial R-Square measures the loss of homogeneity by merging two clusters. When clustering two relatively homogeneous groups together, the loss should be small. In order to achieve the smaller values of semi-partial R-square, three clusters were decided for the 12 species of fishes (Program: SAS Proc Cluster, SAS9.1, SAS Institute, Cary, NC). The maximum depths of each species are listed on the left as references. The cluster analysis separates the 12 species into three different groups, reflecting the importance of vision or hearing to the fishes, depth and habitat, and taxonomy. See text for details.

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