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The bony lung of the coelacanth *Macropoma*

By

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This work is dedicated to my mother Jenny Anderson, sister Louisa Hartley, grandmother Janet Lecompte, and grandfather Oliver Lecompte, each of which has spurred my education on in their own way. I know what hard work excellence are because of you. This is also dedicated Dr. Stan P. Rachootin who had a crazy idea and held on to it.

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ABSTRACT

In 1833, Louis Agassiz described a large flask-shaped structure inside the body of the Cretaceous coelacanth *Macropoma*. Its microscopic structure was analyzed by W.C. Williamson in 1849. Coelacanths have been intensively studied ever since a living coelacanth, *Latimeria*, was discovered in the Indian Ocean in 1938. It became the most famous living fossil, because it was thought to be the closest living relative of the creatures that came on land to become the first amphibians. *Latimeria* is very closely related to *Macropoma*, but it does not have a bony lung, and bony lungs are not a part of the story of the origin of terrestrial vertebrates. My study of the bony lung of *Macropoma* is the first since 1849. I compare its microscopic structure to the much thicker ossified lung of another Cretaceous coelacanth, *Axelrodichthys*, and analogous bony structures in birds and teleost fishes.

The bony lungs in *Macropoma* and *Axelrodichthys* share features such as being composed of plates that overlap and decrease in diameter posteriorly, but are strikingly different in microscopic structure like the possession of osteocytes within the bone matrix. I discuss the formation and possible functions of this curious organ, in the light of some ideas that have come along since 1849: evolution, classification based on genealogy, developmental biology, and biomechanics.

INTRODUCTION

A link that could connect the organization of fishes and terrestrial vertebrates was a scientific concern before the theory of evolution; the South American lungfish *Lepidosiren paradoxus*, the "scaly siren" with both gills and a lung, was a part of the chain of being from its initial description in 1837 and a good example of such a link. Darwin refashioned this fish and also the platypus into "living fossils" in *On the Origin of Species* (1859): they were the sorts of organisms that had to play a part in any account of the diversity of life. Other potential links, such as fossil coelacanths had been found and named slightly earlier than *Lepidosiren,* and though the bones of their fins shared several features with tetrapod limbs, no one compared them, point for point, with terrestrial vertebrates. That changed in 1938, when the first living coelacanth, *Latimeria*, was discovered off the coast of South Africa. Because its fleshy fins contained bones that could be homologized to those in tetrapod limbs (a case that could not be made with the fin bones of living lungfishes), the living coelacanth jumped into the position as the fish that is closest to the tetrapods. *Latimeria* became known as the missing link, and THE living fossil. The features of anatomy and physiology that it shared with the tetrapods were subsequently subjected to close study.

A History of Coelacanths

Early in the 19th century the state paleontologist of Prussia, Count Georg of Münster, discovered a fish with large, fleshy fins in Jurassic deposits. He named the specimen *Undina* (Frickhinger, 1994). His meticulous drawing of this specimen came to the attention of the Swiss ichthyologist and paleontologist Louis Agassiz in 1824 (Beisbart, 2010).

In 1843, Agassiz created the family "Coelacanthes" for *Undina, Macropoma,* and *Coelacanthus* in his five-volume *Recherchez sur Les Poissons Fossiles* (Agassiz, 1843 Vol. 2). These genera were diagnosed by their hollow and unbranched lepidotrichia (fin rays). The lobes at the bases of their fins contained a series of bones, the most proximal of which was a single bone.

Agassiz classified fish by the types of scales that they possessed (Agassiz, 1833 Vol.1). The coelacanths were described as an order of ganoid fishes, or fishes with thick, overlapping, armor-like scales. Coelacanthes were similar with respect to scale types, as well as and pectoral fin structures to the fish in the genus *Holoptychus*, and thus were grouped with them (Agassiz, 1843 Vol.2). The term coelacanth, as used by Agassiz, led to confusion as to what a coelacanth was: a member of Agassiz's new order Coelacanthes, or a fossilized fish with large ganoid scales?

In order to illustrate the characters distinguishing members of the family Coelacanthidae, Agassiz expanded the definition of a coelacanth from a fish with hollow lepidotrichia surrounding the bones at the base of the fins, to a fish with thick skull bones, conical teeth, and thick gular plates. With this wider definition,

Agassiz expanded the family Coelacanthidae to include the genera

Coelacanthus*, *Holoptychus*, *Phyllolepis*, *Hoplopygus*, *Uronemus*, *Glyptosteus*,

Psammolepis, *Glyptolepis*, *Undina**, *Ctenolepis*, *Gyrosteus*, and *Macropoma**

[*denotes members of the original Coelacanthidae that remain as members today]

(Agassiz, 1843 Vol.2).

Agassiz noticed that inside three fishes in his coelacanth group there

appeared to be a sizeable, scaly, and mineralized internal structure. This structure

was first described by Agassiz from a specimen of *Macropoma mantelli* as "un

cylindre squammeux" (Figure 1) (Agassiz, 1843 Vol. 2). He hypothesized that

this cylinder was a stomach, based on its location just below the lateral line of the

fossil fish. This hardened, fossilized structure was like not anything he had seen in

fossilized fish. Agassiz confined his comments to this structure's gross anatomy

(Agassiz, 1843 Vol.2).

In 1849 W.C. Williamson extended Agassiz's investigation of placoid and

ganoid scales including the scales of coelacanths. Williamson investigated the

histology of scales, and bones: lamellar scales were laid down in concentric layers

surrounding a pulp cavity, with a shiny "coating" that created hard outer layers of

such substances as cosmine, dentine, or enamel on the outer surface of each of the

scales (Agassiz had maintained that these substances were found only in placoid

scales), the possession of Haversian canals (the paths of capillaries between

osteocytes within bone matrix) and a lack of lacunae in the outer "coating" layers

(Williamson, 1849).

Figure 1. Louis Agassiz's (1833) lithograph of what he called a hardened stomach in the coelacanth *Macropoma mantelli*. This organ is clearly visible at the center of the fish where it is located ventrally to the notochord.

After discussing histology of coelacanth scales, Williamson (1849) examined the structure that Agassiz called the hardened stomach, including the first account of the histology of the organ. He discovered that the walls were not made of scales, but were plates of bone composed of horizontally oriented lamellae. Within each lamella were lacunae that once housed osteocytes. He reported lacunae with fairly regular shapes joined by cannicular extensions that allowed for communication between the cells in the bone. Williamson also noted that there were undulations on the surfaces of overlapping plates that appeared to correlate with each other (Williamson, 1849).

Williamson proposed an idea for what this perplexing structure in *Macropoma mantelli* might have been. According to Williamson, Dr. Gideon Mantell, the original collector for whom this genus is named, noted this odd bony structure always commenced after the posterior margin of the fish's opercular bone. He noted that this structure was open at its anterior-most point, and came to a close at its posterior. Williamson concluded that the sac-shaped structure was composed of true bone that was open to the front. He suggested that this structure was likely to be an air bladder. He speculated that the bony component of this air bladder would be helpful in resisting rapid changes in pressure from swift descents and ascents (Williamson, 1849).

Williamson (1849) also brought to the family Coelacanthidae a fish with a single ventral lung, the genus *Polypterus*. *Polypterus* met Williamson's criteria for coelacanths: ganoid scales and lobed fins with the lobes containing bones at

the base of the pectoral fins (Williamson, 1849). This addition was widely accepted as a member of this family for almost one hundred years. *Polypterus* was frequently accepted as a living coelacanth until the discovery of *Latimeria*.

One skeptic on *Polypterus* as a coelacanth was Thomas Henry Huxley (1861). Initially, Huxley sought to restructure the entire family Coelacanthidae in order to include the genus *Glyptolameus*. This led Huxley to notice that many genera that were previously classified as members of the Coelacanthidae lacked some of the diagnostic characters described by both Williamson and Agassiz. He further noted that most of these genera had been added to the family simply because of their possession of lobed pectoral fins and ganoid scales. Huxley proposed a reorganization of the fish possessing ganoid scales. He classified many of the fishes that Agassiz had previously added to the Coelacanthidae under the order Ganoidei. This order contained three sub orders: Crossopterygidae (including coelacanths), Chondrosteidae, and Acanthodidae. The Chondrosteidae included fish without ossified vertebral columns like sturgeons, skates, and rays, while the Acanthodidae were extinct fossilized fishes characterized by having a prominent dorsal spine. Of these new sub orders, the Crossopterygidae was the largest with six families. Huxley chose the name Crossopterygidae because it described the very character that had previously caused all of these genera to be classified under one name: their possession of lobed pectoral fins with lepidotrichia extending from beyond the fleshy lobe (Huxley, 1861).

Huxley placed each of the genera that Agassiz and Williamson assigned to the family Coelacanthidae in one of the six families under the new order. He did note that of the many coelacanths that had previously been described, only three were *sensu stricto* coelacanths. These were *Macropoma, Undina,* and *Coelacanthus*. Each of these fossilized fish were united by their overall body configuration: paired and lobed pectoral and pelvic fins, an unossified vertebral column, a three-lobed caudal fin, and an ossified air bladder. Even with only three genera making up the new family renamed as the Coelacanthini, Huxley noted that one species of *Coelacanthus*, *C. münsteri*, would not be included.

Huxley closely examined the body type, scale structure, and fin structure of *Polypterus*, and found that while it had ganoid scales as well as paired and lobed pectoral fins, it lacked lobed pelvic fins and a caudal fin with three distinct lobes. These morphological differences led him to remove *Polypterus* from the coelacanths and place it into its own family, the Polypterini, within the crossopterygians (Huxley, 1861).

Despite Huxley's well thought out argument for removing *Polypterus* from the coelacanths (though not the crossopterygians), it remained the best-known example of a fish with ganoid scales, lobed fins, and a lung. It was not until E. S. Goodrich (1908, 1924) showed that it was an actinopterygian (the other clade of bony fishes), and lacked such crossopterygian features as enlarged median gular plates under the throat region, and pectoral fins with radials centered around an axis of bone, that *Polypterus* was removed from the crossopterygians.

Even as a newly anointed actinopterygian, *Polypterus* was the best representation of the grade of fish from which the tetrapods could have emerged. *Polypterus* replaced the lungfish as the "Living Fossil" (Hall, 2001). The motivation to learn more about the life, development, and morphology of *Polypterus* was so strong that zoologists, such as John S. Budgett, built their careers (and risked their lives annually) to collect and study living specimens (Hall, 2001).

The scientific emphasis on *Polypterus* as a guide to understanding the evolutionary path toward becoming a tetrapod continued until 1938 when the first living coelacanth was discovered by Marjorie Courtnay-Latimer on a dock in East London, South Africa (Thomson, 1991). Almost overnight, this new coelacanth, in her honor named *Latimeria,* took over the position of the "Living Fossil" (Thomson, 1991). *Latimeria* was the only living genus of the Crossopterygii, a class that had been thought to have gone extinct in the late Cretaceous around 60 million years before (Forey, 1998). With scientists around the globe convinced that this fish was a living link to an intermediate evolutionary step from fish to tetrapod, the hunt for more specimens commenced, and every respectable museum around the world had to have its own specimen (Thomson, 1991). The subsequent capture of more specimens enabled the scientific community to perform an exceptionally thorough study of the anatomy, physiology, and development of this fish, and, with the advent of research submersibles, observations on the fish in its habitat.

Almost every character that shed light on the transition from fish to tetrapod received careful study. The aspects of the organism that were idiosyncratic or that pointed away from the transition toward becoming a tetrapod, such as *Lartimeria*'s fatty sac that took the place of the ossified lung of fossil coelacanths, or the lack of an ossified backbone and ribs, were hardly mentioned (Forey, 2009). A sizeable amount of the coelacanth body plan was hardly studied as it was literally not making the evolutionary grade.

The process of determining the structural and functional significance of the structures within the body of *Latimeria* was based on the overall grade of morphological organization rather than specific relationships to other groups of organisms. The emphasis on the features that pre-adapt an organism to a new way of life, even if these features are primitive, was common until 1950 when Willi Hennig's *Grundzüge einer Theorie der phylogentischien en Systematic* was translated from German into English. The translation of this method caused a nearly immediate switch by key ichthyologists to a specifically hierarchical way of classifying organisms according to the derived characters they share with their relatives, and only those characters. Other features, for instance primitive features that might permit them to enter new adaptive zones, were of no consequence in classification.

The previous gradal approach to classifying organisms encouraged researchers to focus more closely on the attributes that an organism had that might have permitted its descendants to go into new adaptive zones—to be a fish out of

water. Many scientists used gradal studies as a way to show an evolutionary transition through small changes in a structure possessed by a series of organisms. Structural comparisons were often completed under the assumption that natural selection was driving evolution toward the best structure possible, and that changes represented the organisms' evolutionary advances (Goodrich, 1924). In the case of the coelacanths, and *Polypterus*, what were emphasized were the characters that could foreshadow the lives of the first primitive tetrapods in the transition from water to land.

With the adoption of the cladistic method, the scientific study of organismal relationships was drastically altered. Rather than focusing on the potential of structures for allowing new adaptations, structures were evaluated as the mark of shared ancestry. This directly impacted the study of the coelacanths because it raised the question of which cladistic branch of the sarcopterygian fishes was the sister group to the tetrapods.

Cladistics was used to resolve the question of whether or not *Polypterus* was a member of the coelacanths or even a part of the Crossopterygii. After examining 75 characters within the dermal skeleton *Polypterus* was placed outside the Sarcopterygii into the Cladista (a new name for the polypterids), a basal group within the Actinopterygii (Lund and Poplin 2002).

At the time when the gradal method was still widely used, the coelacanths were expected to be intermediates to the tetrapods because their pelvic and pectoral fins contained both endochondral bones and dermal lepidotrichia (Forey,

1998). Meanwhile lungfish were rejected as the predecessors to tetrapods due to their lack of ossified bones that were clearly homologous to the limb bones of tetrapods. Still, some had suggested that fossil lungfish shared many other features with the tetrapods (Moy-Thomas, 1939). In an explicitly cladistic analysis Rosen *et al.* (1981) found much evidence for lungfish being closer to the tetrapods than coelacanths, in spite of the autoplasmic state of their appendages. Once cladistic analysis was used to examine the relationship between the tetrapods, coelacanths, and lungfish, four cladistic trees were produced and debated (Takezaki *et al.*, 2004). Today it is widely accepted that the lungfish, rather than the coelacanths, are the extant sister group of the tetrapods (shown in Figure 2).

Though it is meant to help keep clades clearly separated, the notion that one type of fish cannot be compared to another type of fish struck the paleoichthyologist L. Beverly Halstead (1978) as absurd, and a reason to dismiss cladistics. Halstead begins his criticism of the cladistic system by asserting that evolutionary events are better explained by examining structural grades rather than by tracing structures through sister groups. Halstead asserted that grades of organization in organisms show similarities in body plans and in the forces that organismal bodies have to adjust to throughout development and their lifetimes. He further noted that in order to distinguish between ancestral and descendent organisms, both original and derived characters must be differentiable.
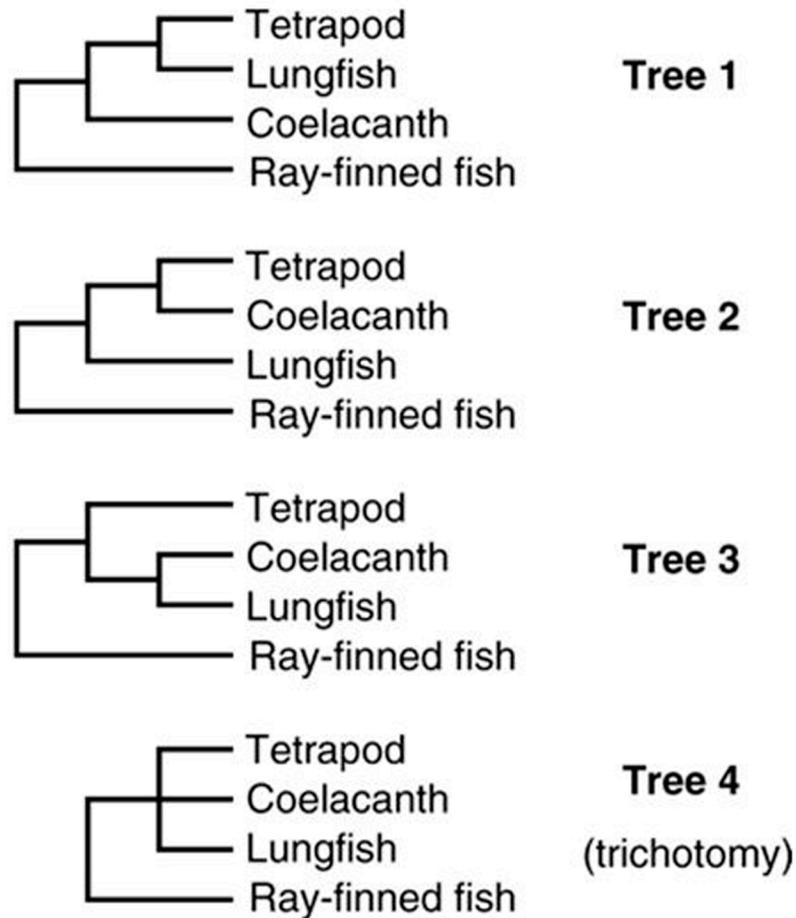
Figure 2. Each of these trees represents possible phylogenetic relationships between lungfish, coelacanths, tetrapods, and ray-finned (actinopterygian) fishes. Tree 1 is the most widely accepted tree, and is supported by whole-genome sequencing. Tree 2 was supported by gradal analysis, while tree 3 depicts a situation in which neither coelacanths nor lungfish are more closely related to tetrapods. Tree 4 shows all three sarcopterygian groups related equally to one another. Trees were taken from Takezaki *et al.* (2004).

This was made more difficult by the fact that cladistics, according to Halstead, hardly took into account the attributes of fossils and the relationships that existed between fossil species. Without using the fossil record, which will always be incomplete, it is impossible to know whether or not a character is the first of its kind, or a derived version passed down from an unknown ancestor (Halstead, 1978).

Halstead's rejection of the validity of the cladistic system brought forth a vigorous response. The exchange became widely known as "The Salmon, the Lungfish, and the Cow." Cladists, such as Colin Patterson, Phillipe Janvier, and Peter Forey, argued that cladograms are meant only to show sister group relationships based on derived characters. They asserted that if one were to try to compare teleosts and sarcopterygians there would be no way to show homology across the clades and that there could be no presumption that similar structures developed or functioned in similar ways (Gardiner *et al.*, 1979).

Phylogeny of the Coelacanthiformes

Today, all coelacanths are classified as one of three subgroups within the Sarcopterygii. The other two members are the Ceratodontiformes (lungfish) and the Tetrapoda. It is accepted now that the members of the Coelacanthiformes are the basal members of the class; they are not the sister group of the Tetrapoda (Nelson, 2006; Rosen *et al.*, 1981).

The order Coelacanthiformes comprises nine families that are cladistically united by 16 characters, and are set apart from the Dipnoi (lungfishes) by the presence of a rostral organ located at the tip of the fish's snout (Nelson, 2006; Forey, 1984). Each of the families comprising the Coelacanthiformes is monophyletic except for Rhabdodermatidae. The families Miguashaiidae and Diplocercidaeare are often placed as the basal families of the order with the Hadronectoridae just above them. Slightly more derived than the Hadronectoridae is the Rhabdodermatidae. The Rhabdodermatidae family is paraphyletic, and is the stem group to all coelacanths within the Coelacanthidae, Mawsonidae, Laugiidae, Whiteiidae, and Latimeridae families (Nelson, 2006; Forey 1998). This means that essentially each of the more derived coelacanth families has been picked out of the Rabdodermatidae in order to emphasize common synapomorphies that connect the genera in that family. The families arising from the Rhabdodermatidae form the crown group of the more derived and best known coelacanths (Donoghue, 2005).

The Latimeriidae is the most derived family within the Coelacanthiformes. This family contains the living species of the genus *Latimeria*, along with members from the extinct genera *Undina, Macropoma, Macropomoides, Lybis, Megalocoelacanthus,* and *Holophagus* (Clément, 2005; Cloutier and Ahlberg, 1996). This family spans from the early Jurassic through the second half of the Cretaceous and is still found today (Forey 1998; Thomson, 1991). Within this family, *Latimeria* and *Macropoma* are sister groups. Between these two genera,

the most striking anatomical difference is the presence of a bony lung in *Macropoma* where a fatty sac is found in *Latimeria*. All of the coelacanth families except for the *Diplocercidae* are represented in Figure 3.

A hard bony lung structure has been described beginning from the family Hadronectoridae in the early Carboniferous period and following through to members of all but one of the more derived families up to the end of the Cretaceous. The only families lacking an ossified lung in the crown group are Whiteiidae and individuals from the genus *Latimeria*. The two individuals in the Diplocercidae and Miguashaiidae have also been found without this structure. This structure is also absent in the genus *Diplurus* from the family Mawsoniidae, both of which are surrounded by genera that possess this bony lung. In both *Diplurus* and *Whitea* it is likely, considering that each of their sister groups had this hardened structure, that they secondarily lost it (Nelson, 2006) (Figure 3).

This is not to say that it is impossible or even unlikely that the more primitive and basal members within the order Coelacanthiformes may have had this bony structure during their lifetime. It is possible that an ossified lung may have existed in the more basal families, but it did not fossilize; It could have been very thin, or dissolved shortly after the death of the fish. This could be due to the organism's geologic surroundings at the time the taphonomic process began. In this regard, it is worth noting that most of the more ancestral families and genera have been described from somewhat incomplete specimens (Forey, 1998). Until more complete and better preserved specimens of these more basal families are

found it is impossible to know exactly how far back the bony lung goes within the coelacanths (Rudwick, 1964).

Despite the initial attention to the existence of the bony air-bladder found in *Macropoma mantelli* by both Agassiz (1833-43) and Williamson (1849) this anomalous structure received little subsequent attention. In each new genus and species found to have this character, this structure would be mentioned in the initial description, but no further work on the formation, histology, or function of this structure was published, despite its presence in over a quarter of the coelacanth genera known today (Forey, 1998). Nothing further has been described about the bony lung besides its existence in members of the genera *Rhabdoderma, Coelacantus, Mawsonia, Laugia, Undina, Holophagus, Polyosteorhynchus, Libys, Cardiostuctor, Hadronector, Allenypterus, Piveteauia, Swenzia,* as well as one species of the genus *Coccoderma* (Brito *et al.* 2010; Forey 1998; Frickhinger, 1994; Clement, 1999; Moy-Thomas, 1939).

The subject of this anomalous bony air-bladder was left untouched for one-hundred and sixty-one years until a group published a study of its structure in the lower Cretaceous genus *Axelrodichthys araripensis* from Brazil (Brito *et al.*, 2010). Their exploration of this bony lung also considered possible functions. They propose that this structure may have been used as a lung for respiration or swim bladder for hydrostatic balance. For hydrostatic balance, the organ would have to be filled with air or oil, since either is less dense than water.
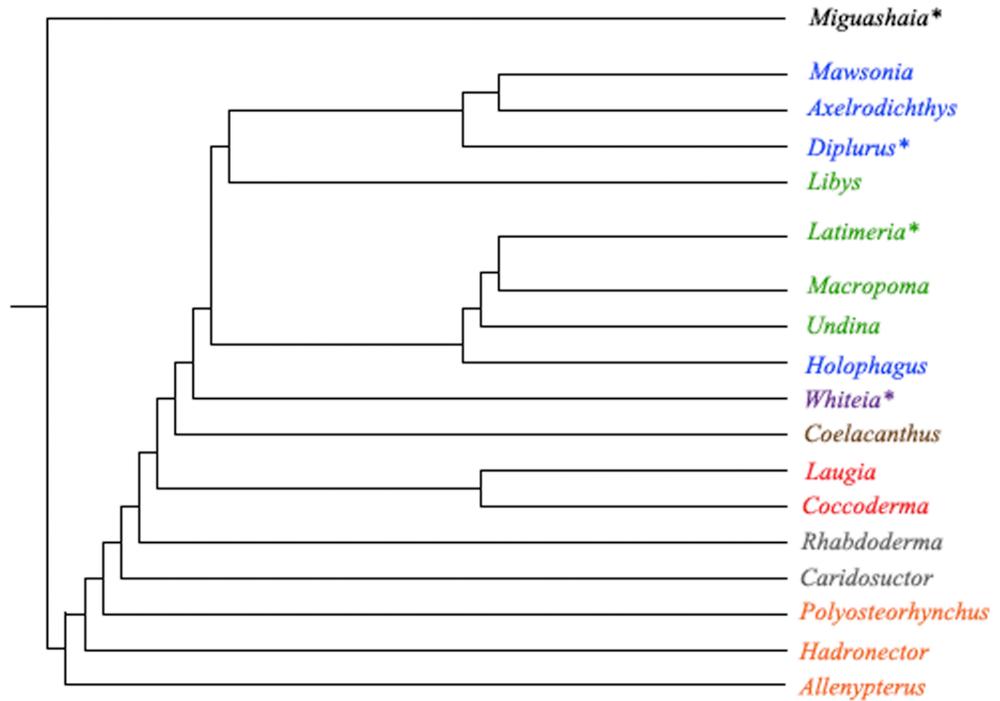
Figure 3. Tree based on Forey (1998) showing coelacanth genera that possess the bony lung. Families are denoted by color: orange is the Hadronectoridae, gray is the Rhabdodermatidae, red is the Laugiidae, brown is the Coelacanthidae, purple is the Whiteiidae, blue is the Mawsonidae, green is the Latimeriidae, and black is the Miguashaiidae. Genera with an * next to their name are genera that have either secondarily lost the bony lung (*Whiteia, Diplurus,* and *Latimeria*), or are the out group (*Miguashaia*), as the least derived of all coelacanth genera. Forey did not include *Piveteauia* (Whiteiidae), *Macropomoides*, or *Swenzia* (both Latimeriidae), all of which have ossified lungs. It is important to note the phylogenetic distance between *Macropoma* and *Axelrodichthys*.

Having some concentration of either air or oil at the center of a body would facilitate the fish's ability to stay at a constant depth. Another of the functions that they propose is that this bony structure surrounds a membranous lung that acts as an auditory organ in the same way that it does in many teleost fishes. This, however, would require a physical linkage to the inner ear analogous to the Weberian apparatus of the teleost superorder Ostariophysi. No such connection is known in any coelacanth. In the cobitid loaches, this direct connection is achieved with a thin and bulbous bone structure that extends from the vertebral column to touch the most anterior-dorsal part of the swim bladder. Also, in *Macropoma* the ossified organ opens anteriorly. Only a closed, fixed volume could be used to pick up sound waves under water. As an alternative, they suggested that the ossified sac could be a sound production organ that may have functioned like one of the many found in teleost fishes. Their reasoning for this was simply that *Latimeria* has been discovered to live in social groups, and an ability to produce sound might be useful for communication (Brito *et al.*, 2010). But socializing by way of sounds would be more convincing if there were an obvious organ of hearing in coelacanths. Indeed, if hearing was an element of sociality in the Cretaceous, why has it since disappeared?

The presence of the fat-filled oily sac in *Latimeria* was noticed upon the first dissection this fish in 1938. This large organ was immediately assumed to be homologous to the swim bladder that would have been integral to the animal's

ability to maintain hydrostatic position in its deep sea habitat (Owen, 1843; Thomson, 1991).

The Bony Lung and Lung Formation

The bony lung found in many coelacanth genera can be compared to other thin and layered bones. What is unique about the bony lung of coelacanths, the Weberian apparatus of some cyprinid fishes, the sound-producing bony organs found in some teleost fishes, along with the sclerotic rings found in amphibians, reptiles and birds is that these structures are composed of true bone (calcium phosphate) rather than a simple calcification (calcium carbonate) of some type of an extracellular matrix.

It is commonly accepted that endodermal tissues (found in the respiratory, digestive, and urinary tracts as well as some elements of the endocrine system) are not associated with bone. Mesodermal tissue (an extracellular matrix) is characterized by having cells with a higher affinity for a ground substance than for other cells of the same type (Arey, 1947; Picken, 1960). This is seen in Figure 4 in cross-sections of mammalian long bones where each osteocyte is surrounded by a self-secreted hydroxylapatite ground matrix and does not touch another osteocyte except through long and thin cytoplasmic processes within the surrounding bone matrix (Kristić, 1985).

All bone is associated with and derived from mesodermal tissue. Endochondral bone is formed by bone cells surrounding previously laid down cartilage, which they secondarily break down and replace with bone cells and a calcium-phosphate matrix (Torrey, 1962). The formation of this bone type requires a cartilaginous scaffolding to be built first (Murray, 1936; Picken, 1960). In fossils of juveniles cartilaginous structures are less likely to fossilize than the bone structures that have not yet replaced them.

Despite this, the location of the bone can oftentimes be used as an indicator of the bone's origin. For example, dermal bone, otherwise known as membranous bone, is usually found in the head region of vertebrates. Dermal bone is formed primarily through the ossification of previously established membranes by osteoblasts, which during ontogeny, subsequently become submerged in the mesoderm below the site of their origin (Arey, 1947). What can be determined from histological examination is whether the bone is cellular (having lacunar spaces where osteocytes previously existed) or acellular (a derived bone type characterized by those lacunar spaces having been secondarily filled with bone after the initial bone formation) (Moss, 1963; Parenti, 1986). A well known example of acellular bone can be seen in the opercular bone of *Fundulus* (Moss, 1961) (Figure 5).
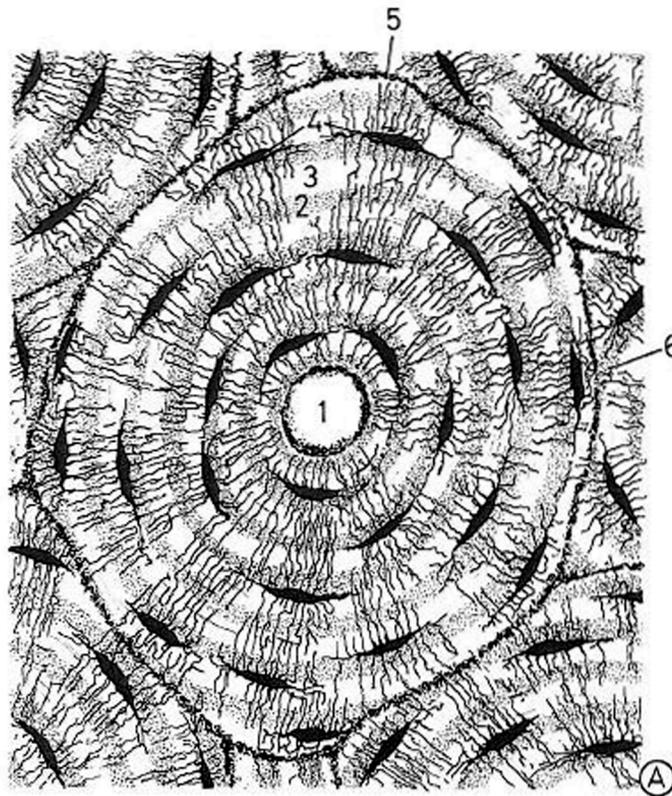
Figure 4. The microstructure of lamellar bone. In this system osteocytes in their lacunae (4) are regularly arranged around Haversian canals (1). They are able to communicate with the cells around them through their canicululular extensions (5). From Kristic (1985).
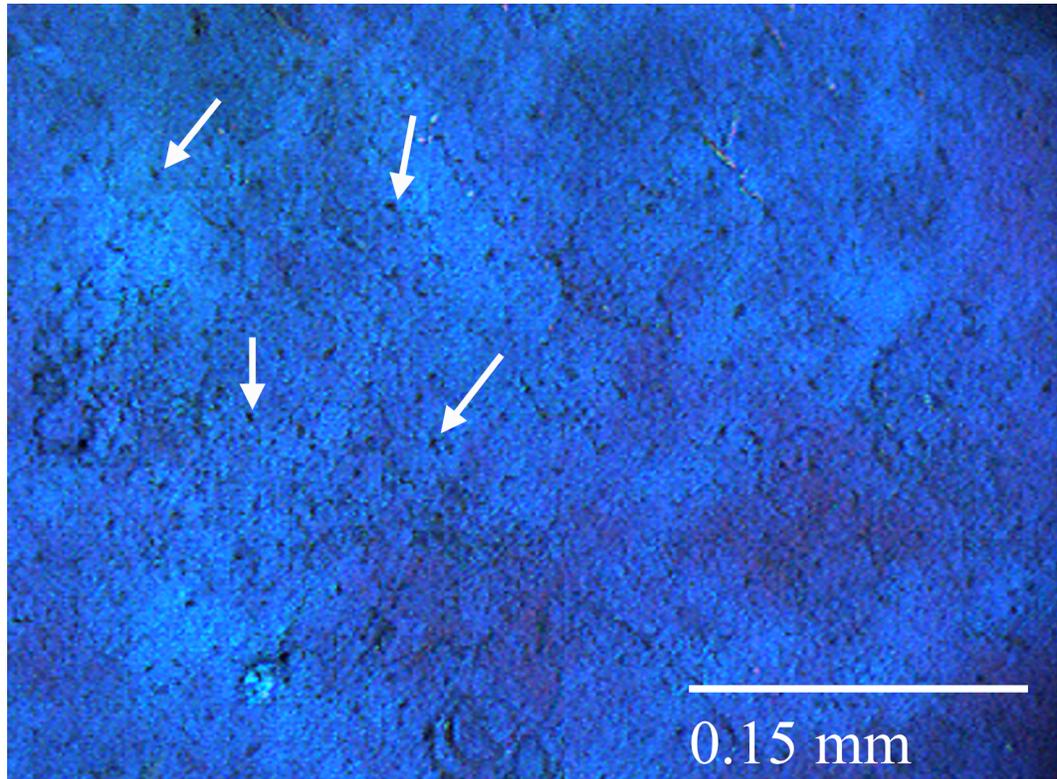
Figure 5. Torn cross-section of *Fundulus* acellular bone from the operculum with arrows indicating the remnants (pinocytes) of secondarily infilled osteocyte lacunae.

The presence of lungs or swim bladders in coelacanths has been widely debated. Williamson (1849) was the first to call this structure an air-bladder while describing a specimen of *Macropoma* showing the plated bone structure. From this point, it was assumed that the air-bladder was possessed by all coelacanth genera, until Brito *et al*. (2010) termed this structure a lung due to its previously described ventral location (Maisey, 1986). The fat-filled sac found in *Latimeria* also inserts ventrally, and was described as homologous to the lung found in the extant lungfishes (Brito *et al.*, 2010).

It is possible that juvenile coelacanths had unossified lungs. These lungs could have been covered in a collagenous membrane that is the first stage of dermal bone formation, or a thin layer of cartilage, if the ossification were endochondral. According to Witzmann *et al*. (2010), a juvenile coelacanth from the genus *Rhabdoderma* (known to possess the ossified lung as an adult) was found without an intact ossified lung. However, this claim was based on a specimen that was poorly preserved and did not have an intact pelvic girdle. Because this specimen was poorly preserved it does not support such a claim.

A well preserved specimen of *Macropomoides* has been found with the ossified lung left intact. Though this specimen is only 126 mm long, about half the length of other reported specimens in the genus, the lung is still present (Figure 5). This size difference suggests that this coelacanth specimen is a juvenile, indicating that it is likely that juvenile coelacanths possessed fully ossified lungs. In order to make an informed conclusion as to whether or not

Figure 6. Juvenile *Macropomoides* specimen. The arrow indicates the presence of the bony lung in this specimen.
Picture from http://stores.ebay.com/PaleoMarket/ April 20, 2011.

juvenile coelacanths consistently possess the ossified lung, a larger sample of juvenile coelacanths will need to be discovered.

Analogy in Biology: the Cobitid, the Coelacanth, and the Cowbird's Eye

Marvalee Wake (1992) argued that there are many valid ways to practice comparative biology. In order to study the function of a morphological character within an organism it is often necessary to look outside the living world to find the most similarly functioning structure possible. Sometimes this means looking to machines in order to find model structures that might operate like a fossilized structure.

This method was used to describe how the Permian brachiopod *Prorichthofenia* would have pumped its upper valve in order to pull food to its lophophore in a way that resembles how a Chinese smelting bellows pumped air into the fire (Rudwick and Cowen, 1968). To look outside the organismal world and into the mechanical realm is a farther leap than looking to an organism that lives and deals with similar living conditions, even if it is in another clade, and independently developed the structures under comparison.

Cladists believe that homology between two structures can only be expressed by organisms within a clade. To circumvent cladistic terminology, Wake began comparing organisms from separate clades under the banner of

evolutionary morphology. This allowed her to compare the flapping patterns of "water wings" in the planktonic gastropod *Clione* and the pigeon and note the similarities of their flights in water and air, in spite of sharing no synapomorphies in their appendages. Their wings are analogous structures, but they provide useful generalizations about flight (Wake, 1992). By working outside the cladistic method, and focusing on using morphology as a descriptive rather than categorical science Wake was able to justify comparing organisms from very different clades.

Comparing across clades is also useful when thinking about similar structures found in other vertebrates and the bony lung in coelacanths. The Weberian apparatus is a derived feature found in most members of the superorder Ostariophysi, the orders Siluriformes, Characiformes, and Cypriniformes, all of which are freshwater fish. These bones amplify and convey sound waves that travel from the swim bladder, where a fixed airspace is compressed as sound waves pass through it. The ossicles are composed of the first four vertebrae behind the skull. Each vertebra has at least two processes that transmit sound waves to the membranous inner ear labyrinth situated above and below the posterior margin of the skull (Brown, 1957). Vibrations are transmitted by the tripus to the intercalarium, then the scaphum, and finally to the claustrum, which finally transmits the energy from the sound waves to the inner ear labyrinth. In the cobitids (Cypriniformes) the tripus takes the form of two hollow spheres that

remain attached to the vertebrae and the anterior surface of the swim bladder (Pough *et al*., 2009).

In other fish, bony structures are used to create sound while using the swim bladder as a resonating chamber. Two examples of this kind of bone-swim bladder relationship can be found in the sonic apparatus of the cusk-eel and the rocker bone in some ophidiform fishes. The sonic apparatus in the cusk-eel, like the Weberian apparatus, is a modified process attached to a vertebra. In this case, the first rib extending from the first vertebra posterior to the cranial region is expanded into a wing-like process on each side of the spinal column that associates directly with the thickened and anterior-most portion of the swim bladder. Sounds are produced when the thickened wall of the swim bladder presses against the extended process (with the help of attached musculature) and is released resulting in a twitch sound (Fine *et al*. 2007). The rocker bone is also found at the thickened anterior-most portion of the swim bladder in some ophidiform fishes. This bone is also used for sound production, but instead of being formed as an extension or modification of a vertebral process, this bone is formed by a collection of spherules below the spinal column. These spherules are composed of bone fibrils and bioapatite that congregate below the second, third, and fourth vertebrae. Subsequently the spherules are covered by a thin outer layer of bone, producing a new kind of bone (Parmentier *et al*. 2008). The kidney-shaped bone grows in front of the swim bladder. Here two structures comprised of bone are directly associated with the endodermally formed swim bladder.

These two sound production organs, in conjunction with the Weberian Ossicles in the Cobitidae, and the ossified lung of some coelacanths, present situations in which bone makes direct contact with an endodermal structure. This kind of mesodermal relationship to an endodermal structure is generally seen where smooth muscle is wrapped around the gut in order to perform peristalsis and in capillaries to facilitate blood movement in places like the gills, though it is rare to find bone associated with an endodermal structure.

All bone structures can be assumed to have originated from mesodermal tissues. The ossified sclerotic rings (which are found in basal Actinopterygii, along with basal and more derived Sarcopterygii such as the Aves) are originally formed in the dermis as membranes that secondarily ossify (Walls, 1942). These bones, following their initial formation, descend into the center of the developing eye after the optic cup engulfs the nascent bony plates (Franz-Odendaal, 2008). Inside the eye these plates form a ring around the cornea. These plates are used, in conjunction with musculature to adjust the lens of the eye. Other musculature in the eye is used to keep the eye spherical and is used along with the sclerotic rings to keep the perceived image focused on the retina (Walls, 1942). By manipulating the shape of the ossified ring during times of varying external pressure applied to the eye, the eye is able to greatly diminish the astigmatism of the image.

Also found in the Aves are cartilaginous rings that are often completely ossified, and surround the inner membrane of the trachea. These fibrocartilage rings interlock in the form of alternated signet rings (McLelland, 1965). When

ossified, these rings maintain the signet ring formation, and a somewhat uniform size. When ossification occurs as it does in Chicken (*Gallus*) it is often at the most posterior portion of the trachea. Only in specific cases like in the trumpeter swan (*Cygnus*) is the entire trachea surrounded with ossified tracheal rings (Hogg, 1982). Both the stiffened fibrocartilage rings and the ossified tracheal rings have been proposed to provide support to the elongated necks of almost all birds (Owen, 1866). In this case, the close association of the ossification surrounding the endodermal trachea and the trachea itself is most likely not induced by the endodermal trachea, but has become canalized to provide a function for the organism.

While the Weberian ossicles, the rocker bone, the sonic apparatus, the sclerotic bone plates found in birds and fishes, along with the ossified tracheae found in members of the Aves are all unusual bone structures that appear to associate at mesodermal-endodermal junctions in odd ways, the lung of many fossilized coelacanths appears to be giving rise directly to a plated bony structure. This ontogenetic relationship is very unlikely because, even taking the aforementioned bone structures into account, endodermal tissue does not historically give rise to either dermal or endochondral tissues.

Though it has not historically been documented elsewhere, ossifications have been known to appear in the pleural membrane and the pleura itself in humans, both of which are endodermal in origin. Though this occurrence is rare, it has been observed often enough to have been published on repeatedly. This

ossification is thought to occur following an injury to the pleura from any number of causes (lung cancer, transplant, and fibrosis) that subsequently requires angiogenesis during the reconstruction and healing processes. As part of the process of activating angiogenesis in the lung, parathyroid hormone is released. Parathyroid hormone, along with the mobilization of calcium from long bones, activates osteogenic protein-1 and vascular endothelial growth factor. The production of osteogenic protein-1 up-regulates alkaline phosphatase activity and consequently activates osteoblastic differentiation in the pleura. Endothelial growth factor is also stimulated by prostaglandin E_1 and prostaglandin E_2 which stimulate bone formation *in vivo* (Chan *et al*., 2002). The fact that a diseased or injured lung can undergo bone formation within the endothelium shows that it is likely that an ancestral form of *Homo sapiens sapiens* (a member of the Sarcopterygii) was able to produce bone from within the endothelium of the lung (Peros-Golubicić and Tekavec-Trkanjec, 2008).

Lungs are believed to have arisen from gill slits that did not break through from the gut to the exterior of the fish (Fox, 1959). They have been reported in placoderm fishes as a pair of pouches (Torrey, 1962). Lungs are formed during ontogeny after the formation of the archenteron as an out-pocketing of the esophagus at the laryngeal groove (Goodrich, 1931). This out-pocketing is formed ventrally to the digestive tract (Kardong, 1995). The lung does not, however, have to remain ventral to the digestive tract throughout the body cavity. This is seen in *Polypterus* where the lung arises ventrally to the digestive tract, but turns up to

become dorsally located over the digestive tract at its posterior-most position (Goodrich, 1931). Swim bladders are a more derived form of the lung. A swim bladder differs from a lung in that it is formed dorsally to the digestive tract, and is often closed off by a sphincter called a pneumatoduct, to the esophagus. This closure allows the swim bladder to be used as a hydrostatic organ as long as it makes up at least 5% of the total body volume in marine fishes, and 7-10% in freshwater fishes (Brown, 1957).

Lungs bring in oxygenated air upon inhalation and release oxygen depleted air during exhalation through the anterior opening in the organ. Two pumping mechanisms have been proposed for ventilating fish lungs in and out of the body cavity in order to achieve the highest possible tissue oxygenation. While all lunged fishes known today use bimodal respiration (gills are the primarily respiratory organ, with air breathing by the lung used secondarily), often when lung ventilation is used, not all of the deoxygenated air from the lung or buccal cavity of the fish is expired (Brainerd *et al*, 1989; Brainerd, 1999).

This is not the case for fishes with swim bladders. With swim bladders, fish often use gills as their only mode of respiration, though some fish like the Cobitidae can gulp air bubbles into the buccal cavity in order to obtain some oxygen when facing hypoxic conditions (Hutchinson, 1941).

My study focused on describing the bony lung in *Macropoma* with respect to its developmental fabrication, functional anatomy. The development of the bony plates around the lung was examined to understand the when, during

development, the bony lung would have been formed. The proposition of possible functions by what can be inferred gives explanations as to what the structure may have been doing, if it was useful, within the fish. The validity of these functions was bolstered by comparing the bony lung in *Macropoma* with the bony lung in *Axelrodichthys* and with other organisms that use bone in direct contact with endodermal organs to deal with changes in pressure or as a way of holding an airspace open.

MATERIALS AND METHODS

Small *Macropoma* Specimen

The specimen contains a portion of the anterior bony lung with fragments of the operculum at one edge. There are also some lepidotrichia at the dorsal margin of this specimen where the dorsal fin would have inserted. This specimen was donated to this project by Dr. Stan Rachootin who has had it in his possession since 1973. This specimen was observed and photographed before it was sectioned one centimeter from its anterior margin. A thin section was prepared by Gerald Marchand by gluing the section to a slide with epoxy, and then grinding it down. Once the section was thin enough for viewing, it was placed under a Leica DM EP polarizing microscope at magnifications of 10x, 20x, and 63x.

To photograph the section under polarized light a Moticam 1000 1.3M pixel camera was placed over the 10x eyepiece of the microscope and was attached to a computer through a USB connection. Photographs were processed using both the Motic software for the Moticam 1000, and using Adobe Photoshop CS4.

The surface of the fossil, which is composed of exposed bony lung plates, was placed in the Quanta 200 Scanning Electron Microscope at Low Vacuum. The EDAX Genesis was used to determine the elemental composition of the bony plates and the surrounding chalk.

Macropoma AMNH Specimen #10368

This specimen was borrowed from the AMNH Fossil Fish collections. It was photographed using a macro lens feature on a Panasonic DMC-ZR3 digital camera. The specimen was observed on an Olympus SZ-STS dissecting microscope, and pictures were taken using a color Pixelink camera attached to the microscope. The bony plates were measured using digital calipers. Photographs were prepared in Adobe Photoshop CS4.

Axelrodichthys AMNH Specimen #12213

This specimen was also obtained as a loan from the AMNH Fossil Fish collections. It was sectioned sagittally at the center of the fish. This section was placed in the Quanta 200 SEM under low vacuum. A thin section was prepared in the same manner as the *Macropoma* section. This section was viewed under polarized light, and was photographed using the same equipment and settings used for the *Macropoma* section. Photographs were processed using Adobe Photoshop CS4.

Hydrostatic Function in *Macropoma* AMNH FF #10368 and *Axelrodichthys* AMNH FF #12212

The volume of each fish was calculated as a cylinder using the average of ½ the head width, ½ width of the body, and ½ the width of the tail as r_1 and the total length of the fish as r_2 in the equation $V = \pi r_1 r_2 h$. The volume of the lung was

calculated using the equation for the volume of the cone: $V=1/3 \pi r_1 r_2 h$ where r_1 was measured as ½ the dorso-ventral diameter of the lung, r_2 was measured as ½ the lateral diameter of the lung, and h was measured as the total length of the entire bone structure.

Fundulus

This specimen was euthanized using MS 222 (Tricane Methane Sulfonate); it was eviscerated and then skeletonized using a colony of sow bugs. The operculum, which is known to be laminar acellular bone, was snapped in half, and observed in cross section under SEM at low vacuum (Moss, 1963). It was also viewed under polarized light using the same polarizing light microscope conditions as used for other specimens.

Cobitidae

Two specimens of *Misgurnis anguillicaudatus* were collected after they were found dead in their fish tanks. For each, the distal abdomen was opened and was left to dry for two days. The specimens were then moved to the sow bug colony for skeletonization. One specimen was photographed using the Olympus dissecting microscope and attached Pixelink camera.

One specimen of *Yasuhikotakia modesta* was found dead in its tank. It was immediately removed and prepared for enzyme clearing and staining using alcian blue and alizarin red. This fish was fixed in 95% ethyl alcohol for two weeks.

Afterward it was transferred through four changes of distilled water, and placed into a solution containing 20mg alcian blue, 160ml of 95% ethyl alcohol, and 40 ml glacial acetic acid for two days. After two days this specimen was put through two changes of 95% ethyl alcohol followed by two hours in, successively, 75%, 40%, and 15% ethyl alcohol. The fish was then transferred to distilled water until it sank, approximately 2 hours later. It was then transferred into a solution containing 60ml aqueous sodium borate, 140ml distilled water and 2 g porcine trypsin enzyme. This solution was made fresh and the fish was transferred into the fresh enzyme solution, until the ribs were clearly visible through the skin. This took four weeks. Once the blue-stained bones were visible through the skin the specimen was cleared in a solution containing 100ml of 0.5% aqueous potassium hydroxide (KOH) solution and 2g of alizarin red. The specimen remained in this solution for 24 hours and was then removed and run through a series containing 100ml of 0.5% KOH and pure glycerin at ratios of 3:1, 1:1, and 1:3. To this series 4 drops of 3% hydrogen peroxide was added to remove remaining dark pigmentation. Once this series was finished the specimen was placed in 100ml of pure glycerin with two crystals of thymol (Clearing and staining protocol by Dingerkus and Uhler (1977)).

This specimen was examined and photographed under the Olympus dissecting scope and was photographed using the attached Pixelink camera.

RESULTS

Macropoma

At first sight the lung of *Macropoma* resembles a shingled cone, open anteriorly and tapering closed at its posterior end. Overlapping plates surround the elongated lung. Each plate appears to form a portion of a ring around a space filled with chalk. The edge of each plate is overlapped posteriorly by its succeeding plate. Each full ring of plates decreases in diameter the more posterior it is, culminating with a conical cap at the very end (seen in Figure 6).

Each plate is curved toward the long axis of the lung with more curvature occurring at the dorsal and ventral edges of the lung. From what can be measured from the articulated *Macropoma* lung specimen (AMNH FF 20368), the exposed portion of each plate averaged 10.91mm over eleven total plates. The length of the exposed portion of each plate decreased as each plate was further from the structure's anterior opening. The bony lung does not span the whole body cavity of the fish, but does reach posteriorly to the point of the caudal fin insertion. This fossil specimen contains at least two-thirds of the whole length of the fish, which is indicated by the presence of both the dorsal and adipose fins above the ossified structure, as is indicated in Figure 7. It is likely that in the portion of this fossil that is no longer present, the plates would have decreased in size, as shown in Agassiz's (1833-44) figure of *Macropoma* (refer to Figure 1).

Figure 7. AMNH Fossil Fish Specimen 20368. This *Macropoma* specimen is a nearly complete bony lung. It is widest at its most anterior point (A), and comes to a point at its most posterior point (P). Each plate overlaps the plate just anterior to it. The final posterior plate forms a conical cap.
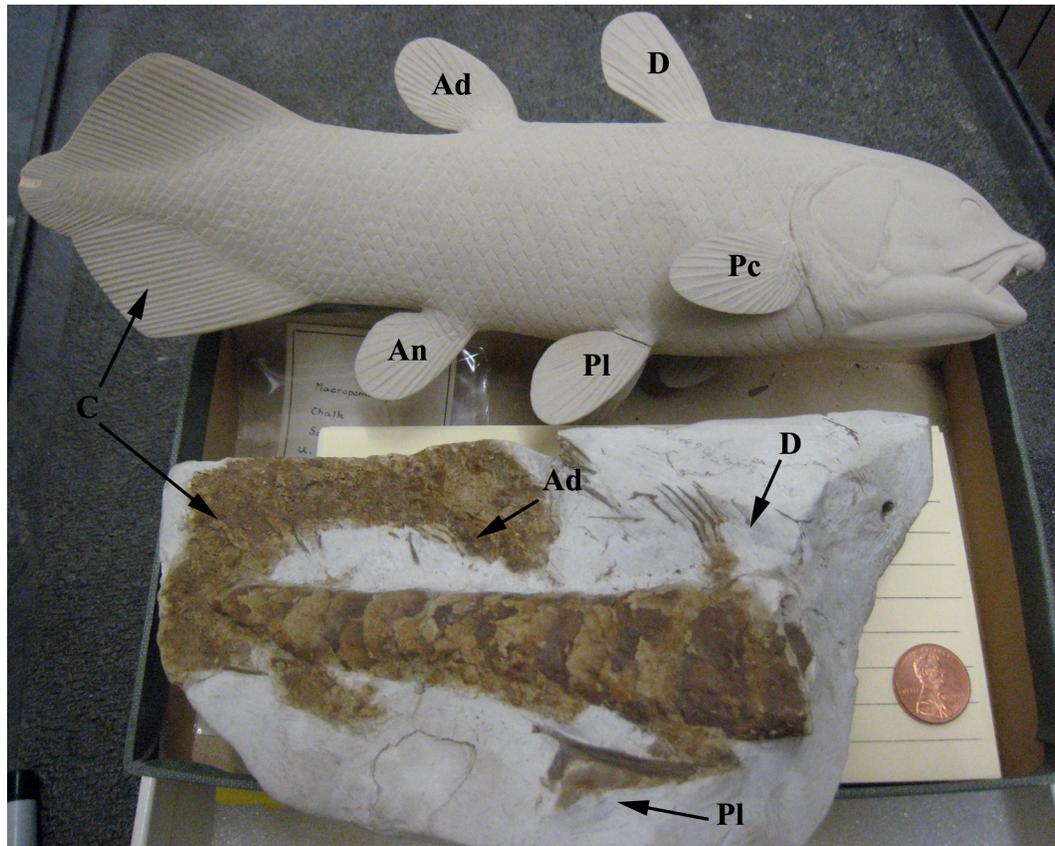
Figure 8. AMNH FF 20368 and model. This life sized *Macropoma* model was scaled to the size of the *Macropoma* fossil below it. Each fin has been labeled to show what parts of the actual fish are represented on the fossil specimen. Ad: adipose fin, D: dorsal fin, Pc: pectoral fin, Pl: pelvic fin, An: anal fin, C: caudal fin.

Every plate in both of the *Macropoma* specimens studied possessed a ridged outer surface (Figure 9). The ridges of one plate line up directly with the ridges of the overlapping plate in a complimentary fashion so that where the ridges of the bottom plate rise, there is a depression of the same dimensions in the overlapping plate, and vice versa. This creates what appears to be a track-like system where each plate would be guided by the plate above and below it. Using these tracks, the plates would be able to slide over one another either slightly expanding or contracting the lung. There seems to be no mechanism that would allow the diameter of the lung to change except slight changes accompanying the intussusception of the plates. The ridges of the plates have an average width of 42.74μm.

EDAX (Energy Dispersive Spectroscopy) was used to determine what elements were present in both the fossil and the substrate surrounding it. It was found that in the chalk surrounding the *Macropoma* lung there was hardly any phosphorous present. In comparison, when the SEM (Scanning Electron Microscopy) beam was aimed at the bony plates within the fossil, phosphorous was easily detected. Both the chalk and the fossil were shown to have high amounts of calcium present. The increased presence of phosphorous as well as calcium in the plates suggests that these plates would have been composed of a mineral salt such as hydroxylapatite ($Ca_5(PO_4)_3(OH)$), the principal mineral in bone (Figure 10).

6/10/2010 | HV | Sig | Spot | WD | Mag | Pressure | ———100.0µm———
3:50:44 PM | 15.0 kV | SE | 4.0 | 14.8 mm | 413x | 0.45 Torr
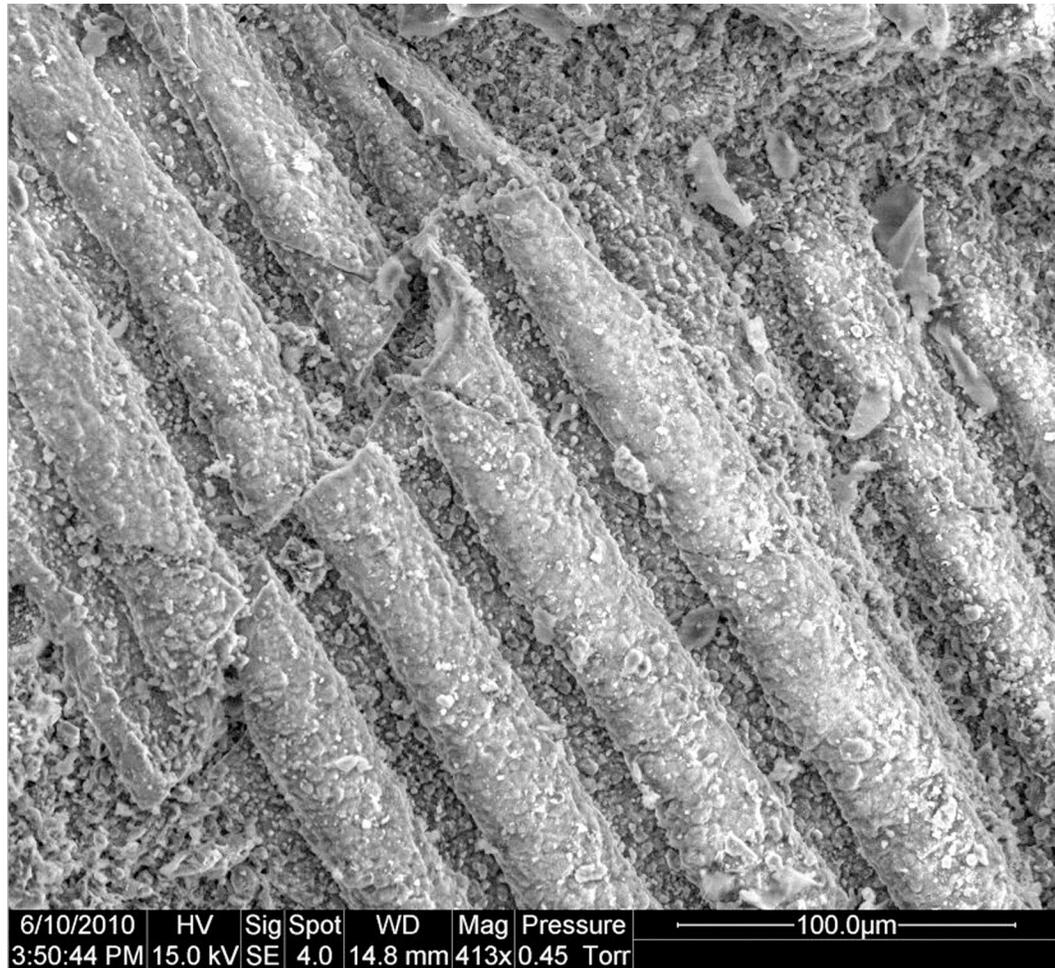
Figure 9. Top view of *Macropoma* bone plates. These plates are parallel to the long axis of the body, and all ridges are marginal extensions that remain attached to the plates they originated from.
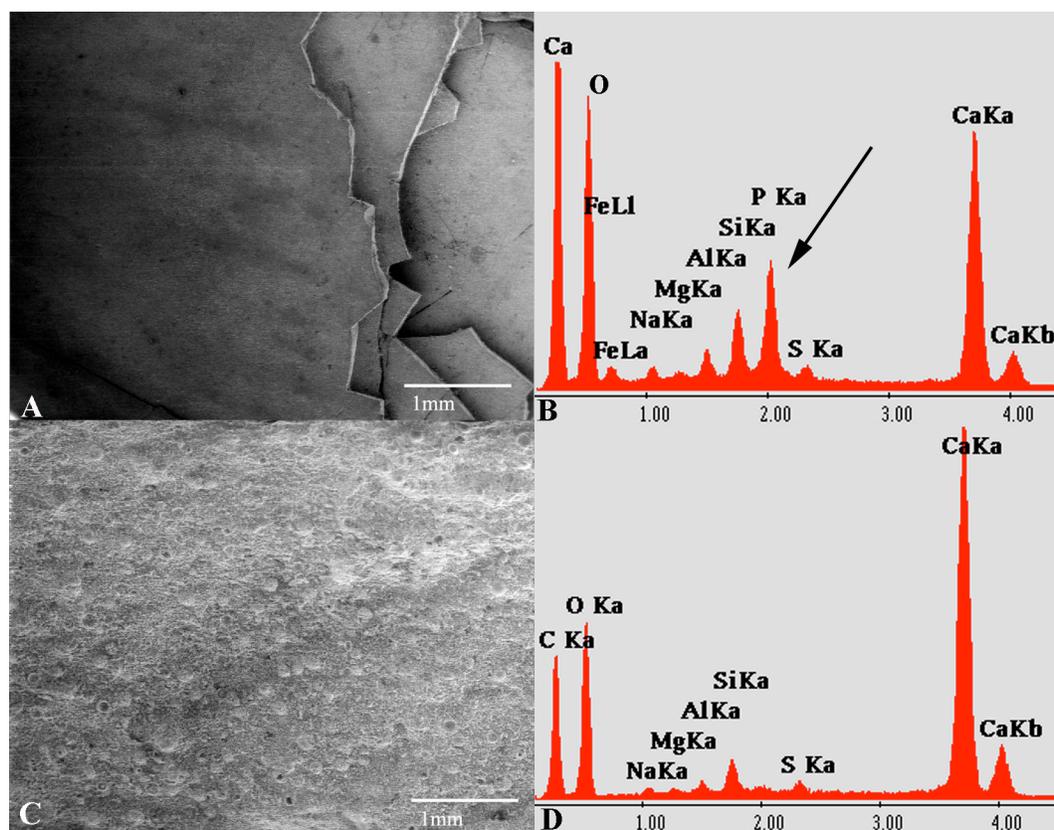
Figure 10. EDAX elemental analysis on *Macropoma* bony lung. The plates (A) from the *Macropoma* specimen and elemental composition from that field of view (B) was taken. The arrow shows the sizeable presence of phosphorus found in these plates. A view of the chalk surrounding the *Macropoma* specimen under SEM and elemental analysis from that field of view (D).

When observed in thin cross-section and under polarized light, the ossified plates averaging 53.3μm with maximum and minimum plate widths of 100μm and 10μm respectively, display lacunae indicative of cellular bone (Moss, 1961). These lacunae often resemble irregularly shaped stars with spindly projections (osteocytic processes) extending away from the cell body (Kristić, 1985). In *Macropoma*, the average cellular volume (using the equation $L(B/2)^2$ from Thomson (1991)) is 952.85μm³ which is small when compared to the range of cell volumes measured in the closely related Dipnoi (Thomson, 1972). This volume, however, is decidedly larger than the average osteocyte volume in these cells also appeared to be very densely distributed throughout the bone matrix. The cellular density of the Macropoma bone layers is 950.27 osteocyte lacunae/mm². This density is comparable to the 942.8 osteocyte lacunae/mm² found in the femoral trabeculae of rats (Mullender *et al.*, 1996). The highest cellularization in the *Macropoma* plates is found under the ridges where one cellular lacunae can be observed under each ridge (Figure 11). These lacunae are identified as osteocytic because they are similar in size and shape to the osteocytic lacunae found elsewhere in the bone matrix. They are also not contiguous with a small amount of bone matrix separating each lacuna. The cells underlying the ridges composed on average 54.12% of the total cells in each layer. These
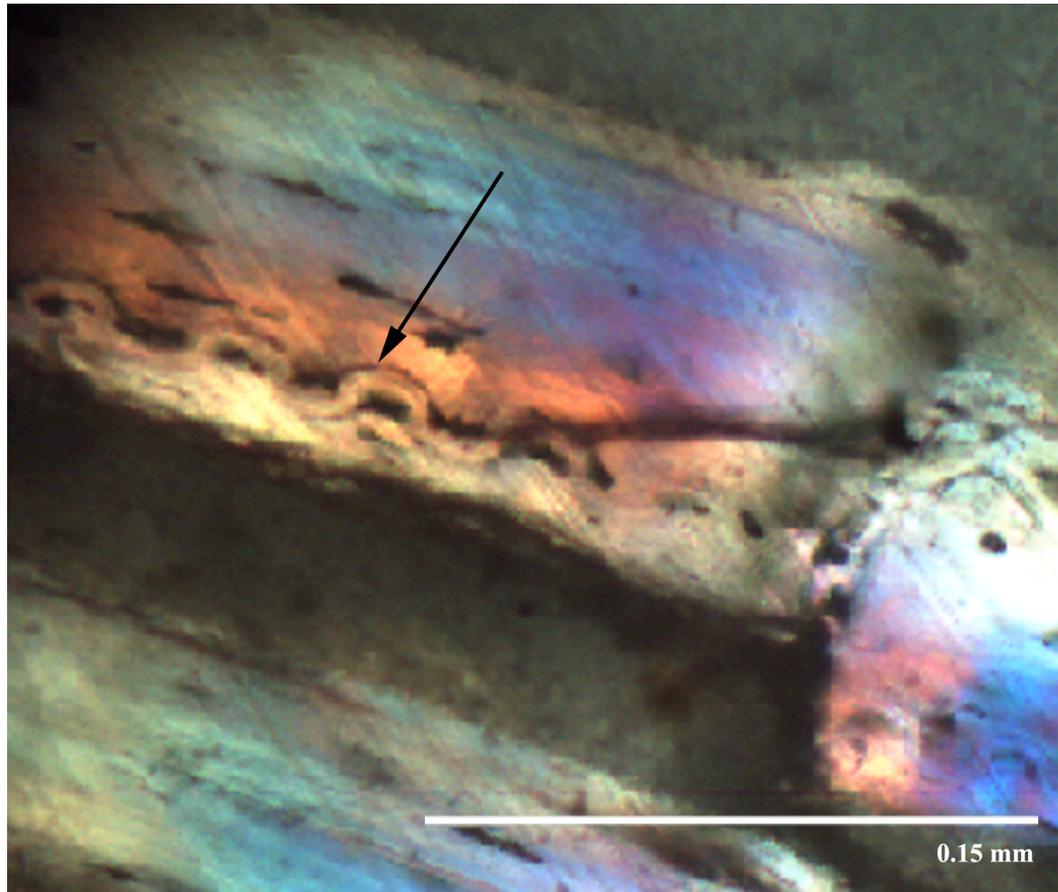
Figure 11. Cross-section of *Macropoma* bone plate under polarizing light. Cells are located under each ridge.

Axelrodichthys

Unlike the *Macropoma* specimens, the *Axelrodichthys* specimen (AMNH FF 12213) has an entire lung structure intact. The lung structure found in *Axelrodichthys* also resembles the lung found in *Macropoma*, but is much larger with two separate lobes. These lobes are clearly identifiable by their ellipsoid protrusion from the otherwise flattened body cavity. The lung in this case is also covered with thin plates of bone. These plates have an average thickness of 310μm which is much more than the average thickness of 53.3μm in *Macropoma* plates. Like *Macropoma* they are each overlapped by the plate immediately posterior, and they appear to form an uninterrupted ring around the lung. In *Axelrodichthys* this space is still filled with air, though a calcite geode has formed at its center (Figure 12). The interior of the lung displays protruding calcite crystals that are also found in the matrix of the rock within which the fish was fossilized, especially at the posterior. Anteriorly this structure appears to have been crushed, but the plates are still visible where the structure protrudes from the flattened fish body cavity (Figure 13).

Figure 12. Cross-section of the posterior portion of the lung in *Axelrodichthys* specimen AMNH FF #12213. Calcite crystals form the walls of the geode that fill some of the lumen. Concentric plates surround the lumen; some plates are delaminating toward its center. The gray infilling through the ventral portion of the lung is automotive epoxy that was injected to stabilize the fossil during excavation. This specimen is 6 cm across.
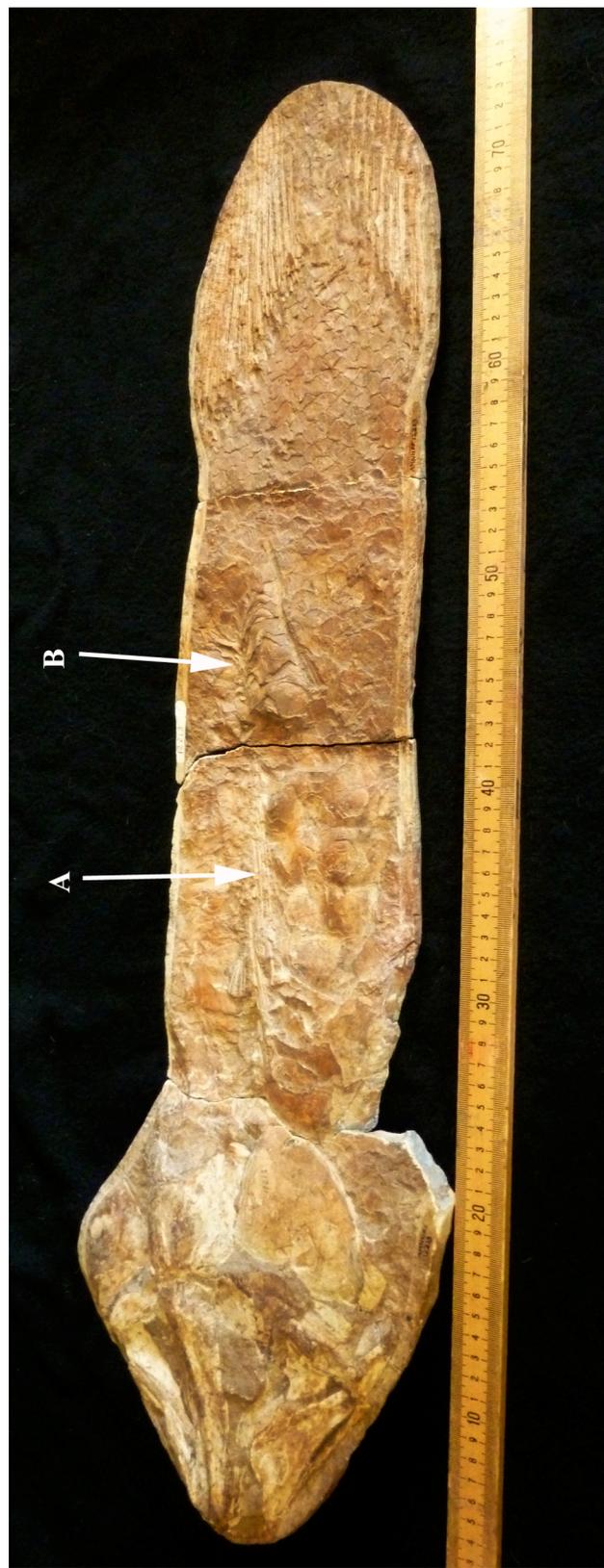
Figure 13. AMNH Fossil Fish Specimen #12213. Whole *Axelrodichthys* specimen with intact bony lung at center. The visceral cavity of this fish was clearly compressed during the taphonomic process, though the lung structure was left intact. The anterior portion of the lung (A) has compacted and is clearly much wider than the posterior portion of the lung that has remained as an intact 3-D structure (B).

Each of the plates on the *Axelrodichthys* specimen was uniformly curved around the long axis of the structure. This structure initially appears just posterior to the opercular bone and slowly increases in diameter for 10cm posteriorly, after which it maintains a constant diameter until the first lobe disappears into the body of the fish at 28.5cm. The second lobe begins 1cm after the end of the first, and continues posteriorly for 80mm more. The organ as a whole is present in 37.5cm, or 50.34% of the 75.4cm length of the body.

Unlike *Macropoma*, these plates appear smooth with no ridges on any of the surfaces. At most, some of the plates appear to have minor depressions that are irregularly reflected in the top or bottom layer (Figure 14).

When viewed using polarized light, the plates covering the lung of *Axelrodichthys* are unlike those found in *Macropoma* (Figure 15). There were very few lacunae in the *Axelrodichthys* plates. Of those that were found, the average volume was 83.90μm³, with an average length of 14μm, and an average breadth of 4μm. When the mean cellular lacunae sizes in both *Macropoma* and *Axelrodichthys* bony plates were examined using a T-test, the cellular volumes were found to be significantly different (t = +5.72, df = 93, P-value <.0001) (Figure 16) .

Between the two genera examined, the plates were found to have drastically different cellularization. In *Macropoma* the average cellular density of the bony plates was 950.27cells/mm², whereas the plates from *Axelrodichthys*
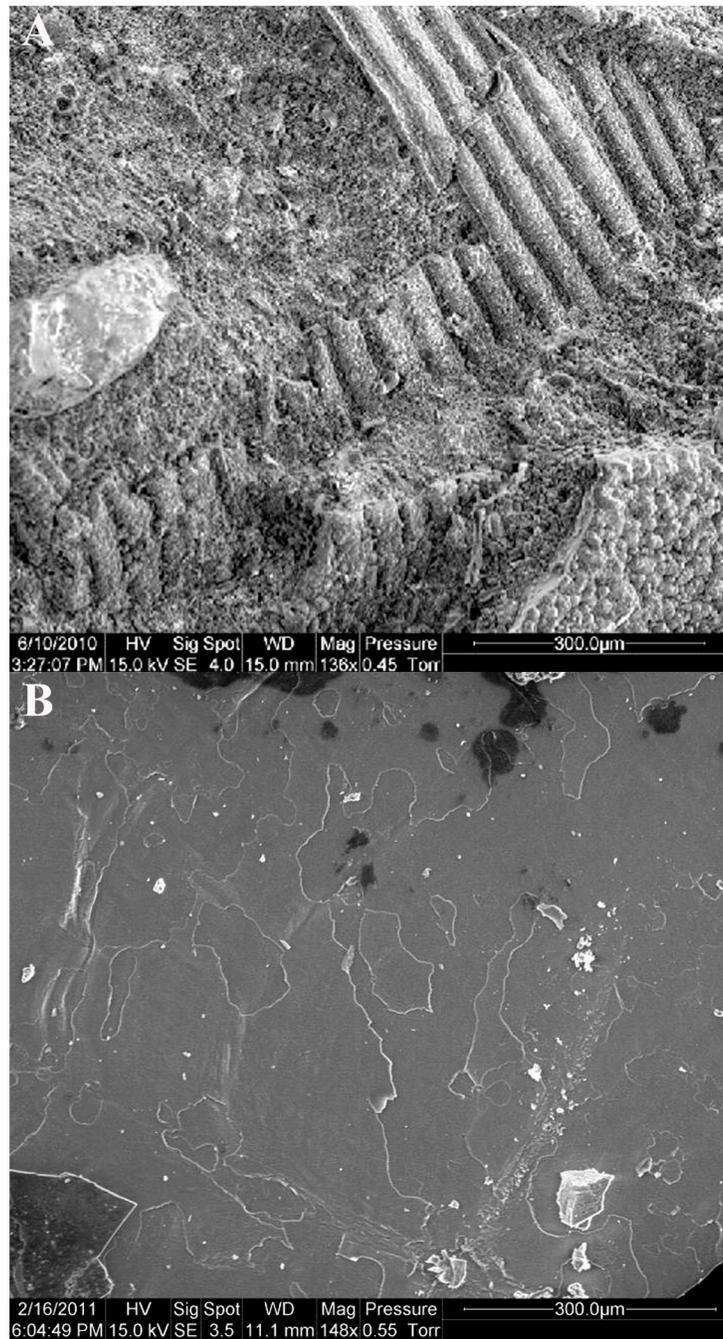
Figure 14. Comparison of the surface of the bony plates in *Macropoma* (A) and *Axelrodichthys* (B).
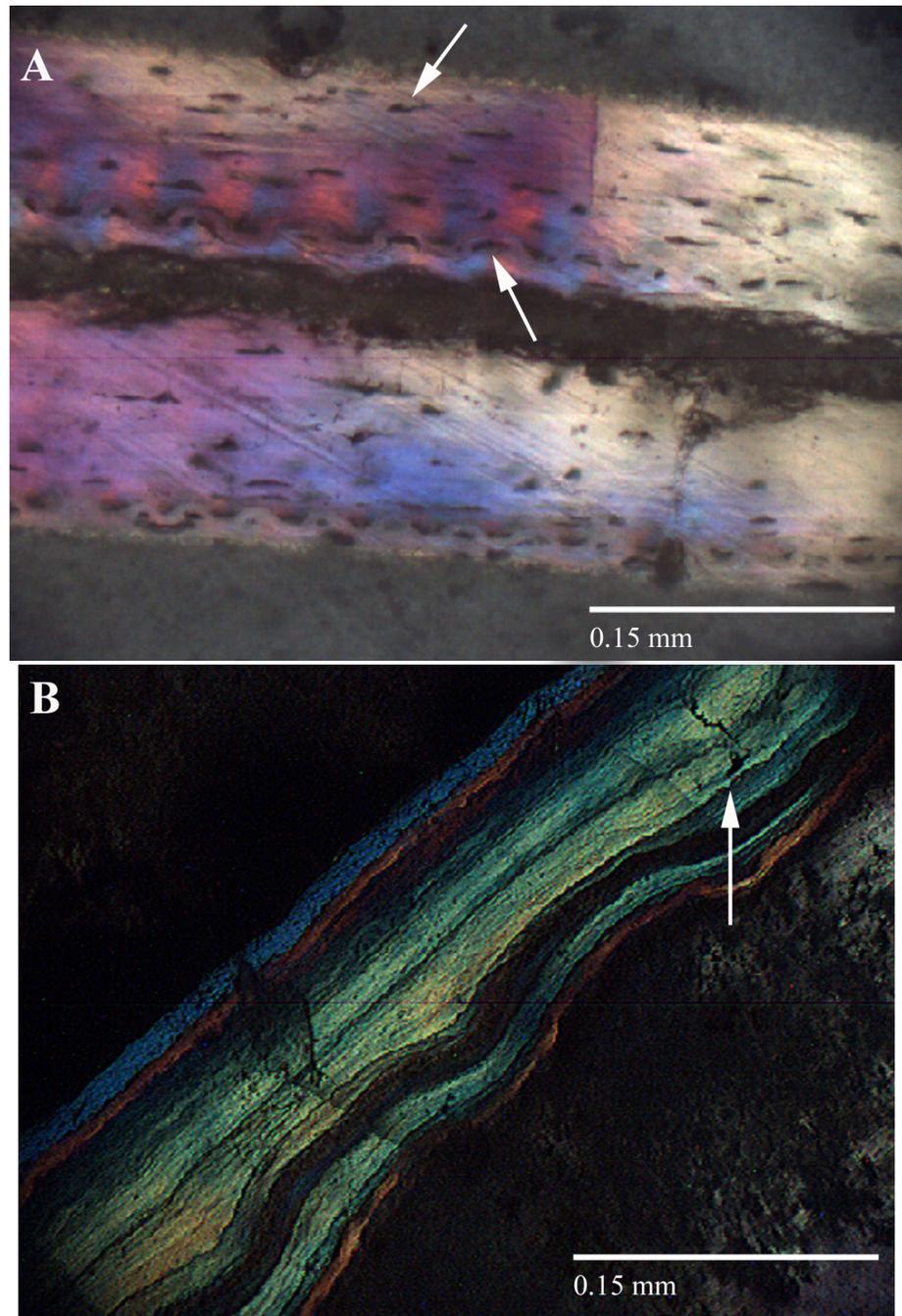
Figure 15. Bony plates surrounding the lung of *Macropoma* (A) and *Axelrodichthys* (B). In *Macropoma*, the plates contain obvious lacunae (arrows), while in *Axelrodichthys*, there is one cavity that may or may not be an osteocyte lacuna.
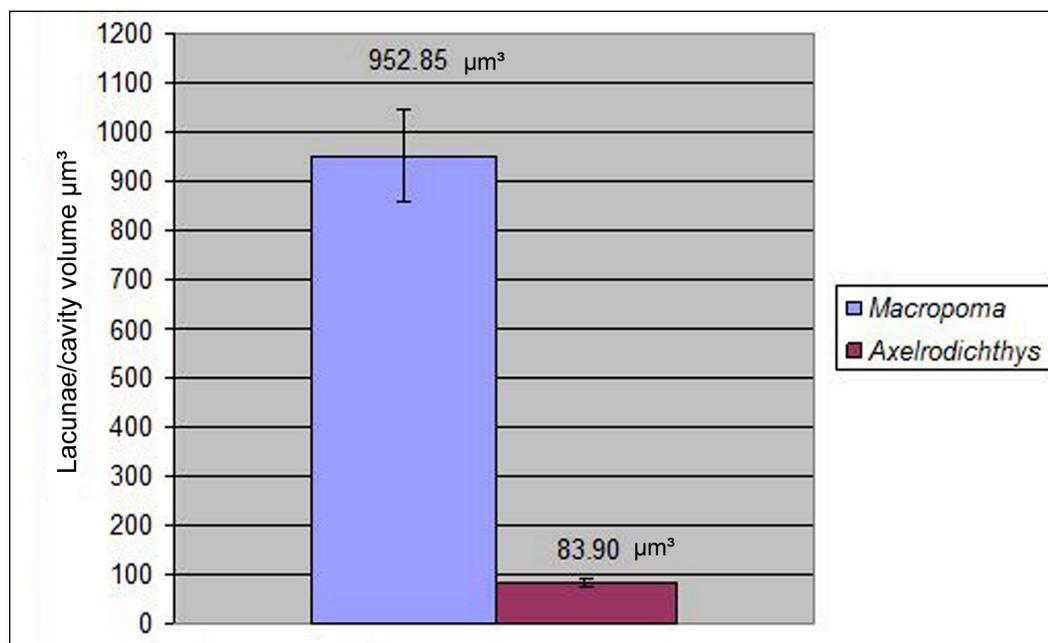
Figure 16. Mean volumes of osteocyte lacunae in *Macropoma* and cavities in *Axelrodichthys*.
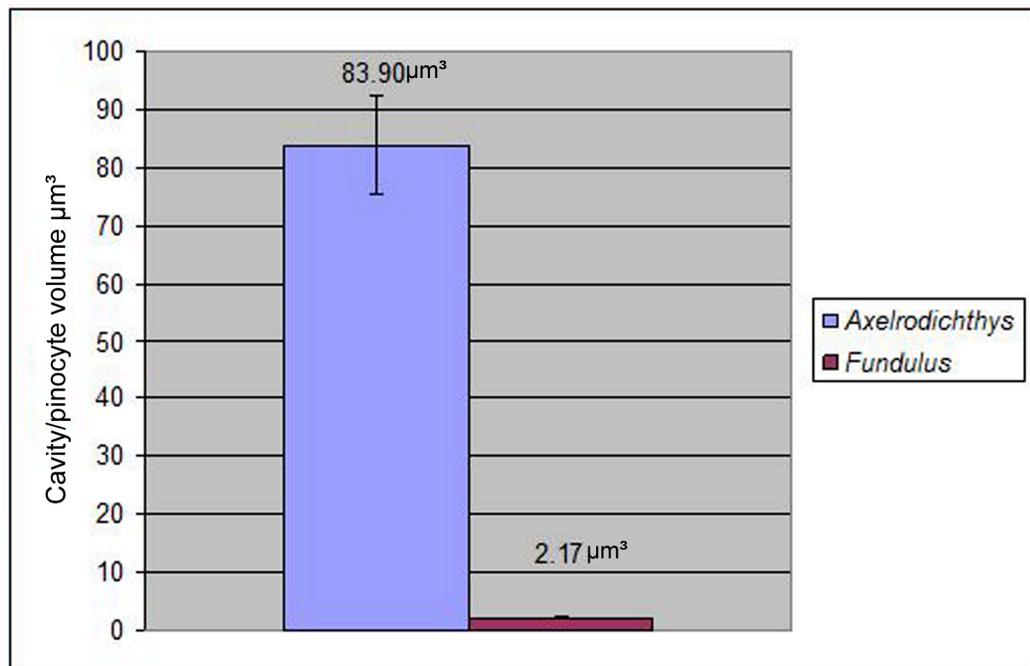
Figure 17. Mean Volumes of the cavities in *Axelrodichthys* and the pinoctyes in *Fundulus*.

have an average cellular density (if these cavities are, in fact, lacunae of entire cells) of 45.97cells/mm². Thus the bony plates of *Macropoma* are over twenty times as cellularized as the homologous plates found in *Axelrodichthys*.

Hydrostatic function/Calculation

Using the formula from Brown (1957), that in order for an organ to be useful as a hydrostatic organ in a marine fish it must occupy at least 5% of the total volume of the animal, the relative volumes of the lung in *Macropoma* and *Axelrodichthys* were calculated. The volume of each fish was calculated using the formula for a cylinder, though I suspect that these fish were quite flat at the posterior end of the body, because the fish could not be removed from their surrounding rock. In *Axelrodichthys* the total volume of the fish was calculated to be 2642.96cm³ while the volume of its lung was 143.13cm³. The volume of the lung was calculated to be 5.42% of the entire body volume of the fish. In *Macropoma* approximately 2/3 of the fish was represented in the specimen from the AMNH. As such, 2/3 of the fish's volume was calculated using the same parameters for r_1 except that the ½ the width of the head was not taken into account because the head was missing in this specimen. When the total fish volume was accounted for (1/3 of the fish's calculated volume was added to the whole volume to attain a value comparable to a whole fish), the volume of the fish was 160cm³ while the volume of the lung was 13cm³. In this specimen, the lung accounted for 8.13% of the projected total volume of the fish.

Fundulus

The *Fundulus* operculum was examined under polarized light. The pinocytes were measured and were found to have an average breadth of 1.5μm, an average length of 3.5μm, and an average volume of 2.17μm³. When compared to the mean volume of the cavities that appear to be osteocytes in *Axelrodichthys*, these pinocytes were found to be significantly different (t = -5.69, df = 46, p<.0001) (Figure 17). The variances between both groups were very small showing that the volume ranges of the cavities in each organism's bone matrices were fairly consistent with the mean.

Cobitidae

The Weberian ossicles in the Cobitidae appear to take on two forms. In the weather fish *Misgurnis anguillicaudatus*, the last ossicle composing the apparatus forms a thin layered bulb that is hollow at its center. There is obvious fusion of the first four vertebrae and their descending processes that culminate in forming this porous bulb have become fused by laminar, but not acellular bone. In the blue botia *Yasuhikotakia modesta,* the same apparatus is flattened and extends backward to meet the anterior portion of the swim bladder (Figure 18). In this case, the portion of the apparatus that associates directly with the swim bladder is
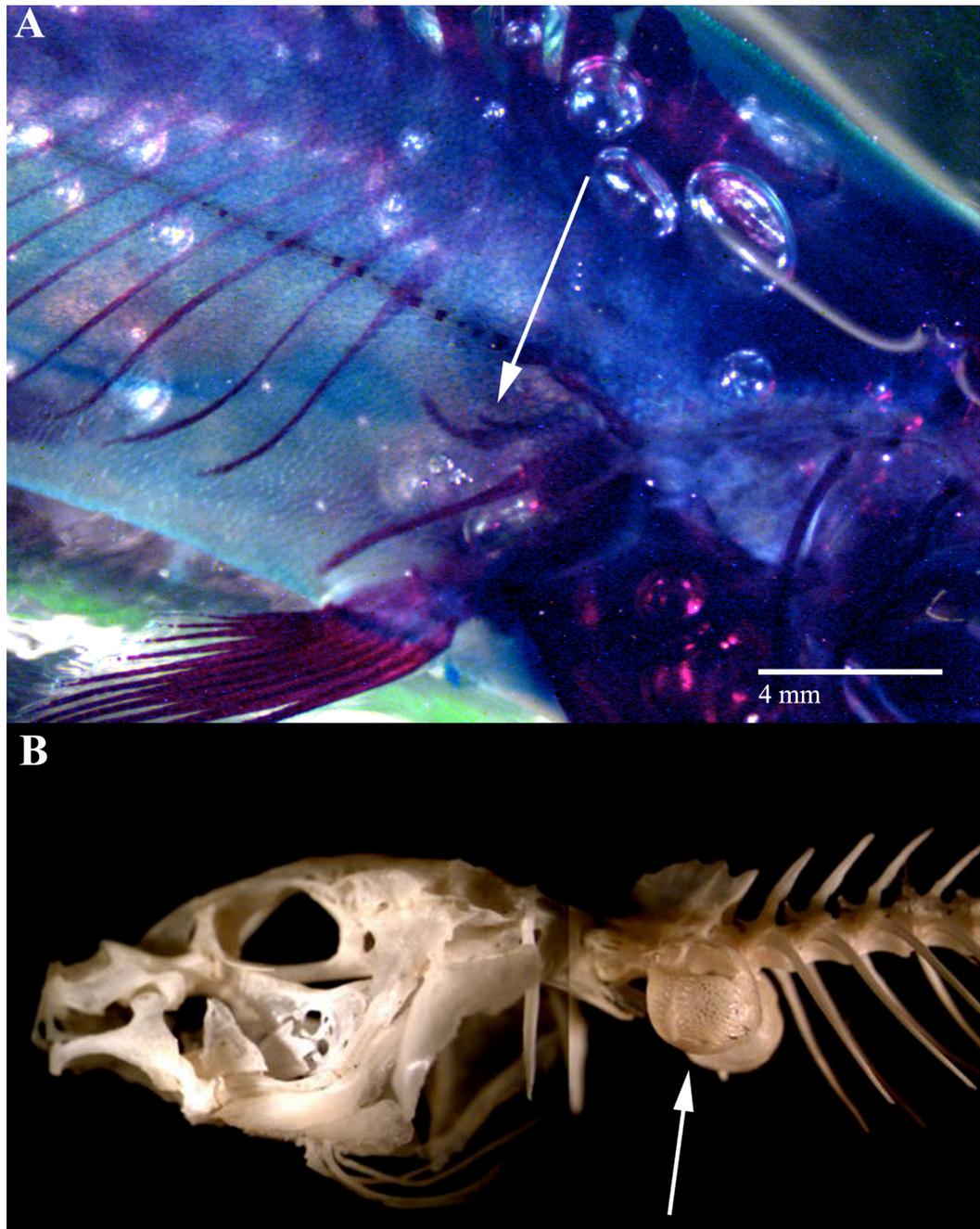
Figure 18. The Weberian ossicles in two cobitids. The fourth ossicle in *Yasuhikotakia modesta* (A) is a sheet of bone that extends posteriorly to meet the anterior portion of the swim bladder. In *Misgurnus anguillicaudatus* (B), the fourth ossicle forms a porous bulb that associates with the anterior portion of the swim bladder. The skull is 1 cm long.

from one vertebra that is not fused to the three anterior vertebrae that compose the ossicles. The vertebral process that extends distally to meet the swim bladder is flattened and appears to be laminar as well.

DISCUSSION

The fundamental questions about the bony lung are:

1. How was it fabricated—what is the interaction of development and material?

2. What did it do? How did anatomy, biomechanics, geometry, and physiology interact in this peculiar structure?

3. To what extent does mapping the presence of the bony lung on the cladogram of coelacanths, the properties of lungs in other groups of fishes, and other causes of plated, bony structures in other vertebrates illuminate the bony lung of coelacanths.

Structure and Type of Bone

The presence of cells within the cell matrix does not indicate whether the bone is endochondral or dermal. In fact, once the bone is fully ossified it is impossible to tell how it was formed (pers. com. William Bemis).

Whereas it is impossible to determine the origin of cellular bone, acellular bone has only been found (in teleost fishes) to arise from dermal bones (Moss, 1963). Because acellular bone originally forms as cellular dermal bone that is secondarily filled in, it is not inconceivable to think that this infilling by hydroxylapatite could occur within endochondral bone as well. Because acellular

endochondral bone has not yet been discovered, bones that are found without osteocytes within their matrices are automatically thought to be dermal bones.

When looked at in thin cross section, the bony plates surrounding the *Macropoma* lung are obviously cellular. The plates had, on average, a cellular density of 950 osteocytes/mm². This level of cellular density can also be found within the sarcopterygiian clade in the femoral trabeculae of rats; they have on average 943 osteocytes/mm² (Mullender *et al.*, 1996). In the case of the bony plates covering the lung in *Macropoma*, 54% of the lacunae in each plate are found directly underneath the ridges at the plates' margins. Under each ridge was a visible cellular lacuna that was clearly separate from the lacunae directly underneath it by changing the plane of focus on the microscope. Only one section of one of the *Macropoma* specimen was taken, and it is highly unlikely that there would be a lacuna under each ridge unless the lacunae in that location were very dense throughout the organ.

In mammals it has been shown that the number of osteocytes in a section of bone correlates with the amount of the remodeling of that bone (Mullender *et al.*, 1996). In the human jaw, the trabeculae in the alveolar bone surrounding the teeth remodel in response to the intense forces transmitted through the teeth, in order to distribute forces more evenly away from the tooth (Clark, 1995). In these cases, the dendritic processes extending away from the osteocyte would be compressed or stretched and would initiate remodeling throughout the bone by way of the piezoelectric properties of osteocytes (Becker and Marino, 1982). In

the lung it is likely that the concentration of osteocytes under each ridge would be consistently remodeled (Burra *et al.*, 2010) in order to keep the surfaces of the sliding plates from experiencing concentrated applied forces at any one location .

Although the bony lung is homologous across coelacanth genera, the details of its histology reveal surprising differences. Whereas in *Macropoma* the bony plates are highly cellularized, thin, and are easily dissociated into thinner layers throughout the fossilized organ, the plates surrounding the lung in *Axelrodichthys* are thick and solid, with a few slits that do not resemble the lacunae seen in *Macropoma*. Because of a lack of cells here, it can be assumed that there would be little, if any, remodeling that would have taken place within this bone. When the volumes of these cavities were compared with the osteocytes in the *Macropoma* plates, the volumes of the lacunae were found to be significantly different than the cavities in *Axelrodichthys* (P-value<.0001).

Nothing about these cavities suggests that they are osteocytic lacunae, or the remnants of osteocytic lacunae. Aside from the volume differential, the shapes of the cavities in the *Axelrodichthys* and *Macropoma* are entirely different. The lacunae in *Macropoma* each have obvious remnants of cytoplasmic processes that extended away from the cell body, that are characteristic of osteocytes. The cavities in the *Axelrodichthys* bone looked like cracks within lamellae rather than cells, and did not show cytoplasmic processes.

Could the small cavities in *Axelrodichthys* be pinocytes (the small cavities that remain after osteocytic lacunae are secondarily filled in)? I found them in the

opercular bone of *Fundulus*, though they are still significantly larger in *Axelrodichthys* (p-value < 0.0001) (Moss, 1963). If cavities in the *Axelrodichthys* lung plates are indeed pinocytes, could this difference be a result of the different cell sizes in *Axelrodichthys* and *Fundulus*? Cell size is proportioned to the amount of DNA in the nucleus (unless there are such added complications as a cell wall or gigantic vacuole). In lungfish, the sister group to coelacanths, the difference in osteocyte volume in Paleozoic fossils and extant genera has been shown to increase by two full orders of magnitude (Thomson, 1972).

Because DNA content is indicative of osteocyte volume, and because the cellular volume in *Macropoma* falls within the range, but towards the smaller side of cell volumes found in fossilized lungfish, it seems plausible that C-values of other coelacanths will also be within the range seen in lungfishes. The smallest fossilized dipnoan C-value is ten times the size of the largest teleost C-value, while the largest is over twenty-seven times larger than the largest teleost C-value (C-values indicate the amount of DNA within a cell)(Gregory, 2011). The C-value of the living coelacanth genus *Latimeria* is only ten times larger than the smallest teleost value, though other groups from the sarcopterygian clade, like amphibians, are known to have C-values equal to that of the highest lungfish value (Gregory, 2001; Helfman, 2009). This variation in comparing C-values and corresponding osteocyte size causes us to be unable to conclude that the cavities in the plates surrounding the *Axelrodichthys* lung are not pinocytes scaled for larger original osteocytes. A larger osteocyte would be filled in by the same

mechanism that a smaller one would be: from the outside margins in. Because the filling in of smaller osteocytes leaves a consistently sized hole at the center of the lacuna, it stands with reason to say that larger lacunae would produce larger pinocytes. With this, the possibility that the bony plates surrounding the lung in many coelacanth genera and, in this case, specifically in *Axelrodichthys* and *Macropoma* are composed of dermal bone rather than endochondral bone cannot be confirmed. If, indeed, the cavities within the *Axelrodichthys* bone were found to be pinocytes, the bone type would immediately be confirmed as dermal bone.

The proposition that the cavities in the *Axelrodichthys* plates are pinocytes is just one possibility. These irregular cavities are few in number, are not concentrated in any particular area within the fossil in the same way that osteocyte lacunae are found in *Macropoma*, and do not have cannicular extensions. It is very possible that these cavities are small cracks that appeared in the bone matrix post-fossilization instead. This section is partly held together by automotive epoxy that was used to glue the entire specimen back together after it was excavated in Brazil. The fact that at one point the entire specimen was in four distinct pieces that were glued back together and later broken apart again, suggests that this fossil specimen has experienced a good amount of stress that may have translated into creating microcracks in the bone matrix of the lung plates.

In both coelacanths the bone surrounding the lungs is arranged in thick (Axelrodichthys) or thin (*Macropoma*) overlapping plates. Plates in

Axelrodichthys were described by Brito *et al.* (2010) as appearing as though they were composed of parallel collagen networks that had been calcified during the taphonomic process. If the hardened structure were only composed of calcified collagen fibers, a uniform surface with no evidence of any type of osteocytic presence within the matrix would be present. However, they did not perform any kind of elemental recognition tests, or thin-section microscopy in order to find whether or not phosphorus or osteocyte lacunae were present in the structure. Also, taking into account the fact that in *Macropoma* this structure is osteocytic in nature, it is rather unlikely that this similar organ in *Axelrodichthys* has a completely different microstructure.

In fact, it is far more likely that what Brito *et al.* (2010) hypothesized were loose bunches of collagen fibers were actually collagen fibers incorporated into the bone matrix. Collected together within the bone these collagen networks create lamellar bone structures (Locke, 2004). These lamellar sheets comprise a special kind of bone called laminar bone.

Laminar bone is sheeted bone that contains large amounts of collagen. It is homologous to the lamellae that surround Haversian canals in endochondral bone, except that instead of circling around one vascular canal, the sheets are laid out flat. Because the bone does not surround one vascular canal, there are many vascular canals or spaces within each sheet in order to have sufficient delivery of nutrients to each of the osteocytes within the bone matrix (De Margerie, 2002; Locke, 2010).

The Lung: Not a Swim Bladder

Lungs and gills are present in most of the basal members of the Osteichthys. The lung diverges from the esophageal tract near the final gill. Usually, lungs branch from the ventral portion of the digestive tract, and remain below it, whereas swim bladders are typically are found dorsal to the digestive tract. This difference in the locations of air-containing organs is likely to be connected to the hydrostatic and often acoustic functions of the swim bladder, and mode of getting air into and out of the lung.

Brainerd (1994) observed that almost no extant fish with a lung depends on it exclusively for breathing. In fact, all lunged fish breathe bimodally with both gills and lungs. There are two types of lung breathing that are differentiated by the number of separate movements required to take in and expel the air stored in the lung; they are known as two and four-stroke breathing cycles. The more primitive two-stroke breathing cycle, is characterized by an outward movement of the operculum, which sucks air into the buccal cavity of the fish while expelled air is pushed out of the lung through the gill slits, and a second muscular movement to push air into the lung. Similar motions have been observed in the living lungfish, and it is thought to be the primitive ventilatory mechanism for all Sarcopterygii. The living coelacanth *Latimeria* can still be observed moving its opercula in and out in this fashion to move oxygenated water over its gills, and it is likely that this

behavior was carried over from ancestral genera to this genus as a way to move water over the gills of the fish despite the loss of the air-filled lung (Brainerd, 1994).

This method of drawing oxygen into the lungs would not have required extra pumping movements because the lung would have needed to have been actively squeezed in order to expel air during exhalation. Inhalation would proceed after under negative pressure in the lung was produced by the expulsion of air, thus creating an empty space that, in conjunction with recoil by the elastic pleural membrane, would cause air to be sucked into the lung. This would indicate that the fish would have had to exhale actively, and would be able to exchange as much or as little of that oxygen store per breath as desired (Brainerd, 1989). The airspace could serve a (varying) hydrostatic function, or that could be used as an oxygen store if the animal encountered hypoxic water. Once under hypoxic water conditions it would be possible for the fish to rely on the air stored in its lung to continue heart function until oxygen was found in the water or at the surface (Brainerd, 1997; Farmer, 1997; Farmer and Jackson, 1998). Neither *Macropoma* nor *Axelrodichthys* (both from the shallow seas of the Cretaceous period) would have been living in water that was likely to have been anoxic, but in the case of a need for rapid ascent from depth, the bony sheath may have countered rapid pressure changes.

In both *Macropoma* and *Axelrodichthys*, the lung extends posteriorly along the ventral margin of the fish. Both *Axelrodichthys* and *Macropoma* meet

the criterion set forth by Brown (1957), that at least 5% of the total body volume of a fish must be occupied by an organ containing a substance with less density than the water around it in order for the organ to be useful for hydrostatic function in marine fishes. As a place for oxygen storage, the lung would have been useful for *Macropoma* and *Axelrodichthys* who both lived in shallow continental seas, unlike *Latimeria* which lives at depths of up to 700m. Its fat-filled sac presumably increases its buoyancy, but obviously has no respiratory function (Smith *et al.*, 1981).

Why is The Lung Covered in Bony Plates?

The bony plates surrounding the lung in *Macropoma* are found in sixteen genera of coelacanths spanning five families. These genera make up over a quarter of the total genera within the order. The question must be asked why an order of fishes that reduces the ossified structures within the body (i.e. the vertebral column and ribs) would add a large unprecedented ossified structure that extends through most of the body.

In *Macropoma*, the lung spans the ventral portion of the body cavity and continues posteriorly until it comes to a point at the insertion of caudal fin. Each plate within this structure has ridges at its internal and external margins that complement those of the overlapping and underlying plates. These ridges are oriented longitudinally, except on the most distal plates. It is possible that each of

these plates was able to telescope over its neighbor, using the longitudinal ridges as guides for regulating. These ridges are only found to be oriented with the long axis of the body, and with these plates only moving in and out along the length of the body, forces would be minimal. Each plate contributes to an overall conical shape for the organ, and it can be assumed that the shape would limit how far each plate would be able to slide. The amount of movement would be constrained by the successively smaller diameter of the posterior overlapping plate over a larger anterior one if an associated membrane, either the pleural membrane or an underlying membrane, did not provide even more of a constraint.

Aside from the final two plates in the lung-plate series, where the ridges are angled at a 45% angle to the longitudinal axis of the organ (and the fish), the ridges on the lung plate parallel the long axis of the fish. This could likely be because longitudinal ridges would allow for predictable loading of the structure when air in the lung caused it to reach its most extreme length with the conical cap reaching the most distal point in the visceral cavity. With ridges oriented in any other direction, the structure would not have the ability to expand or contract, and would only be able to respond to loading within the structure by creating less predictable forces within the visceral cavity of the fish.

Due to the conical nature of the plates, and the fact that they would be limited as to how far inward they would be able to move, it is plausible that this structure would be able to resist pressure changes up to a point, especially under conditions of rapidly decreasing pressure leading to a increased air volume like

what would occur in a lung during a rapid ascent. Though the bony plates would create a minimum length of the bony structure by design, another structure would be needed in order to keep the plates from sliding too far distally with an increase of air volume due to increased pressure within the lung to the point that the plates would jump their tracks (The idea, but not the mechanism of this structure's use for resisting pressure was proposed by Peter Forey (1998)). This structure could be either muscular or an elastic network of connective tissue, or a combination of both.

In *Macropoma*, when the lung is fully extended there is no place left within the body cavity, at least not longitudinally, for the lung to extend. At this point it might be necessary to have some kind of musculature controlling the amount of pressure from the lung that is able to become transferred to the visceral cavity wall. This would be of greater concern to larger coelacanths such as *Axelrodichthys* that have much thicker plates. As there are no visible places of muscle attachment on either *Macropoma* specimens, it could be that the lung is encased in a connective tissue cavity that may be very similar to what is seen in the pleural membrane or pericardium in humans, that is contracted by indirect body wall musculature that would give the fish control over the speed and extension of the lung organ. This musculature may have served as a precursor to the diaphragm. There is no sign of anything on the surface of the lung plates that suggests that muscle attachment (a roughened surface on the plates) has been found.

In order to control the force at which the bony structure moved posteriorly within the body some kind of musculature or elastic connective tissue would need to be able to exert pressure on the lung structure itself. This would only be plausible if the structure were filled with air rather than oil or fat, which would be essentially incompressible. The possession of a bony covering only makes sense if the lung was filled with air in both *Axelrodichthys* and *Macropoma*.

The heavy ganoid scale jacket possessed by all coelacanths may also play a role in air-breathing. In *Polypterus,* the ganoid scales covering the body provide some elastic energy that aids in expelling air from the fish's lung (Brainerd *et al*., 1989; Brainerd and Ferry-Grahm, 2006). In this case, body wall musculature must really only be activated to squeeze air from the lung. When these muscles relaxed negative pressure would have been created that then would have pulled air into the lung. The muscles would have to continue to work to hold air in the lung until exhalation occurs by using the elasticity of the ganoid scale jacket to push the deoxygenated air out of the lung. This type of muscular system may have been present in *Macropoma*.

It is also possible that the body wall musculature, the bony plates covering the lung, and the membrane around the lung, are able to act like an internal jacket of scales that store elastic energy as they are expanded backwards with pressure or with this intake of a breath and later release that energy during the exhalation of deoxygenated air. In this case the lung and the body wall working in conjunction with one another may provide enough resistance that the lung is

unable to expand past a certain point and excess musculature would not be necessary.

Aside from the many implications of the bony envelope on the breathing mechanisms of *Macropoma*, the bony plates may hold space in order to facilitate competing physiological processes that occur within the visceral cavity of the fish. This lung could be used to allot an amount of space to the air-filled lung as it was used to resist pressure changes caused by changes in depth. The preservation of some amount of space for an oxygen store could be used if the fish were to encounter a patch of anoxic water where it would need to use the air stored within the lung to keep its heart functional as it swam for the surface where oxygen would be readily available (Farmer, 1997).

In both *Macropoma* and *Axelrodichthys* the lung would have been large enough to maintain hydrostatic function for the fish once filled with air. In *Macropoma* only 2/3 of the specimen was present, but with adjusting the length of the entire fish to account for the 1/3 missing, the lung present in the 2/3 of the fish available to measure occupied 8.14% of the total fish body volume. This is well above the 5% of the total body volume proposed by Brown (1957) necessary for an organ filled with a substance lighter than water to be useful for hydrostatic function in a marine fish. In *Axelrodichthys* the lung occupies 5.42% of the total volume of the body. These calculations were done without accounting for the amount of compression that the body of either animal may have experienced during the taphonomic process. This may have slightly over estimated the body

size of *Axelrodichthys* due to its obvious compression into a nodule, but would not likely alter the estimation in *Macropoma* which does not appear to have experienced any obvious compression during fossilization.

The lung may also be necessary in order for the lung to be able to expand without being crowded within the body cavity. This could happen for many reasons, but could be especially pertinent if the fish were pregnant. Species of the living coelacanth *Latimeria* have been found to have anywhere from five to twenty-nine embryos inside the body cavity. From this, and the fact that each of the embryos was at a different stage of embryological development led researchers to conclude that *Latimeria* is ovoviviparous (Smith *et al.*, 1975). *Latimeria* and *Macropoma* are each other's closest relatives within the coelacanths, so it is quite possible that their reproductive strategies are the same.

Brito *et al.* (2010) also proposed two other possible functions for the bony lung structure in *Axelrodichthys*, which due to the homology of the organ throughout the coelacanths, might be pertinent to the bony lung in *Macropoma*. They first proposed that this organ could be used for hearing. In this case they postulated that because there is no fossilized remnant of a bony connection between the inner ear and the bony lung structure, that a soft tissue connection would be necessary to convey the energy from sound waves to the inner ear. Here, they did not take into account that a soft tissue connection is likely to dissipate energy through a system rather than convey it to a specific end point. Their mechanism for the conduction of sound to the inner ear recalls a conversation

carried between tin cans connected by a string. Also, the lung seems to be open anteriorly; if this is the case, changes in pressure would push tiny volumes of air out of the lung so that pressure changes would not reach the inner ear. It is not likely that the bony lung is used for auditory functions. In *Latimeria* the presence of auditory nerves and basilar papillae has been found, but a complete auditory structure has yet to be discovered (Fritzsch, 1987).

Brito *et al.* also suggested that the bony plates surrounding the lung *Axelrodichthys* could have been used for sound production. They propose that sound may have been produced due to a hitting of the individual plates against one another, and that the air-filled lung space would be able to function as a resonating chamber. This function, however, is also unlikely because the anteriorly open lung would not provide a closed space for sound to resonate.

Comparisons with Other Organisms

The Weberian ossicles found in ophidiform fishes appear in many forms, but in the Cobitidae they connect to hollow and thinly layered shells of bone that extend downward from the spinal column to make contact with the anterior portion of the swim bladder. This structure does not surround the entire swim bladder, but it touches part of the swim bladder that transmits pressure changes resulting from sound waves passing through the closed off air space to the inner ear of the fish. Because the Weberian apparatus is a bone structure that associates

directly with an endodermal lung derivative that reacts to varying pressure within the air space, it is important to acknowledge it when looking at another bone structure that also likely dealt with pressure changes having to do with the endodermal lung.

The ossified tracheae and the sclerotic rings found in birds share some features with the ossified plates surrounding the coelacanth lung. The tracheal rings keep the airway open as the bird's neck bends. Overlapping bony plates that keep an air filled lumen open also applies to the ossified lung of coelacanths. The sclerotic rings in birds' eyes also give structure to the cornea which they surround while reacting to pressure applied to the eye as a whole. Each sclerotic plate is ossified, with osteocytic lacunae within the matrix (as in the plates surrounding the lung in *Macropoma*), and is able to slide over or under its neighboring plate to keep the eye focused when changes in pressure applied to the eyes are experienced (pers. comm. Franz-Odenaal, 2011). The ability of thin plates sliding over one another is shared between the coelacanth lung and birds' eyes as responses to changes in pressure. For the lung, the pressure changes are experienced within the gas-filled lung, while the pressure changes experienced by the sclerotic ring is in response to air pressure applied to the eye as a whole.

The bony lung was likely an adaptive structure within the coelacanths. Within *Macropoma* and *Axelrodichthys*, the lung structures were remarkably different with variation found macroscopically in the number of lobes seen, and microscopically with the thickness of the plates and presence or absence of

osteocyte lacunae. When structures are not adaptive they do not experience selective pressures, and often do not differentiate or radiate throughout the group. In Figure 3, the radiation of this structure can be seen throughout the Coelacanthiformes. It is possible that there are many variations in the structure of this bony lung throughout the coelacanths that have not been found yet. It is also possible in the genera that have been found without this lung structure did, at some point, possess a bony lung that was not fully ossified, or was too thin to be preserved during taphonomy. Because the bony lung is found in so many genera of coelacanths, and it has at least two distinctly different ways of being formed, it is likely that this structure had a function.

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