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Development and three-dimensional histology of vertebrate dermal fin spines

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Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, Evolutionary Biology Center, Norbyvägen 18A, Uppsala, Monday, 13 June 2016 at 09:00 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Philippe Janvier (Muséum National d'Histoire Naturelle, Paris, France).

Abstract

Jerve, A. 2016. Development and three-dimensional histology of vertebrate dermal fin spines. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1382. 53 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-9596-1.

Jawed vertebrates (gnathostomes) consist of two clades with living representatives, the chondricthyans (cartilaginous fish including sharks, rays, and chimaeras) and the osteichthyans (bony fish and tetrapods), and two fossil groups, the "placoderms" and "acanthodians". These extinct forms were thought to be monophyletic, but are now considered to be paraphyletic partly due to the discovery of early chondrichthyans and osteichthyans with characters that had been previously used to define them. Among these are fin spines, large dermal structures that, when present, sit anterior to both median and/or paired fins in many extant and fossil jawed vertebrates. Making comparisons among early gnathostomes is difficult since the early chondrichthyans and "acanthodians", which have less mineralized skeleton, do not have large dermal bones on their skulls. As a result, fossil fin spines are potential sources for phylogenetic characters that could help in the study of the gnathostome evolutionary history. This thesis examines the development and internal structure of fin spines in jawed vertebrates using two-dimensional (2D) thin sections and three-dimensional (3D) synchrotron datasets. The development of the dorsal fin spine of the holocephalan, *Callorhinchus milii,* was described from embryos and compared to that of the neoselachian, *Squalus acanthias,* whose spine has been the model for studying fossil shark spines. It was found that the development of the *C. milii* fin presents differences from *S. acanthias* that suggest it might be a better candidate for studying "acanthodian" fin spines. The 3D histology of fossil fin spines was studied in *Romundina stellina*, a "placoderm"; *Lophosteus superbus*, a probable stem-osteichthyan; and several "acanthodians". The 3D vascularization reconstructed from synchrotron radiation microtomographic data reveal that "acanthodian" and *Lophosteus* spines grew similarly to what is observed in chondrichthyans, which differs slightly from the growth of the *Romundina* spine. Chondrichthyans and "acanthodians" also share similarities in their internal organization. Overall, *Lophosteus* and *Romundina* spines are more similar in terms of morphology and histology compared to chondrichthyans and "acanthodians". These results support the current hypothesis of gnathostome phylogeny, which places "acanthodians" on the chondrichthyan stem. They also emphasize the need for further study of vertebrate fin spines using 3D approaches.

Keywords: fin spine, paleontology, early vertebrate, histology, development

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To Ignatius J. Reilly

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I   **Jerve, A**, Johanson, Z, Ahlberg, PE, & Boisvert, C. (2014) Embryonic development of fin spines in *Callorhinchus milii* (Holocephali); implications for chondrichthyan fin spine evolution. Evolution and Development 16(6): 339-353.

II   **Jerve, A**, Qu, Q, Sanchez, S, Blom, H, Ahlberg, PE. (2016) Three-dimensional paleohistology of the scale and median fin spine of *Lophosteus superbus* (Pander 1856). Submitted to *PeerJ*.

III   **Jerve, A**, Sanchez, S, Ahlberg, PE, & Haitina, T. (2016) Vascularization and odontode structure of the fin spine of *Romundina stellina* Ørvig 1975. *Manuscript*.

IV   **Jerve**, **A**, Bremer, O, & Sanchez, S. (2016) Morphology and histology of acanthodian fin spines from the Silurian Ramsåsa E locality. *Manuscript*.

In paper I, AJ made and imaged thin sections, produced figures, and wrote the manuscript with the other authors. In papers II, III, & IV, AJ segmented the fin spine data, produced all fin spine figures, and wrote the manuscript with the other authors.

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Contents

Introduction

The fossil record of vertebrates dates back to the late Ordovician period (485-444 million years ago) of the Paleozoic era (541-252 million years ago). The majority of the early vertebrates were jawless "ostracoderms", but definite remains of jawed vertebrates (gnathostomes) first occur in rocks of Silurian age (Janvier 1996). Extant gnathostomes are split into two monophyletic groups: the chondrichthyans (cartilaginous fish including sharks, rays, and chimaeras) and the osteichthyans (bony fish and tetrapods). There is little doubt to the monophyly of these clades, which is supported by both morphological and molecular data (Maisey 1986; Chen et al. 2012). However, it is unclear where the two extinct groups of gnathostomes, the placoderms (armored fish) and acanthodians ("spiny sharks"), sit in relation to chondrichthyans and osteichthyans (Brazeau & Friedman 2014). Traditionally, each of these groups were considered to be clades, both being defined by a tenuous set of morphological characteristics (Miles 1973; Denison 1978, 1979; Goujet 1984). However, the discovery of fossils that show a combination of morphological characters that were previously used to define the traditional gnathostome clades, has led to a re-evaluation of early vertebrate relationships (Zhu et al. 1999, 2009, 2013; Brazeau 2009; Davis et al. 2012).

Fin spines, in particular, have been central to this discussion. The Acanthodii ("spiny sharks") was traditionally defined as a clade by the presence of spines in front of all fins (Denison, 1979; Janvier 1996). These include several paired and median spines positioned along the body that exhibit an array of morphologies. However, recent discoveries suggest that fin spines are primitively present in all four major groups of jawed vertebrates, and challenge the monophyly of acanthodians. Both median and paired fin spines have now been described from early chondrichthyans with articulated skeleton (Miller et al. 2003). Moreover, median and paired fin spines have recently been described from fossil osteichthyans, which have similar spine-bearing shoulder girdles to many placoderms (Zhu et al. 1999, 2009). Along with other morphological data from braincases and jaws, placoderms have been split up into a paraphyletic group and acanthodians have consequently been placed on the chondrichthyan and osteichthyan stems (Brazeau 2009; Davis et al. 2012). These ideas continue to change as new data are brought forth; for instance, currently all acanthodians sit on the chondrichthyan stem as part of the chondrichthyan total group (Figure 1) (Zhu et al. 2013; Dupret et al; 2014; Giles et al. 2015; Brazeau & Friedman 2015; Long et al. 2015; Burrow et al. 2016).
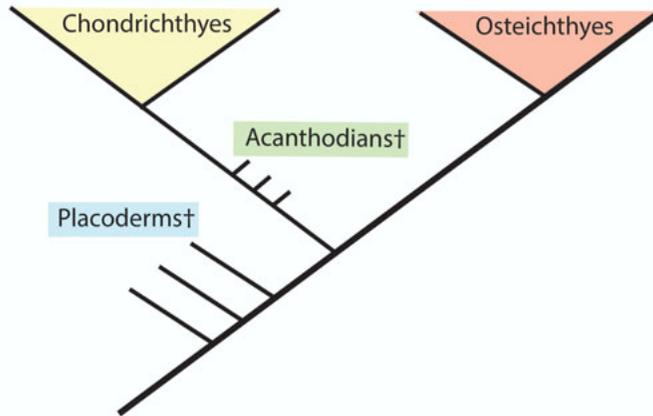
Figure 1. Simplified phylogeny of early vertebrates based off of Giles et al. (2015). Daggers represent extinct taxa.

Placoderms and osteichthyans are covered in an array of large dermal bones (called macromery), a characteristic that has recently been shown to be potentially homologous between placoderms and osteichthyans, and if true, this would create an evolutionary continuum between them, in addition to providing support to the position of acanthodians on the chondrichthyan stem (Brazeau & Friedman 2014; Zhu et al. 2013).

In fossils, fin spines show a reasonable amount of variation in morphology, ornamentation, and histology, and have been used for studying taxonomy and phylogenetic relationships (Burrow & Turner 2010, Burrow et al. 2016). Considering their high preservation potential, variable morphologies, and broad distribution in early vertebrate phylogeny, fin spines provide a crucial dataset in the current reassessment of the interrelationships and evolutionary history of the earliest jawed vertebrates. Previously isolated fin spines are usually assigned to acanthodians (e.g., Gross 1973) and occasionally chondrichthyans (e.g., Sansom et al. 2005), but the discovery of fin spines in other groups is challenging such assignments. Moreover, fin spines are usually the only large dermal structure present in chondrichthyans and acanthodians, which generally do not have ossified endoskeletons and have bodies covered in small denticles, or scales (called micromery). As such, further study on both isolated fin spines and fin spines from articulated specimens is necessary to understand early vertebrate evolution in general.

One of the earliest investigations into the morphology and evolution of chondrichthyan fin spines studied growth of the structure in *Squalus acanthias* (Markert 1896). Markert (1896) was able to provide a description on the development of the spine based on a limited number of specimens. This assessment was later revised by Maisey (1979), which was, in fact, originally part of his doctoral dissertation on fin spine growth and evolution, but focused mainly on neoselachian (Maisey 1979) and fossil hybodont sharks (Maisey 1978). Since then, there have been a several papers focused on the growth and

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evolution of chondrichthyan fin spines, including xenacanth shark cranial spine growth (Soler-Gijon 1999; Beck et al. 2004), the symmoriiform spine brush complex (Zangerl 1984; Coates et al. 1998; Maisey 2009), and using the histology from sinacanth spines to investigate their phylogenetic significance (Zhu 1998; Sansom 2005). These studies illustrate the diversity in morphology, histology, and growth of chondrichthyan fin spines, in addition to highlighting the importance of studying these structures from other jawed vertebrates.

This thesis will study the morphology and histology of fin spines from extant and fossil representatives from across gnathostome phylogeny using classic histological methods **(Paper I)**, as well as three-dimensional data obtained using synchrotron radiation microtomography **(Papers II, III, & IV)**. Fin spine development from extant chondrichthyans will be compared and microtomographic datasets will be utilized to study the 3D morphology and histology to address questions relating to growth in fossil dermal spines.

Fin spines

Distribution

Today, fin spines are known from two groups of neoselachians (sharks), the heterodontiforms (horn sharks) and the squaliforms (dogfish sharks), and all holocephalans (chimaeras). Holocephalan fin spines are found anterior to the first dorsal fin and neoselachian spines are usually located anterior to the first and second dorsal fins (Maisey 1979; Clarke & Irvine et al. 2006; Didier et al. 2014). Squaliform sharks typically have a long spine that sits in the skin at an angle and curves posteriorly (Maisey 1979) while Heterodontiforms have a straighter spine that is positioned more upright (Tovar-Ávila et al. 2008). Batoids (rays and skates) have a barbed poison spine associated with their tails, but this is regarded as a unique character for the group and will not be discussed further in this thesis (Nelson 2006; Marmi et al. 2010).

There is a larger diversity in the morphology of fossil chondrichthyan fin spines compared to those from extant taxa. Paleozoic and Mesozoic hybodont and ctenacanth sharks are known to have very large and heavily ornamented fin spines that are reminiscent of those from modern neoselachians (Maisey 1978, 1981, 1982). Some fossil spines are more triangular in shape, like those from *Cladeoselache* (Maisey 1977) and sinacanth sharks (Zhu 1998, Sansom et al. 2005), but some can be oddly shaped, including the unusual double-pronged spines from the fossil holocephalan, *Erismacanthus* (Stahl 1999). Xenacanth sharks, which range from the Devonian to the Upper Triassic (419-201 million years ago), have almost completely unornamented cranial spines that are attached to the back of the neurocranium (Soler-Gijon 1999). These spines are homologous to other fossil spines and structurally similar to those found in modern sharks (Soler-Gijon 1999; Beck et al. 2004). Pectoral fin spines have been identified on the early chondrichthyan *Doliodus*, and possibly *Antarctilamna* (Miller et al. 2003).

Fin spines from acanthodians are extremely common, as was already mentioned in the introduction. They can have both multiple median and paired spines along their bodies, including ones in front of the anal and pectoral fins (Denison 1979; Brazeau 2009). Acanthodian spines exhibit a wide variety of morphology and symmetry depending on where they are located on the body, with some being symmetrical along the midline and recurved (median dorsal spines), some being symmetrical, short and straight (anal spines), some have an asymmetrical stubby cone-like appearance (intermediate spines), and some

are long, asymmetrical and curved (pectoral spines) (Denison, 1979). This is, of course, a generalization, and variations exist among them; for example, the Lower Devonian form, *Climatius* (Burrow et al. 2005), have median dorsal spines that appear to be short and stubby cones compared to the very long and slender median fin spine from another acanthodian of the same age, *Parexus*, whose spine is more than half of its total body length. (Burrow et al. 2013).

Fin spines in osteichthyans are not common. To date, there are only a handful of fossil taxa with fin spines, which are *Lophosteus* (Gross 1969), *Psarolepis* (Zhu et al. 1999), and *Guiyu* (Zhu et al. 2009). Paired fin spines are reported on *Lophosteus*, but it is difficult to say how many and from which part of the body they come as this taxon is known only from isolated material (Gross 1969; Schultze & Märss 2004). The median dorsal spines of *Guiyu* and *Psarolepis* resemble each other slightly, but the base of *Guiyu* is more flared and stout in morphology compared to *Psarolepis* (Zhu et al. 1999, 2009) and shares some similarities to the composite spines of placoderms (Zhu et al. 2009). The pectoral spines of *Psarolepis* and *Guiyu* are large and not described in great detail, but the one from the former has morphological similarities to those found in acanthodians and placoderms (Zhu et al. 1999).

Spines are also present on extant and fossil siluriform fish (catfish) and include poisonous spines that are found along the leading edge of the dorsal and pectoral fins (Lagler 1977). However, these spines are modified lepidotrichia that fuse during development and are non-homologous to the dermal fin spines discussed in this thesis (Reed 1924).

Most placoderms have paired spinal plates that connect with the shoulder girdle (Denison 1978). Median spines, like in *Romundina* **(Paper III)**, comprise the posterior portion of a composite structure of two median dorsal plates (Goujet & Young 2004). Composite spines are also identified on some yunnanolepids and antiarchs placoderms (Zhang 1978; Goujet & Young 2004).

Some early jawless stem-group gnathostomes ("Ostracodermi"), namely the heterostracans, had large median bony spinal plates (Halstead 1973; Elliot 1985; Botella & Farina 2008) that superficially resemble the dermal spines associated with the spine-like median dorsal plate of placoderms (stem-group jawed vertebrates), like *Romundina* **(Paper III)**. These spinal plates are one of many medially placed modified scales that do not sit anterior to fins (Halstead 1973; Janvier 1996).

Morphology

Fin spines are variable in morphology, but they all have the same general features. They are typically long and recurved structures that are round to triangular in cross-section and taper away from the body (Figure 2). The apex of the spine, or tip, is the most distal portion, and the base is the most proximal and is the part where the spine attaches to the body of the animal. The main

part of the spine is composed of two lateral surfaces that meet anteriorly to create the anterior ledge, or leading edge, and widen posteriorly and are connected by a posterior surface. The anterior ledge of a fin spine
can be thicker in some taxa, this is called the keel. The posterior surface can have a small groove along the midline for attachment of the fin.
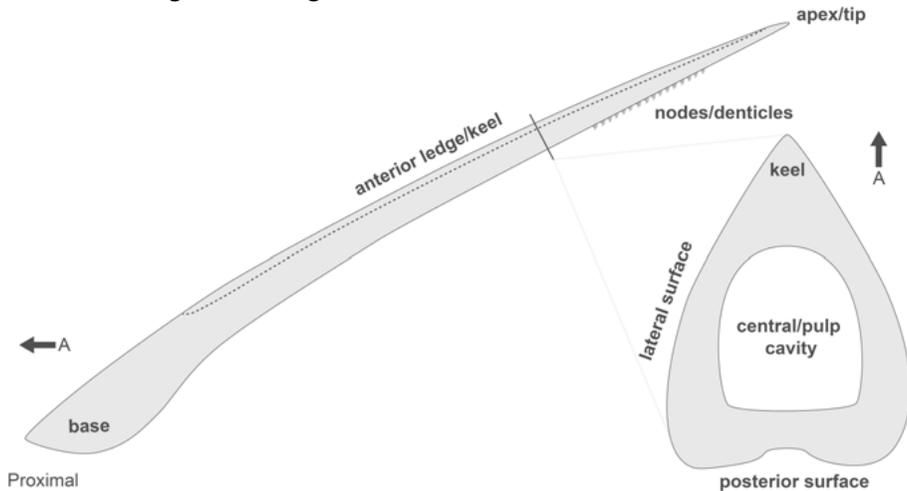

Figure 2. Generalized morphology of a holocephalan fin spine.

Fin spines can have shallow or deep insertions, though it seems that most of the deep inserting spines belong to elasmobranchs (Maisey 1978; 1979). Neoselachian fin spines are deeply inserted into the body and held in place by a portion of the basal cartilage of the fin. Holocephalan spines are similar to these, but are slender with shallow insertions (Maisey 1979; Clarke & Irvine 2006). Acanthodians have both deep and shallow inserting spines depending on where they are found on the body. Fin spines from extant taxa have a central cavity that can accommodate a supporting cartilage. In sharks, the anterior portion of the fin cartilage (or basal cartilage) splits and inserts into the base of the spine, but in chimaeras, this cartilage becomes a long, rod-like extension along the anterior portion of the basal cartilage (Maisey 1979).

A common feature to all fin spines is the presence of some sort of dentine ornamentation that can be stellate, nodal, ridged/ribbed, serrated, or a combination of these. The individual units of ornament are called odontodes, usually associated with dermal bone, but the term can also be applied to fin spines (Ørvig 1977). The terms "tubercles" and "denticle" are commonly used to describe the ornamentation on chondrichthyan and acanthodian fin spines (see Maisey 1978), and are distinguished by their morphology (a denticle, for example, is tooth-shaped).

Neoselachian spines are not elaborately ornamented, but there can be a thickening of tissue at the anterior keel on deep water species, such as

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Etmopterus (Clarke & Irvine 2006; Irvine et al. 2006). Paleozoic and Mesozoic chondrichthyans are known to have fin spines that are heavily ornamented with longitudinally arranged ribs that can become ridged or noded baseward (Maisey 1978, 1981, 1982). *Asteracanthus*, a Paleozoic hybodont shark, has a fin spine that is covered in longitudinal rows of tubercles (Maisey 1978). Rounded nodes and noded ribs can also be found on fin spines from some fossil holocephalans (Stahl 1999).

The most common type of ornamentation on acanthodians is straight, parallel ribbing that converges apically. The width and number of ribs vary between taxa resulting in several characters relating to ribbing being used for phylogenetic analyses (see Burrow & Turner 2010; Burrow et al. 2013, 2016). Thick noded ribs, rounded and ridged, are also present on acanthodian dorsal spines, like those from gyracanthids (Turner et al. 2005; Burrow et al. 2008, Warren et al. 2000). Individual ribs, including the anterior keel, where present, can be composed of several closely set ornamented nodes, and the bases of some pectoral spines can be noded as well (Miles 1973).

The posterior surface of fin spines from fossil chondrichthyans and some acanthodians can have posteriorly facing retrose nodes (denticles), which has been suggested as a possible total group chondrichthyan feature by Brazeau & Friedman (2014) when acanthodians are considered stem-group chondrichthyans. These are usually deposited in somewhat regular intervals and are present in two parallel to slightly offset rows (Maisey 1978; Burrow et al. 2013). Retrose nodes are usually small features on the posterior surface of the spine, but can sometimes be rather large and comprise a single row (*Recurvacanthus*, Duffin 1981). This feature is retained by extant holocephalans but is not present on the fin spines of extant neoselachians (Sullivan 1977; Maisey 1979). Additionally, fossil holocephalans, including *Myriacanthus* and *Metopacanthus*, can have nodes that are tightly positioned together creating a serrated edge along the basal and middle portion of the leading edge of the fin spine (Stahl 1999). This type of node is also found along the posterior surface of some acanthodian pectoral spines (Miles 1973).

The dermal spine of the placoderm *Romundina* is ornamented with stellate odontodes that can be round or elongated (Goujet & Young 2004; **Paper III**). The probable stem-osteichthyan *Lophosteus* has fin spines that can also be ornamented with odontodes (Gross 1969, 1971; **Paper II**). *Psarolepis* and *Guiyu* fin spines have parallel ribbing but with no anterior keel (Zhu et al. 1999; Zhu et al., 2009).

Function

Fin spines are used for defense in extant chondrichthyan fishes (Maisey 1979). Venom producing tissue is associated with fin spines in *Heterodontus*, *Squalus*, and the caudal spines of batoids (Southcott 1977; Dehghani et al. 2010).

Tests performed on mice with venom that was extracted from *Hydrolagus colliei* suggest that there is a mild toxicity to it (Halstead & Bunker 1952). Didier et al. (2014) report that some chimaera fin spines may have venomous spines, but its toxicology of is unknown. In *Squalus* embryos, glandular tissue is very well developed throughout ontogeny (see **Paper I** for images). This could mean that a poisonous spine in hatchlings, which are fully developed at birth, is necessary for protection immediately after being born (Maisey 1979).

Given that fin spines in extant chondrichthyans have a defensive purpose it is reasonable to say that this function was the same for the median dorsal spines in fossil taxa. It has also been suggested that fin spines may have a stabilization and locomotory function (Maisey 1979; Coates et al. 1998), which is also suggested by the *Lophosteus* fin spine presented in **Paper II**. Zangerl (1981) proposed that *Stethacanthus*, a Paleozoic shark with a spine anterior to a rather unusual denticulated dorsal fin, called a "spine-brush complex", could have used it for courtship. The spines and the spine-brush complex are not yet found on females (Maisey 2009), suggesting that they might be sexually dimorphic, which has also been reported in some fossil chimaeras (Lund 1977; Lund & Grogan 1997).

Composition of hard tissues

Vertebrate hard tissues can be classified into a variety of types based on their mineral composition, microstructure, and growth patterns. These classifications form a comparative framework that is essential in studies of early vertebrate interrelationships and evolution. The three principle tissue types found in fin spines are bone, dentine, and enameloid, which will be introduced here.

Bone is a skeletal tissue made from mineralized (hydroxyapatite) and organic (mainly collagen type I) extracellular matrix (Hall 2005; Sire et al. 2012). It is found in almost all living vertebrates, with the exception of lampreys, hagfishes, and most living and extinct chondrichthyans, whose endoskeletons are entirely cartilaginous (Janvier 1996; Donoghue et al. 2002; Donoghue et al. 2006; Sire et al. 2012). Bone is deposited by osteoblasts (when enclosed in matrix, these are called osteocytes) and can form from mesenchymal condensations (intramembranous bone) to make dermal bones (such as fin spines), or from a cartilage precursor (endochondral bone) to make long bones (Francillion-Vieillot et al. 1990; Hall 2005; Sire et al. 2012). The process of bone deposition may also vary: lamellar bone is deposited in thin layers as alternating sheets of aligned collagen fibers in apposition, parallel-fibered bone is formed by parallel collagen fibers, fibrous bone is formed by collagen fibers that are oriented in all directions (Sire et al. 2012). Bone can also be vascularized, with primary canals forming around blood vessels, but the vascularization may be secondarily filled in or remodeled with inward growing lamellar

bone that fills in vascular canals to create osteonal bone (Francillion-Vieillot et al. 1990; Hall 2005).

Dentine is the main tissue of true teeth (enamel + dentine) in extant animals (Hall 2005; Nanci 2012). It is a tubular tissue that has a composition of 75% inorganics (hydroxyapatite crystals), 20% organics (mainly collagen fibers of type I), and 5% liquids. Dentine matrix is deposited by mesenchymal cells called odontoblasts, and is later mineralized with hydroxyapatite (Francillon-Vieillot et al. 1990).

Francillon-Vieillot et al. (1990) divides dentine into two different categories: *true dentine* and *dentine tissues*. *True dentine* is acellular and split up into two types that are called *orthodentine*, which is compact and surrounds a main pulp cavity and has long cell processes that extend through the dentine tubules, and *osteodentine*/*trabecular dentine*, which is made from a mesh of parallel-fibered tissue that may or may not have bone cells with vascular canals that are filled in with lamellate dentine (denteons) – reminiscent of primary osteons in bone (Ørvig 1951; Francillion-Vieillot et al. 1990; Smith & Sansom 2000). *Dentine tissues* are observed in fossil vertebrates and usually have cell spaces trapped in the matrix. *Semidentine*, mainly known from arthrodire placoderms, has polarized tear-drop shaped cell spaces located at the end of long cell processes (Halstead 1974; Denison 1978; Janvier 1996; **Paper III)**. *Mesodentine* is characterized by having odontoblast cell spaces that are trapped within the dentine matrix to create a network of multiple branching interconnected tubules of irregular shapes, similar to what is seen in bone (Ørvig 1951; Francillion-Viellot et al. 1990; Smith & Sansom 2000; Donoghue et al. 2002; Sire et al. 2012). This tissue is commonly described from acanthodians and jawless stem-vertebrates (Halstead 1974; Sire et al. 2012).

Enamel is a prismatic hypermineralized tissue that covers the surface of teeth and scales in actinopterygians, sarcopterygians and tetrapods, and is almost completely composed of inorganic material (Hall 2005; Sire et al. 2012; Qu et al. 2015b). Ameloblasts are responsible for depositing the matrix and are identified as being large and bulbous to prismatic cells with polarized nuclei that line the inner surface of epithelial tissue (Hall 2005; Nanci 2012). Once the enamel matrix has been deposited on top of the dentine matrix, the organic component begins to degrade and is replaced by hydroxyapatite (Sire et al. 2012).

Enameloid is a clear hypermineralized tissue that covers the surface of some teeth and scales from chondrichthyans and actinopterygians (Gillis & Donoghue 2007). Enameloid is similar to enamel in that they both rely on epithelial-mesenchymal interactions and they both function as a resistant outer layer on exoskeletal structures (Gillis & Donoghue 2007). It is still deposited by ameloblasts, but a large part of the matrix is deposited by odontoblasts, which

leaves behind a poorly organized crystalline structure after mineralization occurs. In histological sections, the boundary between enameloid and dentine is uneven. Enameloid is also referred to as mesodermal enamel, modified dentine, vitrodentine, or durodentine (Francillon-Vieillot et al. 1990).

Fin spine composition and distribution

Fin spines can be broken down into different components based on their composition. This can differ greatly depending on the group, but the general organization of these structures is as follows: a main tissue, either bone or dentine, that is covered in dentine ornament that may or may not have a hypermineralized layer of tissue associated with it (Figure 3).

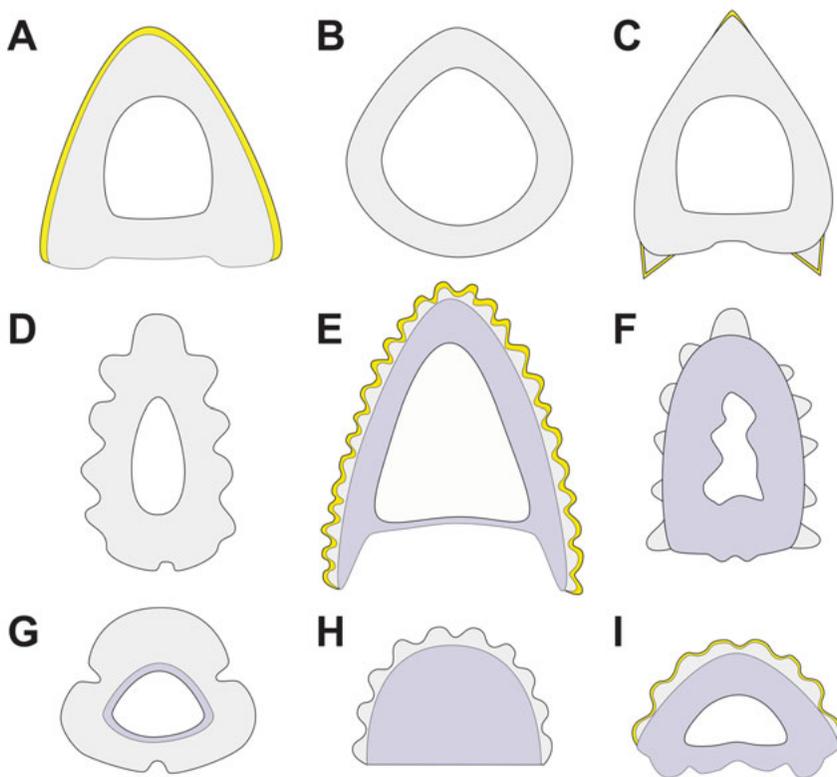*Figure 3*. Diagram illustrating the general compositions of fin spines in A), *Squalus*, B), *Oxynotus*, C), *Callorhinchus*, D) acanthodian morphotype E from **Paper IV**, E), *Romundina*, F), *Lophosteus*, G) acanthodian morphotype D from **Paper IV**, H) acanthodian morphotype A from **Paper IV**, and I), *Psarolepis*. Tissues are grey: dentine; blue: bone; yellow: enamel/enameloid. Fin spines are not to scale.

Extant neoselachian fin spines are fully dentinous and can be divided into different structural units, which will be discussed in more detail later in this thesis. The main structural component of these fin spines is usually composed of inward and outward growing lamellar orthodentine that sits around the pulp cavity (central cavity in fossils) of the spine (Maisey 1979). This is generally the same case with holocephalan fin spines, but the spine also includes a region of trabecular dentine that is located anterior to the orthodentine (Halstead & Bunker 1952; **Paper I**). Fossil chondrichthyan fin spines show an increase in the presence of trabecular dentine, with some being constructed solely of trabecular (osteo-)dentine, including hybodonts (Maisey 1978), symmoriiforms (Zhu 1998; Coates & Sequeira 2001; Coates et al. 2008; Maisey 2009), and stem-holocephalans (Patterson 1965). Fossil chondrichthyan fin spines can have a thin inner layer of lamellar dentine around the pulp cavity (Maisey 1978; Zhu 1998; Maisey 2009).

Acanthodian fin spines are interesting in that they can have both bone and dentine comprising their spines, with some being fully bony like in *Nostolepis* (Denison 1979) and *Ptomacanthus* (Brazeau 2012), but most have spines that are composed of both dentine and bone (Burrow & Turner 2010, Burrow et al. 2013, 2015, 2016; Turner et al. 2005). In these taxa, there is usually a thin layer of lamellar bone or laminar dentine at the core with a thicker outer portion of trabecular dentine. The fin spine of *Machaeracanthus*, is composed only of trabecular dentine (Burrow et al. 2010).

Placoderm spines, like the one from *Romundina* described in **Paper III**, are made of dermal bone, as are the fin spines from the phylogenetically problematic stem-osteichthyan *Lophosteus* **(Paper II)**. The *Romundina* spine has a thick middle layer of osteonal bone and a thinner inner layer of bone around the central cavity. Preliminary results from synchrotron scan data of the dorsal spine from the osteichthyan *Psarolepis* show that the main tissue is a thick inner layer of lamellar bone, which is not present in *Romundina* or *Lophosteus* (Figure 4).

Dentine ornamentation on fossil fin spines has been reported to be composed of semidentine in placoderms, and mesodentine and orthodentine in all other jawed vertebrates, depending on the taxon (Ørvig 1967). Ornamentation can have a layer of hypermineralized tissue, such as enamel or enameloid. *Psarolepis* fin spines have a layer of enamel (pers. comm. Qu 2015) and most extant chondrichthyans fin spines are partially covered in enameloid. Some diplacanthid acanthodians have a "thin outer layer" that covers the ribbing and ornamentation in some of the spines (Burrow et al. 2016). This layer is distinct from the trabecular dentine that makes up the main part of the spines and lacks tubules, vascular canals, or cell lacunae (Burrow et al. 2016). In *Diplacanthus crassinus* this outer layer is composed of orthodentine (Burrow et al. 2016), in *Gyracanthides* it is described as being 'pallial' dentine (after Ørvig 1951), and in *Rhadinacanthus*, it is described as durodentine (=enameloid) (Burrow et al. 2016).
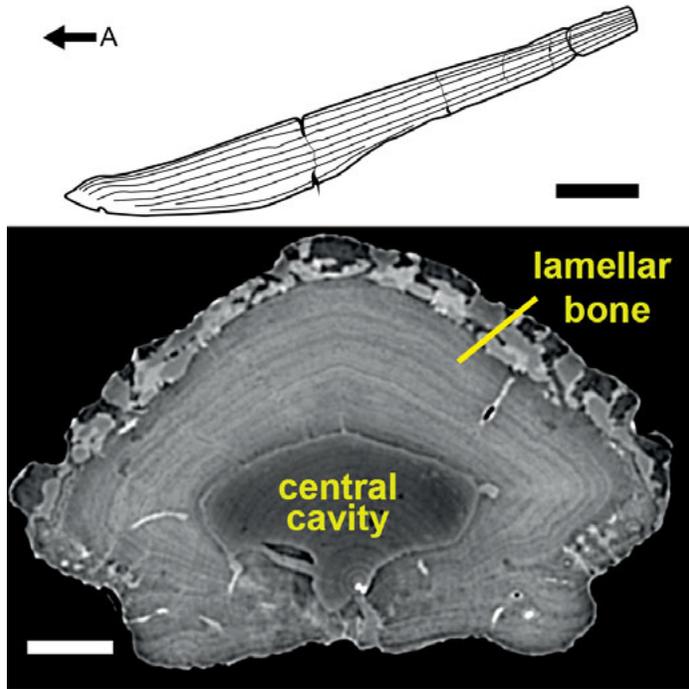
Figure 4. Illustration of the lateral view of the *Psarolepis* fin spine and synchrotron scan slice showing lamellar bone. Scale bar for illustration is 2.5 mm and the virtual thin section is 550 µm.

When some of the tissues discussed above are considered within an evolutionary context, the bony fin spines that are represented by *Romundina* and *Lophosteus* (**Papers I & II**) sit at one end of the spectrum, while fully dentinous spines, represented by chondrichthyans, end up at the other. The fin spines of acanthodians tend to be a mixture of dentine and bone, with the main tissue type being trabecular dentine. Trabecular dentine is also present in fossil chondrichthyans, but is reduced in holocephalans and lost in extant neoselachians (Patterson 1965).

Aims of thesis

The purpose of this thesis is to develop an understanding of fin spine morphology and evolution through a comparison of examples from fossil and living taxa. In this thesis, I apply modern microtomographic analysis, in conjunction with classical histological methods to:

1. provide a detailed description of the embryonic development of the dorsal fin spine of the extant holocephalan, *Callorhinchus milii*, and how it compares to fin spines from the neoselachian *Squalus acanthias*. **(Paper I)**

2. investigate the development of fin spines of *Callorhinchus* as a model for studying growth in fossil fin spines. **(Paper I)**

3. describe and analyze the morphology and histology of fossil fin spines and to identify potentially informative characters relating to growth using 3D datasets. **(Papers II, III, & IV)**

Taxa described in this thesis

The extant taxa studied in **Paper I** are the chondrichthyans, *Callorhinchus milii* (Holocephali), and *Squalus acanthias* (Neoselachii). **Papers II, III, & IV** deal with fossil taxa sampling from each of 'the traditional' groupings of early gnathostomes. Their characteristics, biology, and paleontology are briefly summarized below.

Extant taxa

Callorhinchus milii (Bory de Saint-Vincent 1823)

Holocephalans, also known as chimaeras, are a group of cartilaginous fish that are found worldwide (Didier et al. 2012). These animals are defined by a number of unique characters including smooth skin lacking scales, except during development; holostylic jaw suspension; one gill opening anterior to the pectoral fin; absence of a spiracle; and hypermineralized ever-growing tritural toothplates (Patterson 1965; Didier 1998). Chimaeras generally live at a water depth of 200-2000 meters and tend to live near the bottom of the shelf and slope (Inoue et al. 2010; Didier et al. 2012). However, they reproduce near shore, laying up to two eggs, sometimes in estuarine waters (Didier 1995, 1998).

Fossil chimaeras date back to the Upper Carboniferous with fossils of members of Callorhynchidae going back to the Upper Cretaceous, but these fossils are only known from teeth and spines (Patterson 1965). Some *Ischyodus* (a fossil callorhynchid) specimens are known from complete specimens and date back to the Upper Jurassic (Stahl 1999). *Callorhinchus* fossils date back to the Cretaceous and are represented by fin spines and in some exceptional cases, egg sacs (Dean 1909). Due to their unusual skeleton and overall appearance, chimaeras have been linked to ptyctodont and rhenanids placoderms (Stensiö 1925; Ørvig 1960). However, the morphological similarities used to unite these groups with holocephalans have been shown to be either convergent or primitive (Patterson 1965). It has been suggested that chimaeras are a paraphyletic assemblage that share cranial and dental features (Lund and Grogan (1977), but it is generally accepted that holocephalans are the sister group to sharks based on both morphology and molecular biology (Zangerl & Case 1973; Didier 1995; Inoue et al. 2010; Maisey 1986, 2012).

Due to its placement as the sister group of other extant holocephalans (Didier 1995; Venkatesh et al. 2007; Inoue et al. 2010) and its recently published genome (Venkatesh et al. 2014), *Callorhinchus* has been used as a model organism to study the evolution and development of morphological structures in fossils (Cole et al. 2011; Gillis et al. 2011; Johanson et al. 2015). It has been argued that modern chondrichthyans are in many ways too derived to be used as a model organism (Rücklin & Donoghue 2015, Donoghue & Rücklin 2016), but the many similarities between spines from *Callorhinchus* to those of fossil chondrichthyans and acanthodians suggest that they may be a good basis from which to study the evolution of dermal spines **(Paper I)**.

Squalus acanthias (Linneaus 1758)

The Neoselachii include all living sharks, rays and skates and are defined by many characters, including fins with radials that do not extend in to the distal webs of fins; left and right pectoral girdle fused/articulated at the ventral midline; fused left and right halves of the pelvic girdle; elongated ethmoid region; modern tooth enameloid; and simplified dermal denticles (Compagno 1977). Neoselachians are divided into the galeomorphs (horn sharks, white sharks, mako sharks, and carpet sharks) and the squalimorphs (dogfish, sawshark, angelsharks, and batoids), with *Squalus* belonging to the latter group (Compagno 1977). The genus *Squalus* lacks an anal fin and has a dorsal fin spine in front of the anterior and posterior dorsal fins (Compagno 1984). It is a migratory taxon, common to coastal and temperate oceans (Campana et al. 2006) that has a very wide distribution and lives between 10-200 m in the water column, depending on size and sexual maturity. *Squalus* is ovoviviparous, having litters of 1-20 pups with a gestation period of 18-22 months (Compagno 1984).

Neoselachians have been shown to be a well supported monophyletic group (Maisey 2004), dating back to the early Triassic. Their fossil record is known mainly from isolated teeth, but rare skeletal remains can also be found. This group of sharks quickly diversified into the Mesozoic, but their true diversity is unknown due to a bias in the fossil record for larger teeth and skeletal elements (Underwood 2006).

Squalus represents one of two closely related groups of neoselachians that possess fin spines. As previously mentioned, the growth model based on the fin spine of Squalus that was provided by Maisey (1979) has been used to study others from both extant and extinct fossil taxa (Soler Gijon 1999; Beck et al. 2004; Irvine et al. 2006; Tovar-Ávila 2009). The histology of the fin spine from a developing *Squalus* is investigated in **Paper I** to provide a comparable descriptive source of its development when compared to the data from *Callorhinchus*.

Fossil taxa

Lophosteus superbus (Pander 1856)

Lophosteus superbus is a late Silurian (Pridoli) probable stem-Osteichthyan (Botella et al. 2007) known from isolated remains, including scales and spines (Gross 1969, 1971a). The fossils were originally collected from the Ohesaare Cliff locality in Estonia and described by Pander (1856). Gross (1969, 1971a) provided the most detailed description of *L. superbus* based on an assemblage collected from the same locality. *Lophosteus* was identified from fin spines and rhombic scales with a peg and socket base that are ornamented with stellate odontodes (Gross 1969). The material discussed in **Papers II** was collected as part of a collaboration between Uppsala University and the Institute of Geology at the Tallinn University of Technology (GIT) in Estonia. All of the material was chemically prepared out of the rock with acetic acid by Git Klintvik Ahlberg at Lund University in Sweden. The type and figured material is held at the GIT in Estonia and the rest of the material is being stored at the Museum of Evolution at Uppsala University, Sweden. 3D printed models of both specimens have been catalogued in the collection.

Since it was first discovered, several other species of *Lophosteus* have been described from localities in North America (Märss et al. 1998), Australia (Burrow 1995), and central and eastern Europe (Märss 1997; Botella et al. 2007; Cunningham et al. 2012) indicating that its distribution was relatively worldwide. It is known from an array of bone fragments, scales, and spines, but no articulated material has been found, making it difficult to determine precisely where it fits in gnathostome phylogeny. Historically, *Lophosteus* was shown to have affinities with actinopterygians (ray-finned fish) (Rohon 1893), acanthodians (Schultze & Märss 2004), and placoderms (Burrow 1995). *Lophosteus* was assigned as a stem-osteichthyan based on its osteichthyan-like marginal jaw bone, and lack of shedding tooth rows that are characteristic to most crown-osteichthyans (Botella et al. 2007; Cunningham et al. 2012). This assignment makes it one of the oldest reported stem-osteichthyans, highlighting the importance of understanding the morphology and histology of the isolated remains referred to this taxon.

The *Lophosteus* material described in **Paper II** provides the first detailed histological description from the scales and the fin spines from this taxon. There are up to eight different types of spines associated with *Lophosteus* (Gross 1969, 1971), and so there is the possibility that this specimen could be one of a couple different types of median spine (anal or dorsal spine). The high-resolution scan was taken at the apex of the fin spine and does not include the entire specimen. However, the fin spine from **Paper II** appears to be a symmetrical fin spine and has a leading edge composed of elongated, smooth odontodes, similar to the *Lophosteus* median dorsal spine that was described by Otto (1991).

Romundina stellina (Ørvig 1957)

Romundina is an acanthothoracid placoderm from the earliest Devonian (Gedinnian stage, Lochkovian age) that was originally described by Ørvig (1975). The material was collected from the Prince of Wales Island in the Canadian Arctic by members of the Geological Survey of Canada (GSC). The type material is held at the GSC, but many other specimens are held in Europe, including at the Muséum National d'Histoire Naturelle in Paris, France (MNHN) (Goujet & Young 2004; Dupret et al. 2010; Dupret et al. 2014), and Naturhistoriska Riksmuseet in Stockholm, Sweden (NRM) (Giles et al. 2013; Rücklin & Donoghue 2015; **Paper III**).

In some phylogenies, *Romundina* is grouped together with *Brindabellaspis* and *Murrindalaspis* as an acanthothoracid placoderm, and placed as the sister group to all other placoderms when considered a clade (Goujet 1984; Goujet & Young 2004), or as the sister group to other jawed vertebrates when they are considered paraphyletic (Giles et al. 2013). This opinion has changed with the shifting idea of placoderm paraphyly, but the morphology of *Romundina* still retains primitive characteristics and interesting anatomical differences compared to other more crownward placoderms, such as arthrodires (Dupret et al. 2014).

Placoderm skeletal histology, including descriptions on bony plates and scales, has been studied by Ørvig (1951, 1957, 1967), Downs & Donoghue (2009), Dupret et al. (2010, 2014), Giles et al. (2013), and Rücklin & Donoghue (2015), but none of these focus on paired spinal plates or median spines. The *Romundina* median spine in **Paper III** is the first dermal spine from a placoderm to be described using synchrotron radiation tomography.

Ramsåsa acanthodian fin spine assemblage

The Upper Silurian Ramsåsa site E locality in Skåne, is one of a few localities in Sweden that yields larger fragments of gnathostome fossils (Vergoosen 2004). Among the material collected are scales and bone fragments from jawless stem-group gnathostomes, in addition to many scales, fin spines, and shoulder girdles from acanthodians (Vergoosen 2004). According to Vergoosen (2004), the material from the Ramsåsa site E assemblage was probably collected by Carl Pleijel. The collection was never described and is currently uncatalogued at the NRM.

The Ramsåsa fin spines are difficult to study because they consist of mostly fragmentary, isolated remains that have no anatomical or taxonomical data associated with them. In **Paper IV**, these spines are divided into seven different groupings based on differences in morphology and it was found that most of them resemble the median fin spines from climatiid and ischnacanthid acanthodians. These assignments are based on descriptions of fin spine frag-

ments and scales provided by Gross (1971b) and Vergoosen (2004). It is currently not possible to provide more specific taxonomical assignments without articulated acanthodian material from the Ramsåsa site. However, the broader taxonomical assignments given to them in **Paper IV**, along with histological information provided from three-dimensional datasets, make it possible to study the growth patterns of acanthodian fin spines.

Methods

Fin spine development in extant taxa was studied using traditional histological techniques, including both ultra-thin sectioned decalcified and paraffin-embedded material from developing embryos and adult spines, and ground thin sections of epoxy embedded adult material (not decalcified). Fossil histology was studied using synchrotron scan datasets. Summaries of these techniques are described below.

Embryo acquisition

The *Callorhinchus milii* embryos used in this thesis were between 32-36 weeks of development (staging by Didier 1998). Adult females were collected and transferred to a holding facility using the protocol developed by Boisvert et al. (2015). Specific details and permit numbers are provided in **Paper I**. Adult *Callorhinchus* spines were obtained from the few females that died in captivity. *Squalus* embryos were donated by Dr. J. Sulikowski of the University of New England. Adult fin spines from *Callorhinchus callorhynchus* and *Hydrolagus colliei* were donated to the project from Dr. L. Ferry-Graham, but these were not used for this thesis. These specimens are currently being held at the Evolutionary Biology Center, Uppsala University.
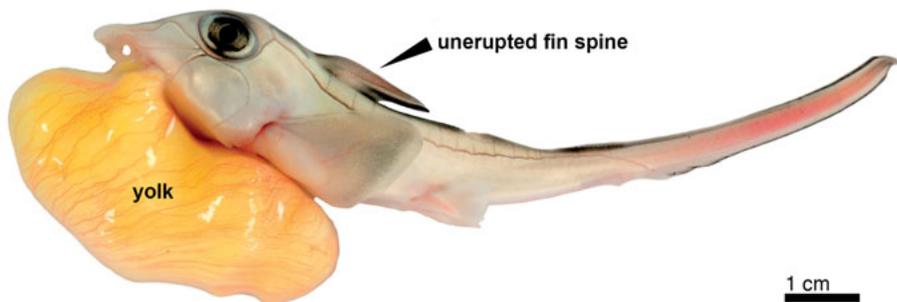

Figure 5. Stage 34 embryo of *Callorhinchus milii*.

Histology

Ultra-thin sections for the majority of *Callorhinchus milii* and *Squalus acanthias* specimens used in **Paper I** were prepared using the paraffin embedding and eosin-hematoxylin & alcian blue staining protocol followed by the histology laboratory at Monash University in Melbourne, Australia. Any of the paraffin blocks that were not fully cut were saved and cut with microtomes at the Natural History Museum in London (Sackler Imaging Center) and Uppsala University Evolutionary Biology Center between 2012 and 2015. The staining protocol used during this time was roughly the same as that followed at Monash. The formulas for the stains were from Humason (1967). Sections were cut at 5, 8, and 10 µm. Ground thin sections of adult *Callorhinchus* spines were sent away to the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP) for thin sectioning and were cut at 50-80 µm.

Imaging

Images of the thin sections were produced using a Micro-Leica CTR 6000 microscope and a Leica MZ 95 dissection microscope (Leica DFC 420C and 290 cameras; Leica, Wetzlar, Germany) at Uppsala University. Nomarski Differential Interference Contrast and polarized light were used when imaging the ground sections. All macrophotography for **Paper I** was produced using a Nikon D200 and all RAW files were rendered in Adobe Photoshop 5 or 6. All drawings in the manuscripts and in this thesis were drawn by hand and then digitized using Adobe Illustrator 6. All histology sections are currently being held at Uppsala University and Imperial College London.

3D Synchrotron microtomography

Synchrotron radiation microtromography produces volume-based datasets that have been used to study structures from a wide variety of fields including, but not limited to, biology, paleontology and geology. This tool, developed by Tafforeau et al. (2006), has been used extensively by paleontologists to study the microstructural details of different bones, scales, teeth, and related structures (e.g. Tafforeau et al. 2007; Sanchez et al. 2012, 2013, 2014; Qu et al. 2013, 2015; Cunningham et al. 2014; Rücklin & Donoghue 2015). This method is useful as it is nondestructive and allows for fragile or hidden microstructural components to be reconstructed and manipulated using 3D printing software (Tafforeau et al. 2006). Propagation Phase-Contrast Synchrotron X-Ray Microtomography (PPC-SRµCT) can use resolutions down to less than a micrometer and has been shown to resolve histological microstructures really well (Tafforeau et al. 2006; Sanchez et al. 2012; Qu et al. 2015a). The

material described in Papers **II, III, & IV** was scanned at the European Synchrotron Radiation Facility in Grenoble, France (ESRF), in 2010 and 2011 as part of proposal EC688 using this technique. Please refer to **Papers II, III & IV** for exact scan parameters. A preliminary low-resolution dataset for a Ramsåsa spine was segmented in Mimics v.10 three-dimensional printing software (developed by Materialise in Leuven, Belgium). The primary software used to segment the datasets acquired at the ESRF was VG StudioMax 2.2 (developed by Volume Graphics Inc. in Heidelberg, Germany).

Fin spine growth and development

Callorhinchus milii

Paper I used a developmental staging table to identify the stages of fin spine development in the holocephalan *Callorhinchus milii.* Maisey (1979) divided shark fin spine development into six stages, which are: the development of the dermal papilla, differentiation of the spine primordium, reduction of the spinal epithelium and deposition of enameloid, maximum growth of mantle and trunk, exertion of the spine through the epidermal bulb and formation of the trunk transitional layer, and irregular deposition of the mantle in adults (Maisey 1979). The developmental data from **Paper I** show that while the *Callorhinchus* fin spine develops similarly to *Squalus*, there are also crucial differences between them, which are summarized below.

Maisey (1979) reported four different dentine components that create the structure of the fin spine based on the development of *Squalus*: the trunk, the mantle, the enameloid cap, and a transitional layer (Figure 6A). Trunk dentine is the innermost layer of dentine and makes up the bulk of the spine in all extant chondrichthyans and is composed of lamellar orthodentine (Maisey 1979). The trunk begins to form when dentine matrix is deposited by odontoblasts in an inward and outward direction from a mesenchymal tissue called the trunk primordium ("stammtheil" of Markert, 1896; Maisey 1979). The initial shape of the trunk primordium of *Callorhinchus* is 'H'-shaped (Figure 6B, dotted-H) in cross-section and the one that forms *Squalus* is U-shaped (Figure 6A, dotted-U). The open parts of these primordia become closed in different ways: in *Callorhinchus*, the upper part of the 'H' is closed by mantle dentine at the very tip of the keel while the lower part grows together posteriorly, and in *Squalus*, the 'U' closes anteriorly (Figure 6). The horizontal part of the *Callorhinchus* trunk primordium forms the anterior part of the dentine that borders the central cavity (which houses the cartilage rod) of the fin spine. The anterior arms of the 'H' deposited inward and outward growing dentine to create the thin outer border that is in contact with the trabecular dentine (Figure 6B). *Squalus* and *Heterodontus* do not have trabecular dentine, and therefore, have simpler trunk primordia than that observed in *Callorhinchus*.
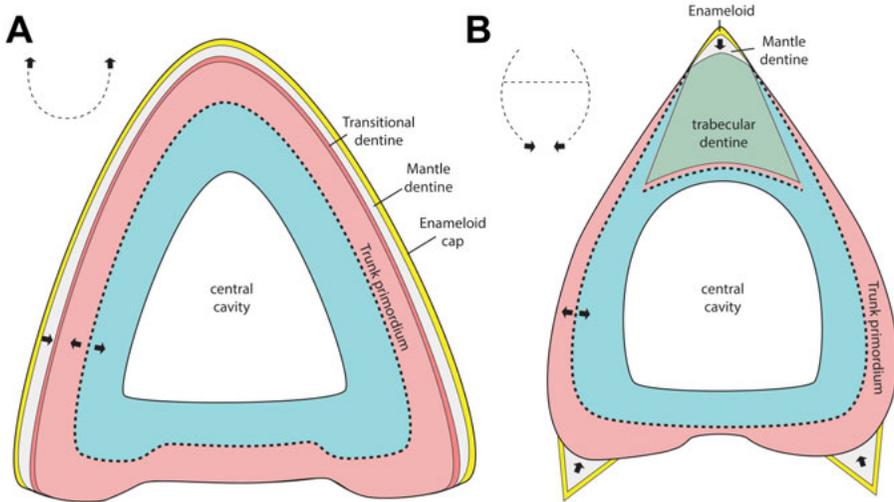
Figure 6. Differences in the internal organization and development of A) *Squalus acanthias* and B) *Callorhinchus milii* (right). Red is outward growing trunk dentine, Blue is inward growing trunk dentine, Grey is mantle dentine, Green is trabecular dentine, dark pink is transitional dentine to mantle, and Yellow is enameloid. Dotted lines indicate the position of the trunk primordium. Arrows indicate direction of growth.

Trabecular dentine (=osteodentine) is located in the anterior portion of the spine and is only present in extant holocephalans (Figure 6B, green). It is much more common in fossil fin spines from chondricththyans and acanthodians, and it has been shown that there is a general trend towards loss of trabecular dentine in chondrichthyans (see Patterson 1965; Maisey 1976, 1977a, 1977b; Coates et al. 1998). This type of dentine forms from a mesh located between the anterior portion of the trunk primordium and grows inward toward vascular canals and is capped anteriorly by mantle dentine. The presence of trabecular dentine makes *Callorhinchus* particularly valuable as a guide to understanding the ontogeny of fossil chondrichthyan and 'acanthodian' spines.

Mantle dentine, when present in extant taxa, is a thin layer of inward growing dentine that rests between the trunk and the enameloid cap (Figure 6, grey and yellow respectively). Mantle dentine in *Callorhinchus* is restricted to the anterior keel and the ornamentation on the posterolateral margins on *Callorhinchus* (**Paper I**). In *Squalus* and *Heterodontus* the mantle is continuous over the lateral faces of the spines (Maisey 1979, Tovar-Ávila et al. 2008). Maisey (1979) reports a transitional layer of trunk dentine in *Squalus* and *Heterodontus* fin spines, but this refers to and outward growing dentine, transitional to the mantle layer, that helps to connect the two layers together (Figure 6A, dark pink).

Enameloid is well-developed on the spines of *Squalus* and covers the anterior surfaces of the fin spine, but in *Callorhinchus* it is restricted to the anterior keel and the posterior facing sharp nodules that are present on the posterolateral margins of the spine. The enameloid cap on the keel of adult spines of *Callorhinchus* is difficult to identify on adult specimens in **Paper I**, and so it could be that the layer of tissue that caps the keel of the spine is slightly different from the enameloid on shark spines.

The results of **Paper I** provide a valuable comparative model for studying fossil fin spines. Much of what we know about fin spine growth and development comes from either mature organisms, fossil material, or based on neoselachian growth models (Markert 1896; Maisey 1978, 1979; Soler-Gijon 1998). Understanding the cellular organization of chondrichthyan fin spines during early ontogeny has shown that there are variations in the original cellular organization between the living taxa, in addition to emphasizing that some growth processes are not observable in fossil material. Understanding the differences observed between the development of the *Callorhinchus* and *Squalus* dorsal fin spines is vital for the continued study of fossil fin spines.

Romundina stellina

The dorsal spine of *Romundina*, which is described in **Paper III**, differs from the fin spines of extant chondrichthyans in having a base of dermal bone, and an external covering consisting of several superimposed generations of small odontodes rather than a single-generation mantle dentine. The 3D paleo-histological data from **Paper III** show that the bone vascular canals of *Romundina* form a pattern comprising two distinct regions: a region of longitudinal canals encompassing the middle and distal part of the leading edge of the spine, and a much larger surrounding region that includes the sides and proximal part of the spine, and is characterized by radially arranged vessels. The first region is the area of origin of where the spine began to grow, apparently as a long splint-like bone primordium. Growth continued from this area to create the spine with new tissue being added proximally and posteriorly. The multiple generations of odontodes on the surfaces of the spine suggest that it was covered by epithelium during most of its development. This contrasts with chondrichthyan spines, which have only a single generation of mantle dentine, form within the epithelium but eventually erupt from the skin, after which growth only occurs proximally.

Lophosteus superbus

It is difficult to assess the growth of *Lophosteus* as the synchrotron data described in **Paper II** only includes the distal tip of a median dorsal spine, but

some useful comparisons with other gnathostomes can still be made. The spine of *Lophosteus* is most similar *Romundina* in its composition and internal organization, but the longitudinal vascularization of the spine tip suggests that the distal part of the spine is the oldest. *Lophosteus* also bears partially buried odontodes at the tip, and so was probably either partially or wholly covered by epithelium during the animal's lifetime. The attachment surface along the posterior side of the spine is bony with many Sharpey's fibers that extend almost to the tip, suggesting that this spine was attached to the fin along its whole length and functioned as support for the fin **(Paper II)**. This contrasts greatly with chondrichthyan fin spines, which have a long, free tip that is not attached to the fin apically and is thought to be used for defense. Three-dimensional data of the middle and proximal parts of median spines from *Lophosteus* is required to provide more detailed information relating to its ornamentation and growth.

Ramsåsa acanthodians

Burrow et al. (2015) suggests, on the basis of morphological and 2D thin section data, that *Climatius* spines grew as a folding of a plate that is fused along the edges. This assessment was based on the presence of a median groove along the posterior side of the spine, but it is entirely possible that the groove could also have been for fin attachment. The synchrotron data of the vascularization of the median dorsal and anal spines of acanthodians that are described in **Paper IV** show that they have longitudinally running vascular systems, without a radially organized region like that in *Romundina*, which indicates that growth in these spines is very similar to chondrichthyans and not like that of a folded plate. If these had grown by fusion of a single plate along the posterior side, the canals would indicate a growth origin leading away from the anterior ledge. The overall morphology of the intermediate spine (Morphotype B) described in **Paper IV**, which is long and smooth with a very short point, is suggestive of a growth pattern more similar to what is seen in *Romundina*, but synchrotron data of the vascularization is required to confirm this.

Acanthodian fin spines usually have a compositional organization that can be divided into three major parts (Denison 1979; Burrow et al. 2005): an inner lamellar/laminar basal layer; a middle thicker trabecular layer; and outer dentine ornament. Denison (1979) and Gross (1971) note that in mature parts of the spines, the central cavity is filled in with inward growing tissue (either dentine or bone) that becomes thicker distally. **Paper IV** shows that this organization is similar to the internal organization in chondrichthyan spines (Figure 7). Trunk dentine exhibits both inward and outward growth from the trunk primordium in *Callorhinchus* and *Squalus*, which is the same as what is

seen with the combination of the basal layer and the in-filled central cavity in acanthodians.
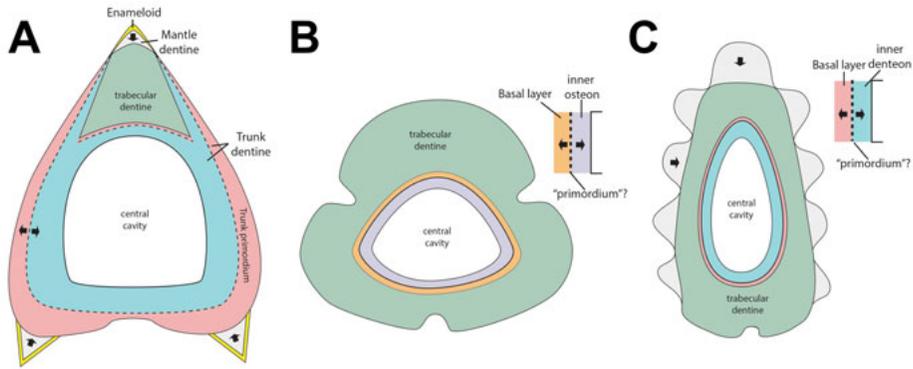

Figure 7. The internal composition of acanthodian fin spines compared to chondrichthyans showing the distribution of tissues. A) *Callorhinchus milii*, B) acanthodian morphotype D from **Paper IV**, and C) generalized version of acanthodian morphotype F from **Paper IV**. Red is outward growing trunk dentine, Blue is inward growing trunk dentine, Grey is mantle dentine/ornament dentine, Green is trabecular dentine, Yellow is enameloid, Purple is inner growing bone (osteon) and Orange is basal layer. Dotted line is trunk primordium and arrows indicate direction of growth.

The trunk layer in *Callorhinchus* is thick distally and becomes thinner proximally, which is also suggested by the synchrotron data in **Paper IV**. The principal difference between acanthodian and chondrichthyan spines is that the inner layer in the former can be composed of bone whereas in the latter it is always dentinous. Diplacanthids and ischnacanthids have a central cavity and several accessory cavities running longitudinally in their spines. Described as accessory pulp cavities by Burrow et al. (2016) and "stachelhöhle" by Gross (1971), these tend to be present in fin spines that are tall and narrow and so may have been necessary to create their distinctive shape, but more data are necessary to support this. Additionally, the extent of trabecular dentine can vary in chondrichthyans and acanthodians, with some having fully trabecular spines, including *Helodus* (Stahl 1999), *Hybodus* (Maisey 1978), and *Machaeracanthus* (Burrow 2010a), while others have both trunk dentine and trabecular dentine as in *Callorhinchus milii*. It is possible that mostly or fully trabecular spines lack a trunk layer, but **Paper I** shows that some of the depositional layers (i.e., the transitional layer) are difficult to discern in adult spines even though they can be clearly seen during development.

Overall, the developmental data clearly show that the spines of *Romundina* and *Lophosteus* resemble each other in terms of construction and growth mode, whereas the spines of acanthodians are much more similar to those of chondrichthyans except in containing varying amounts of dermal bone. This supports the assignment of acanthodians to the chondrichthyan stem group, as suggested by the majority of recent phylogenetic analyses.

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Growth of ornamentation

In chondrichthyans, the epithelial mantle fold and its associated mesenchyme are responsible for the formation of this ornamentation by inward growth of dentine and deposition on to the main body (trunk) of the spine. In *Squalus*, the mantle dentine is continuous over the surface of the spine and in *Callorhinchus* **(Paper I)** it is restricted to the anterior keel and the posterolateral margins. Among other spine-bearing extant sharks, the mantle dentine can also be restricted or absent, like in *Oxynotus* (Maisey 1979). When mantle dentine is present in extant chondrichthyans, it is covered by a layer of enameloid (Maisey 1979; Clarke & Irvine 2006; Irvine et al. 2006). *Romundina* **(Paper III)** has odontodes that are covered in a hypermineralized layer, *Psarolepis* fin spine ribbing is covered in enamel, and *Lophosteus* **(Paper II)** odontodes are bare, suggesting that odontodes may or may not have a hypermineralized cap.

The mantle dentine in extant chondrichthyans is associated with a series of vascular canals, which are called mantle canals (Maisey 1979) (Figure 8A, B, &C). Mantle dentine is deposited toward these canals, and in mature spines they are closed or partially closed. In **Paper IV**, almost all of the Ramsåsa fin spines have ornament canals associated with every individual rib (which are
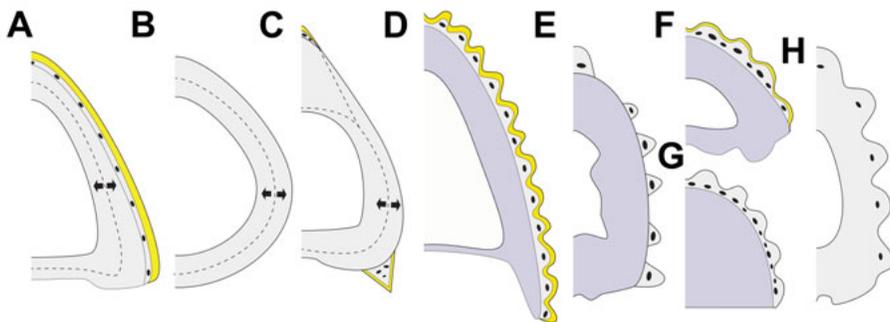

Figure 8. Simplified diagram of fin spines showing the positions of the vascular canals responsible for ornament deposition from A), *Squalus*, B), *Oxynotus*, C), *Callorhinchus*, D) *Romundina*, E), *Lophosteus*, F), *Psarolepis*, G) acanthodian morphotype A from **Paper IV**, and H) acanthodian morphotype F from **Paper IV**. Dotted line is trunk primordium and arrows indicate direction of growth.

figured by Gross, 1971) (Figure 8G & H), and the retrose nodes on the morphotype C spine have ascending canals within them, similar to those in *Lophosteus* **(Paper II)** (Figure 8E). *Romundina* odontodes also have a small network of vascular canals associated with individual odontodes (Figure 8D) and Psarolepis has a layer of canals in the ornament layer (Figure 8F). This means that the odontodes on dermal bone and other types of ornamentation is similar enough in terms of growth and topology that it might be worth re-evaluating and standardizing the terminology relating to them. The major difference between the odontode skeletons of *Romundina* and *Lophosteus* on the one hand,

and acanthodians and extant chondrichthyans on the other, is that the former have multiple generations of superimposed odontodes, whereas in the latter there is only a single generation and the addition of further odontodes is precluded by the eruption of the spine.

Conclusions & Future Perspectives

The purpose of this thesis was to investigate the morphology and histology of fin spines from extant and fossil jawed-vertebrates. To do this, a detailed histological study on the development of the extant holocephalan, *Callorhinchus milii*, was carried out, then compared to the 3D paleohistology provided from microtomographic datasets from different early vertebrates. The following outlines the major conclusions and ideas introduced from these data.

1. Trabecular dentine, found in extant holocephalan spines, is also present in fossil chondrichthyans and acanthodians, making the histological organization of the developing spine of *Callorhinchus* a good framework from which to study fossil fin spines. **(Paper I)**

2. Growth processes that are not visible in fully developed spines can be observed from developing extant spines, and as such, care must be taken when studying growth and development in fossils. **(Paper I)**

3. Three-dimensional datasets provide a great deal of morphological information that cannot be observed from 2D thin sections. Features of the vascular network, ornamentation, and cellular organization are shown to be variable among the few taxa described in **Papers II, III, and IV** and could be useful for extracting phylogenetic characters as more 3D datasets become available.

4. The 3D data collected from fin spines provide another way to study and compare the growth of gnathostomes whose skeletons are dominated by bony plates (osteichthyans and placoderms) and those without (chondrichthyans and acanthodians) **(Papers II, III, & IV)**.

The findings in this thesis underscore the importance of the continued study of gnathostome fin spines. The utilization of synchrotron microtomography to study fossils has provided a great deal of new morphological and histological information. Currently, the use of these datasets is limited by the lack of taxonomic diversity, but as more scan data becomes available it will be possible to study the morphological diversity of microstructural characters that are difficult to assess from 2D thin sections, including, for example, the vascularization, distribution of cell spaces, and Sharpey's fibers. It will, furthermore, be

necessary to test the utility of the morphological characters identified from these data.

Acanthodian fin spines are morphologically and compositionally diverse, in addition to being phylogenetically important. It appears as if they have much in common with chondrichthyan spines in terms of longitudinal growth and the presence of trabecular dentine, but some also retain bone and may grow more similarly to the spine of *Romundina*. They also have unique characteristics, including the presence of accessory pulp cavities. Thus, understanding the morphological and histological variation in acanthodian spines and how they relate to those from chondrichthyans and bony fish is integral to the continued study of fin spines. It is likewise important to undertake a detailed study of the interesting fossil osteichthyans, *Psarolepis* and *Guiyu*. The overall morphology of fin spines from *Psarolepis* is superficially similar to acanthodians and the presence of lamellar bone in *Psarolepis* departs from the composition of *Lophosteus*, illustrating how variable these structures can be. Moreover, acquiring more histological information from the fin spines of symoriiform sharks (Sansom 2005; Coates et al. 2008, Maisey 2009) and other early chondrichthyans, such as *Cladeoselache* (Maisey 1977) and the paired and median spines from *Doliodus* (Miller et al. 2013), will ultimately aid in increasing our understanding of fossil fin spines.

The 3D data of the *Romundina* spine have provided valuable information relating to its growth and morphology. It would be useful to study the growth of fin spines from juvenile *Romundina*, which could confirm the ideas presented in **Paper II** in addition to providing information on the development of the overall morphology and ornamentation, which at the moment can only be inferred from the 3D vascularization in an adult spine.

The possibilities for studying fossil spines using 3D techniques seems endless when the starting point is fewer than five taxa, but this should not take away from the developmental aspect of this thesis. *Callorhinchus milii* has proven itself to be a model organism for studying the development of fossil fin spines **(Paper I)** and could therefore be useful for investigating questions relating to hard tissue homology and evolution. The recently sequenced genome of *Callorhinchus milii* does not have any annotated genes for dentine, bone and enamel. (Venkatesh et al. 2014). These tissues are identified in the *Callorhinchus* and *Squalus* fin spines, with the former possessing trabecular dentine, a tissue that is also found in fossils fin spines. This makes the *Callorhinchus* fin spines a good candidate for examining hard tissue homology and evolution. For example, the presence of ameloblasts interacting with odontoblasts in the histological sections in **Paper I** leads one to believe that what is being deposited is enameloid. However, the tissue is difficult (but not impossible) to discern in ground sections of adult spines. The hypermineralized tissue covering *Romundina* also does not have the same qualities of enameloid when viewed in thin section (Qu et al. 2015b; **Paper III**), but it is clearly different from the semidentine comprising the odontodes. Hard tissue

homology is a large and difficult topic that lies outside of the scope of this thesis, but understanding the processes and mechanisms that drive the deposition of dentine and hypermineralized tissues in vertebrates may help to answer some of the questions raised in this thesis.

Svensk sammanfattning

Introduktion

De nu levande käkförsedda ryggradsdjuren (gnathostomerna) delas in i två monofyletiska grupper: broskfiskar (hajar, rockor och helhuvudfiskar) och benfiskar (inklusive tetrapoder). Monofylogenin för dessa klader styrks av en mängd morfologiska och molekylära data. Det har dock varit oklart var de två utdöda grupperna av gnathostomer, nämligen "pansarhajar" (placodermer) och "taggpansarhajar" (acanthoder), hör hemma i ryggradsdjurens fylogeni. Dessa två grupper har traditionellt ansetts vara monofyletiska och definierades då av ett antal morfologiska karaktärer, något som kommit att ifrågasättas av nya fossilfynd. Fentaggar har varit centrala för denna utveckling. Förekomsten av ett antal pariga fentaggar och mittlinjefentaggar hos "taggpansarhajar" ansågs tidigare som en unik egenskap för denna grupp. Pariga fentaggar och mittlinjefentaggar har nu identifierats även i fossila broskfiskar och benfiskar, och dessutom har upptäckten av tagglika element hos vissa "pansarhajar" påvisat att fentaggar förekommit inom alla gnathostomgrupper. Tillsammans med nya morfologiska karaktärer i kranium och käkar har gruppen "pansarhajar" blivit uppdelad i en parafyletisk grupp av stam-gnathostomer och "taggpansarhajar" har fördelats på både broskfiskarnas och benfiskarnas stam. De senaste analyserna ger starkt stöd åt "pansarhajar" som en parafyletisk grupp och placerar alla "taggpansarhajar" som stam-broskfiskar.

Fentaggar

Fentaggar är stora dermala strukturer som sitter framför fenor längs mittlinjen och framför pariga fenor på nu levande samt utdöda gnathostomer. De byggs upp av samma hårdvävnad som tänder och fjäll. Den huvudsakliga hårdvävnaden utgörs av dentin eller ben, och ytskiktet har ett lager, eller flera lager, av dentinornamentering som kan vara täckt av hypermineraliserad vävnad (emalj eller emaljliknande vävnad). Fentaggar är de enda stora dermala strukturerna hos broskfiskar och "taggpansarhajar", som i allmänhet annars saknar ett förbenat skelett och har en kropp täckt av små fjäll (mikromeri). Detta står i kontrast till "pansarhajar" och benfiskar, som karaktäriseras av stora dermala benplattor som täcker delar av kroppen (makromeri).

Idag finns fentaggar endast hos ett litet antal taxa, inklusive squaliforma och heterodonta hajar, samt hos alla helhuvudfiskar. Dessa taggar är generellt

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långa och smala, och saknar kraftig ornamentering, men det finns små morfologiska skillnader mellan grupperna. Fossila broskfiskars mittlinjefentaggar kunde dock vara mycket större och kunde ha en kraftigare ornamentering jämfört med dagens hajar.

Fossila fentaggar är mycket vanligare och uppvisar en stor variation i morfologi, ornamentering, och histologi jämfört med taggar hos nu levande former. Tack vare detta har fentaggar kunnat användas för att studera taxonomi och fylogenetiska släktskap. Tagglika element återfinns även hos käklösa stam-gnathostomer, kallade pansarpirålar, men dessa taggars natur är fortfarande oklar. "Pansarhajar" kan ha pariga, taggiga plattor associerade med skuldergördeln, och vissa har en dermal tagg i anslutning till medianplattan (liksom *Romundina*, **Artikel III**). De bäst kända fossila fentaggarna tillhör "taggpansarhajarna", som kunde ha ett antal mittlinjefentaggar och pariga taggar längs med kroppen. Fentaggar har hög bevaringspotential, variabel morfologi, och är vanliga bland tidiga vertebrater, vilket innebär att de kan utgöra en nödvändig informationskälla som kan bidra till en ökad förståelse av släktskapen och den evolutionära historien bland de tidigaste käkförsedda ryggradsdjuren. Tillväxten och morfologin hos fentaggar har studerats i detalj tidigare, men majoriteten av vår kunskap är baserad på utvecklingen av dessa strukturer hos levande hajar, såsom *Squalus acanthias*. De studier som presenteras här illustrerar diversiteten i morfologi, histologi, och tillväxt hos hajarnas fentaggar, samt påvisar deras betydelse för framtida studier.

Material och metoder

I det här projektet har morfologin och histologin hos fentaggar från nu levande och utdöda representanter från olika delar av gnathostomernas släkträd studerats. Fentaggens utveckling hos den nu levande helhuvudfisken *Callorhinchus milii* studerades med klassiska histologiska metoder (**Artikel I**) och jämfördes med fentaggen hos hajen *Squalus acanthias*. Utvecklingen av *Callorhinchus* fentaggar har, till skillnad från fentaggarna hos *Squalus*, tidigare inte studerats i detalj. Redan publicerade tunnslip visar dock att den inre organisationen av fullvuxna taggar från *Callorhinchus* skiljer sig från *Squalus*, vilket gör dem viktiga för att förstå skillnader i deras tillväxt. Resultaten från **Artikel I** jämfördes med histologiska data från fossila fentaggar som skannades med faskontrast-tomografi vid synkrotronanläggningen ESRF (European Synchrotron Research Facility) i Grenoble, Frankrike **(Artikel II, III, & IV)**. Denna teknik skapar volymbaserade dataset med en upplösning på mindre än en mikrometer och visar tydligt histologiska egenskaper. Med hjälp av mjukvaran VG StudioMax 2.2 kunde dessa dataset behandlas för att studera tredimensionell (3D) morfologi och histologi. De fossila fentaggar som har beskrivits på detta sätt i den här avhandlingen inkluderar exemplar från *Lophosteus* (en trolig stambenfisk från Estlands silurlager, **Artikel II**), *Romundina*

(en "pansarhaj" från arktiska Kanada, **Artikel III**), samt isolerade, fragmenta-riska fentaggar från "taggpansarhajar" från silurlager i Skåne (**Artikel IV**).

Fentaggarnas tillväxt och utveckling

Resultaten från detta arbete visar att det finns avgörande skillnader mellan utvecklingen av fentaggarna hos *Callorhinchus* och *Squalus*, trots att det all-männa tillväxtmönstret är likartat. Taggarna hos *Callorhinchus* består av samma vävnader som hos fossila broskfiskar och "taggpansarhajar", vilket re-flekteras i deras vävnadsorganisation. Detta antyder att *Callorhinchus* fentagg är en bättre kandidat för att studera fossila fentaggar från "taggpansarhajar" (**Artikel I**).

Den tredimensionella vaskulariseringen i de återskapade dataseten från synkrotrontomografin visar att det finns två olika typer av tillväxt hos de fen-taggar som har studerats i det här projektet. Den ena exemplifieras av brosk-fiskar, vars taggar växer då ny vävnad läggs till vid basen. Detta innebär att taggens spets utgör den äldsta delen (**Artikel I**). Den andra typen av tillväxt representeras av fentaggen hos *Romundina*, vilken växer bakåt från en smal ben-kil som utgör den övre delen av taggens framkant (**Artikel III**). De tredi-mensionella rekonstruktionerna visar att majoriteten av "taggpansarhajarnas" fentaggar (**Artikel IV**) växte på ett liknande sätt som hos broskfiskar, men antyder även att vissa taggar växte på ett liknande sätt som hos *Romundina*. Resultaten i denna artikel visar också att broskfiskar och somliga "taggpansar-hajar" delar likheter i sin inre organisation. Data från **Artikel II** antyder att *Lophosteus* taggspets är den äldsta delen av taggen, men att den generella hi-stologin och morfologin liknar *Romundinas* fentagg.

Fentaggarnas betydelse för studier av gnathostomernas utveckling

De fentaggar från både fossila och nu levande arter som presenteras i den här uppsatsen demonstrerar att den tillväxtmodell som är baserad på *Callor-hinchus* är mycket bättre lämpad för jämförande studier av fossila fentaggar. Synkrotrondataseten visar att 3D-histologin hos fentaggar kan ge betydligt mer information än konventionella, tvådimensionella tunnslip, inklusive aspekter relaterade till taggarnas tillväxt och utveckling. Tillgången till data om taggarnas tillväxt ger möjlighet att göra direkta jämförelser mellan struk-turer som finns i både mikro- och makromeriska djur, något som annars ofta är ett problem. De jämförande resultaten mellan nutida och fossila fentaggar i den här uppsatsen visar att "pansarhajarnas" och benfiskarnas fentaggar är mer lika varandra än vad de är lika broskfiskarnas och "taggpansarhajarnas" tag-gar. Detta ger stöd åt den nuvarande teorin om gnathostomernas fylogeni som placerar "taggpansarhajarna" på stammen till broskfiskarna.
Fynden i den här studien understryker vikten av att fortsätta studera gnat-hostomernas fentaggar. För tillfället begränsas användandet av 3D-data av den taxonomiska tillgängligheten, men i takt med att mer data samlas in

kommer det bli möjligt att studera den morfologiska och histologiska diversiteten även bland mikrostrukturella karaktärer. Det kommer framförallt bli nödvändigt att studera fentaggar från artikulerade exemplar av "taggpansarhajar" för att kunna jämföra dessa mot tillväxten hos fragmentariskt och isolerat material såsom det som presenteras i **Artikel IV**. Det är även nödvändigt att undersöka diversiteten av fentaggar hos benfiskar, vilket indikeras av *Psarolepis* som har taggar som innehåller lamellär benvävnad. Att förstå fentaggar och de vävnader som bygger upp dem, samt de processer och underliggande mekanismer som ligger bakom deras deposition kan också hjälpa till att besvara frågeställningar kring dessa vävnaders distribution bland tidiga vertebrater.

Acknowledgments

"You could tell by the way he talked, though, that he had gone to school a long time. That was probably what was wrong with him."

from *A Confederacy of Dunces* by John Kennedy Toole

In the past decade (understand the quote now?), I have had the honor of meeting and working with some outstanding individuals to whom I must express my gratitude.

Per Ahlberg, *Supervisor*, thank you for the support and patience over the years. I can't believe you stuck by me. I appreciate all of your efforts and I will forever be grateful.

Martin Brazeau, *Supervisor*, the opportunities and experiences that you have bestowed upon me have changed my life considerably, and the unwavering faith that you have for my ability is astounding. I am still a little terrified by your "genius", but I have learned a lot from it and am glad for it. Thank you for being there for me through all of the years.

Tatjana Haitina, *Supervisor*, we both know that this thesis would not exist without your involvement. Your initiative to set me on a realistic path toward finishing and our many hours spent in conversation were what I needed to get my head back in the game. For this I am very thankful.

This thesis would not have been possible without the help from my many collaborators, including:

Qingming Qu, your support as a friend and colleague helped to put me back on track an endless number of times. I truly appreciate all of the hard work and help you provided for me through the years.

Sophie Sanchez, for your vast knowledge on all things relating to synchrotron scanning and histology, in addition to having a rock solid work ethic and enthusiasm that I could only hope to strive for.

Catherine Boisvert, for the access to the elephant shark embryos and help developing the histology project, and for facilitating my visit to Monash.

Zerina Johanson, for being supportive and showing me how things are done even though it was not your job to do so.

Oskar Bremer, for those two days of acanthodians, helping with the translation, and being all-around supportive during this time.

Henning Blom, for providing material and the initial funding (VR # 2006-5558) for this PhD project.

Min Zhu, for providing material for scanning and hosting my visit to the IVPP in 2010.

Many people within the department should be named, including,

Daniel Snitting, for your incredible knowledge, technical support, and engaging conversation over the years.

Helena Malmikumpu, for all of the lab help and friendly conversation over the years.

Henrik Viberg and Irene Söderhall, for your assistance with departmental matters over the years.

Afsaneh Ahmadzadeh, Tove Broberg, and Rose-Marie Löfberg for administrative assistance.

My deepest gratitude goes out to Qingming Qu, Daniel Snitting, Marco Castiello, Richard Dearden, Thomas Guillerme, Oskar Bremer, and Allison Daley for providing comments and proof-reading this thesis.

I would like to thank my opponent, Philippe Janvier, and my committee for their participation in this process.

Many thanks to all of the past and present lab members at the EBC and to the staff and students at Imperial College London.

Lastly, but definitely not least, a big thank you to all of my amazing friends and family members—good job standing by me through the years.

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Editor: The Dean of the Faculty of Science and Technology