

Skeletal Histology of *Bothriolepis canadensis* (Placodermi, Antiarchi) and Evolution of the Skeleton at the Origin of Jawed Vertebrates

Jason P. Downs^{1,2*} and Philip C.J. Donoghue³

¹Department of Geology and Geophysics, Yale University, New Haven, Connecticut 06511

²Academy of Natural Sciences of Philadelphia, Philadelphia, PA 19103

³Department of Earth Sciences, University of Bristol, Bristol BS8 1RJ, UK

ABSTRACT We used light microscopy and scanning electron microscopy to compile a complete histological description of the dermal skeleton of the antiarch placoderm, *Bothriolepis canadensis*. Placodermi is most often cited as the sister group of crown group Gnathostomata, but some recent authors propose that placoderms instead represent a paraphyly of forms leading to the crown. In either phylogenetic scenario, comparative analysis of placoderm and gnathostome histological data allows us to address the primitive condition of both the gnathostome skeleton and the jawed vertebrate skeleton. The results of this work support the interpretation that the external skeleton of *Bothriolepis canadensis* is comprised exclusively of cellular dermal bone tissue. The unique stratification of the antiarch thoracic skeleton that has led to controversial interpretations in the past is explained by the nature of the articulations between adjacent elements. Skeletal features long thought to be gnathostome innovations are instead discovered to arise along the gnathostome stem. These innovations include secondary osteons, the systematic reconstruction of the skeleton in response to growth, and unfused, overlapping joints that enable marginal growth while maximizing the area of the articulation surface. The extensive evidence for spheritic mineralization agrees with a model of the skeleton as one capable of a high growth rate and active remodeling. Dermal skeletal development in both placoderms and osteichthyans is primarily skeletogenetic with only a minor odontogenetic contribution in some taxa. This demonstrates the problem inherent with assuming a broad application for those hypotheses of dermal skeletal evolution that are based on a chondrichthyan model. Our results highlight the importance of anatomical and ontogenetic context in the interpretation of fossil tissues. *J. Morphol.* 270:1364–1380, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: vertebrate; placoderm; histology; dermal skeleton; skeletogenesis; bone

INTRODUCTION

Chondrichthyans are generally perceived to be the most basal clade of vertebrates with a mineralized skeleton and, thus, they have been influential in attempts to uncover the nature of the primitive vertebrate skeleton (Donoghue, 2002). This has

been especially true in debate over the primacy of bone versus cartilage, and the developmental evolution of the dermal skeleton. Though they may be among the oldest extant lineages of skeletonizing vertebrates, it has long been recognized that chondrichthyans are far removed, both temporally and phylogenetically, from those extinct vertebrates in which a mineralized skeleton first evolved and in which skeletal developmental systems were established (Heintz, 1929; Romer, 1942). This recognition stems from the discovery that the mineralized skeleton first arose in extinct jawless relatives of the jawed vertebrates, to whom they are related by degree (Janvier, 1981). The study of these extinct stem gnathostomes (see Fig. 1 for meaning of taxonomic concepts) has revealed that peculiarities, such as the chimeric embryological composition of the vertebrate skeleton, betray its piecemeal evolutionary assembly over a protracted episode of early vertebrate phylogeny (Donoghue and Sansom, 2002; Donoghue et al., 2006). Thus, the vertebrate skeleton may be considered more appropriately and accurately as a series of distinct skeletal systems characterized by their distinct developmental and evolutionary origins: the dermal skeleton, neurocranium, splanchnocranium (viscerocranium), appendicular and axial skeletons (Donoghue and Sansom, 2002).

Considerable effort has been expended in understanding the early evolutionary assembly of the vertebrate skeleton, but we remain no closer to

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*Correspondence to: Jason P. Downs, Academy of Natural Sciences of Philadelphia, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103. E-mail: downs@ansp.org

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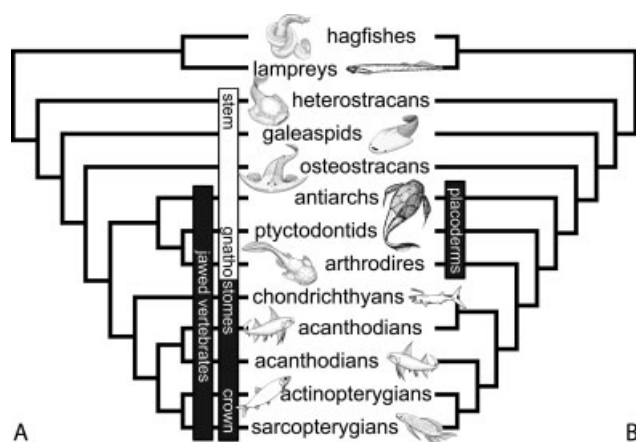


Fig. 1. Evolutionary relationships among the principal groups of stem- and crown-gnathostomes, applied following Hennig (1981) and Jefferies (1979), effecting an explanation of the concepts of gnathostome stem and crown, and how they relate to the concept of a clade of jawed vertebrates. Trees differ only in terms of the relations of placoderms and acanthodians and the topology of the tree otherwise follows Donoghue et al. (2000) and Donoghue and Smith (2001). (A) The traditional view of placoderm monophyly as promulgated by Goujet and Young (1995, 2004), Young (1986), and Janvier (1996). (B) The hypothesis of placoderm paraphyly as promulgated by Brazeau (2009) and Johanson (2002). Icons of representative fishes after Janvier (1996).

understanding the condition from which the skeletons of chondrichthyans and osteichthyans departed. In large part, this occurs because so little is known concerning the nature of the skeleton in the most basal jawed vertebrates, the placoderms. Placodermi is an extinct clade or grade of jawed vertebrates that first appears in the mid Silurian (Wenlock, circa 428 Ma) and goes on to dominate the Devonian (417–354 Ma) vertebrate fossil record (Carr, 1995). Though the systematic position and interrelationships of the group remain points of contention, recent analyses place Placodermi as either the sister group of crown Gnathostomata (Fig. 1A; Young, 1986; Goujet and Young, 1995; Janvier, 1996) or a paraphyletic grade leading to the crown (Fig. 1B; Johanson, 2002; Brazeau, 2009). In either phylogenetic context, placoderms are integral to understanding the condition of the skeleton at the origin of jawed vertebrates and immediately before the emergence of chondrichthyans and osteichthyans.

There is no good explanation for the dearth of information on the composition of the skeleton in placoderms. Placoderms are well represented in the fossil record, with over 700 recognized species (Carr, 1995). However, it is perhaps because placoderms are so richly represented in the fossil record and possess such complex skeletons that palaeontologists have not resorted to skeletal histology, an approach that has historically been associated with the most desperate attempts to classify fossil vertebrates known only from scraps of bone.

The aim of this study has been to remedy this situation, to completely characterize the skeletal histology of a placoderm, providing a benchmark for future studies that will seek to establish the diversity of skeletal histology within the group. Our study focuses on the antiarch, *Bothriolepis canadensis* (Whiteaves, 1880). This is not because it is representative of placoderms (though they may be representative of the very earliest jawed vertebrates; Johanson, 2002; Brazeau, 2009) but because *Bothriolepis* has proven previously to be the best source of histological data for placoderms. There is an abundance of specimens representing all stages of ontogeny, many of which are fully articulated and preserve histological microstructure with great fidelity. Thus, *B. canadensis* represents the best possible candidate for establishing skeletal composition in placoderms.

Previous Research Into the Skeletal Histology of Antiarch Placoderms

The difficulty in obtaining ontogenetic data for fossil taxa continues to generate controversial tissue identifications and developmental interpretations for Antiarchi. Past interpretations of the clade's skeletal microstructure are marked by a lack of anatomical and developmental context.

Goodrich (1909) provided a diagrammatic figure of the gross structure of the dermal skeleton of *Bothriolepis canadensis* which he described as composed of true bone. He noted that, although the surface is ornamented by tubercles, there is no evidence that they were formed from fused denticles. Goodrich recognized a vascular middle layer and a typically lamellated basal layer and his figure (p. 206) shows clear evidence of planar discontinuity in the middle vascular layer.

Heintz (1929) provided some of the earliest published microscopic images of the antiarch external skeleton. The description, limited to a single section through an unidentified “trunk armor plate” (Pl. XXIV: Heintz, 1929), demonstrates the particular stratified nature of the external skeleton in *Asterolepis* sp. Heintz (1929) designated four different zones of tissue without sharp distinctions among them, a superficial and a basal compact, lamellar bone tissue and two unique middle cancellous bone tissues. The superficial and basal cancellous tissues were respectively designated the Maschen-schicht (mesh layer) and the Kanalschicht (channel layer).

Though he did not publish microscopic images, Erik Stensiö did observe thin sections to interpret the skeletal tissues in *Bothriolepis canadensis* and *Remigolepis* sp. and to describe the internal features of those skeletons. Stensiö (p. 111: 1931) noted the presence of a “perichondral layer of lime-bearing tissue ... intermediate between true bone and calcified cartilage” in an unidentified ele-

ment of the pectoral limb endoskeleton. Though seemingly not based on observations, Stensiö (1931) argued that *B. canadensis* is the only antiarch species with a mineralized component to the endoskeleton. His description of the mineralized tissue matches that of "globular calcified cartilage," an observation first noted by Ørvig (p. 410: 1951), who used topological relationships to further propose that this zone of calcified cartilage is the homologue of the "subperichondral calcified layer" observed in other placoderm species, *Plourdosteus canadensis* and *Coccosteus decipiens*.

Walter Gross's (1931) monograph on *Asterolepis ornata*, makes reference to three stratified layers in the antiarch external skeleton: the superficial Tuberkelschicht (tubercle layer), the Spongiosa, and the basal Grundlamellenschicht (basic lamellar layer). In unspecified elements of the thoracic skeleton, deep to the Tuberkelschicht, Gross (p. 11: 1931) identifies a second cancellous zone without a definitive association with either the Tuberkelschicht or the Spongiosa. This is likely a reference to the Maschen-schicht of Heintz (1929).

In a vertical cross section of the anterior median dorsal element of *Asterolepis ornata*, Gross (1935) identifies an acellular, lamellar tissue zone within the Spongiosa that divides the cancellous tissue zone into basal and superficial components. No further mention was made of a distinction between the two stratified components of Spongiosa.

Ørvig (1968) recognized that *Bothriolepis canadensis*, by possessing two stratified zones of cancellous tissue, demonstrates an unusual histological organization. Clear evidence was presented for spheritic mineralization within the basal cancellous tissue and the author properly noted that such a mode of mineralization is atypical of bone. To match his observations to the traditional diagnosis for a dermal bone organ, Ørvig (p. 377: 1968) tentatively designated the peculiar tissue "globular bone."

Burrow (2005) proposed that the spheritically mineralized, lower middle cancellous tissue in the external skeleton of *Bothriolepis canadensis* represents bone trabeculae that separated large spheres of calcified cartilage. The mineralized spherites of this tissue were interpreted as the lacunae of osteoblasts from the bone trabeculae and chondroblasts of the cartilage. Burrow interpreted this tissue as the precursor to prismatic calcified cartilage, proposing that the bone trabeculae separating the spaces of the lower middle cancellous tissue in *B. canadensis* are lost in Chondrichthyes and are replaced by the interlocking prisms that are characteristic of prismatic calcified cartilage.

This brief but comprehensive review demonstrates that to date, attempts to characterize the skeletal histology of antiarchs have been cursory and often contradictory. Furthermore, the suggested presence of cartilage in the external skele-

ton (Burrow, 2005) perhaps unwittingly challenges the dogma that there is a fundamental dichotomy between the dermal skeleton and endoskeleton, both in terms of their development and evolution (Patterson, 1977; Smith and Hall, 1990; Donoghue and Sansom, 2002). There have been isolated accounts of developmental integration of the endo- and dermal skeleton, although it is perhaps notable that they are invariably based on fossil material where development cannot be observed. For instance, Scheyer (2007) described a cartilaginous bone from the integument of extinct placodonts (a group of sauropterygian reptiles). It might be anticipated that such phenomena should be manifest early in the evolutionary establishment of skeletal developmental programs, but there is no evidence of this among extinct jawless vertebrates (Smith and Hall, 1990; Donoghue and Sansom, 2002). However, placoderms are the first vertebrate clade in which all vertebrate skeletal systems are manifest (Donoghue and Sansom, 2002) and so it is possible that during this integral episode of vertebrate skeletal evolution, some of the distinctions that we observe in living vertebrates did not yet hold.

Thus, it is integral that we obtain a more comprehensive understanding of the composition of the mineralized skeleton of *Bothriolepis canadensis* and this article provides an histological analysis and interpretation of its skeletal biology.

MATERIALS AND METHODS

The present study is based on a growth series of 13 near-complete specimens of *Bothriolepis canadensis* from the Frasnian-age exposures of the Escuminac Formation at the Miguasha field site in Quebec, Canada. The Escuminac Formation outcrops exclusively in the Baie des Chaleurs area, on the southwestern shore of the Gaspé Peninsula. The sedimentology and stratigraphy of the formation indicate deposition of terrigenous detritus in a marginal estuarine environment (Hesse and Sawh, 1992; Prichonnet et al., 1996). Each specimen was preserved in three dimensions within a calcium carbonate concretion that was collected as float and therefore without specific stratigraphic context.

Following the methodology developed by Jeppsson et al. (1999), the individual external skeletal elements were isolated from the concretions using repeated baths of 7% acetic acid buffered with calcium acetate to a pH of 3.6. The pH was chosen to maximize dissolution of the calcium carbonate in the concretion while preventing the dissolution of the fossil material.

All material to be sectioned was first embedded in Struers Serifix polyester resin. Where possible, transverse sections were cut through skeletal elements of the external head skeleton (premedian, lateral, postmarginal, paranuchal-marginal, postpineal, nuchal), thoracic skeleton (anterior median dorsal, anterior dorsal lateral, mixilateral, posterior median dorsal, anterior ventral lateral, posterior ventral lateral) and pectoral appendage (dorsal central 1, ventral central 1). The samples were cut in two using a diamond wafering blade mounted on a bench-top Buehler ISOMET low speed saw. Cut surfaces were then cleaned in water using an ultrasonic cleaner and impregnated with Buehler EPO-THIN resin. The specimen surfaces were manually ground using grit sizes ranging from P1200 to P4000 and polished using 1.0 and 0.1 μm deagglomerated alpha alu-

mina powder and finally 0.04 μm colloidal silica solution. Each specimen was affixed to a petrographic slide using the EPO-THIN resin as an adhesive. A Ward's Natural Science Ingram Thin-Section Cut-Off Saw was used to remove all but the last millimeter of the specimen from the slide. The slide was then ground to a thickness of $\sim 300\ \mu\text{m}$ using a Buehler ECOMET 4 grinder/polisher. Finally, each thin section was ground to $\sim 50\ \mu\text{m}$ and polished using the manual grinding/polishing method described earlier.

For each element, we prepared two serial sections for the purposes of comparative imaging using transmitted light optical microscopy and scanning electron microscopy (backscatter electron and secondary electron imaging). The scanning electron images were generated using a JEOL JXA-8600 electron microprobe and a Philips Field Emission Gun Environmental Scanning Electron Microscope. Following the methodology of Sundström (1968), before study, the scanning electron samples were etched for 1 h in a bath of 0.5 weight percent solution of chromium (III) sulfate [$\text{Cr}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$] brought to pH 3.5 with the addition of sodium hydroxide (NaOH). Immediately before imaging, a carbon coat was applied to each sample using a Cressington "208carbon" Carbon Coater.

RESULTS

Ordinarily, we would provide a direct interpretation of skeletal histology; however, given that it is the interpretations of these tissues that has been the source of contradiction and controversy we have chosen instead to provide a neutral description of the histological tissues, followed by an interpretation of this evidence. The following description is conducted according to the following three a priori assumptions: 1) the material represents elements of a vertebrate skeleton, 2) the gross morphology of the elements may be used to determine anatomical direction and to identify the elements of the head and thoracic skeletons, and 3) osteocytes, canaliculi, and vascular canals are recognizable by their distinctive morphology.

Histological Description

All of the tissues of the *Bothriolepis canadensis* external skeleton are cellular. The elements of the external skeleton are characterized by a distinct horizontal zonation or stratification. In elements of the thoracic skeleton, at least three zones of tissue are discernible: a superficial lamellar tissue with a compact component and a cancellous component; a basal, compact lamellar tissue; and a middle zone of woven-fiber cancellous tissue between them (Fig. 2A). The boundary plane between the superficial and middle cancellous tissues forms the zone of overlap between articulating elements of the thoracic skeleton. In other words, in each of the overlapping joints of the external thoracic skeleton, the superficial tissue zone of one element overlaps with the basal (woven-fiber + lamellar) tissue complex of another. In the external head skeleton and proximal pectoral appendage, where such overlapping joints do not occur, compact superficial and basal lamellar tissue zones bound a deep zone of woven-fiber, cancellous tissue. In this

section, we separately describe the microstructural anatomy of the external thoracic, head, and pectoral appendage skeletons. Each description remarks on the features common to all observed elements of the respective skeletal component.

External thoracic skeleton. The most superficial tissue of the external thoracic skeleton includes the tuberculated ornament and is composed of a cellular, lamellar tissue without evidence for pulp cavities. This zone of tissue is composed of approximately parallel superimposed laminae and varies little in depth within a single element. Individual lamellae average $\sim 10\ \mu\text{m}$ in thickness. Lacunae lie within and between the lamellae and are often round or star-shaped with a slight tendency toward a flattened, elongate shape in the most superficial lamellae (Fig. 2B). Relative to those of the basal lamellar tissue, the shapes of the lacunae are far more variable. Primary processes, or canaliculi, issue from the lacunae roughly parallel to the tissue lamellae and many additional ramifying processes form a dense network throughout the tissue. The degree to which the primary processes are parallel with the layers of tissue growth is another measure that increases with proximity to the superficial surface. The tissue's primary vascular canals are aligned in the plane of the skeletal plates and feature simple reticulating canals (Fig. 2C). Open spaces in the tissue frequently truncate lamellae. In many instances, these spaces are lined by concentric lamellae of cellular tissue (Fig. 2D). The lacunae enclosed in the concentric lamellae are spindle-shaped with long axes parallel to the lamellae. The frequency with which these open spaces appear is highest in the deepest part of this tissue. This cancellous component of the superficial lamellar tissue zone corresponds with the Maschenschicht of Heintz (1929). A sharp division separates this tissue zone and the middle cancellous tissue deep to it. The boundary itself is planar and is continuous with the plane of overlap at the margins of thoracic skeletal plates (Fig. 2E). Burrow (2005) described thin but distinct bone lamellae within this boundary; we find no evidence in our own material or in any of the material presented by Burrow (2005). Between plate margins, the boundary is discontinuous due to interruptions imposed by open spaces that extend between the two cancellous layers. These spaces, too, are lined by concentric lamellae of cellular tissue. Because of the abundance of these open spaces, in certain positions, the boundary between the superficial and the underlying tissue zones is not obvious and the entire region between the superficial and basal lamellar tissues takes on the appearance of a single, gradational cancellous tissue zone. Of final note, the superficial tissue exhibits mineralized spherites. Within this tissue zone, they are most commonly observed surrounding the smaller cav-

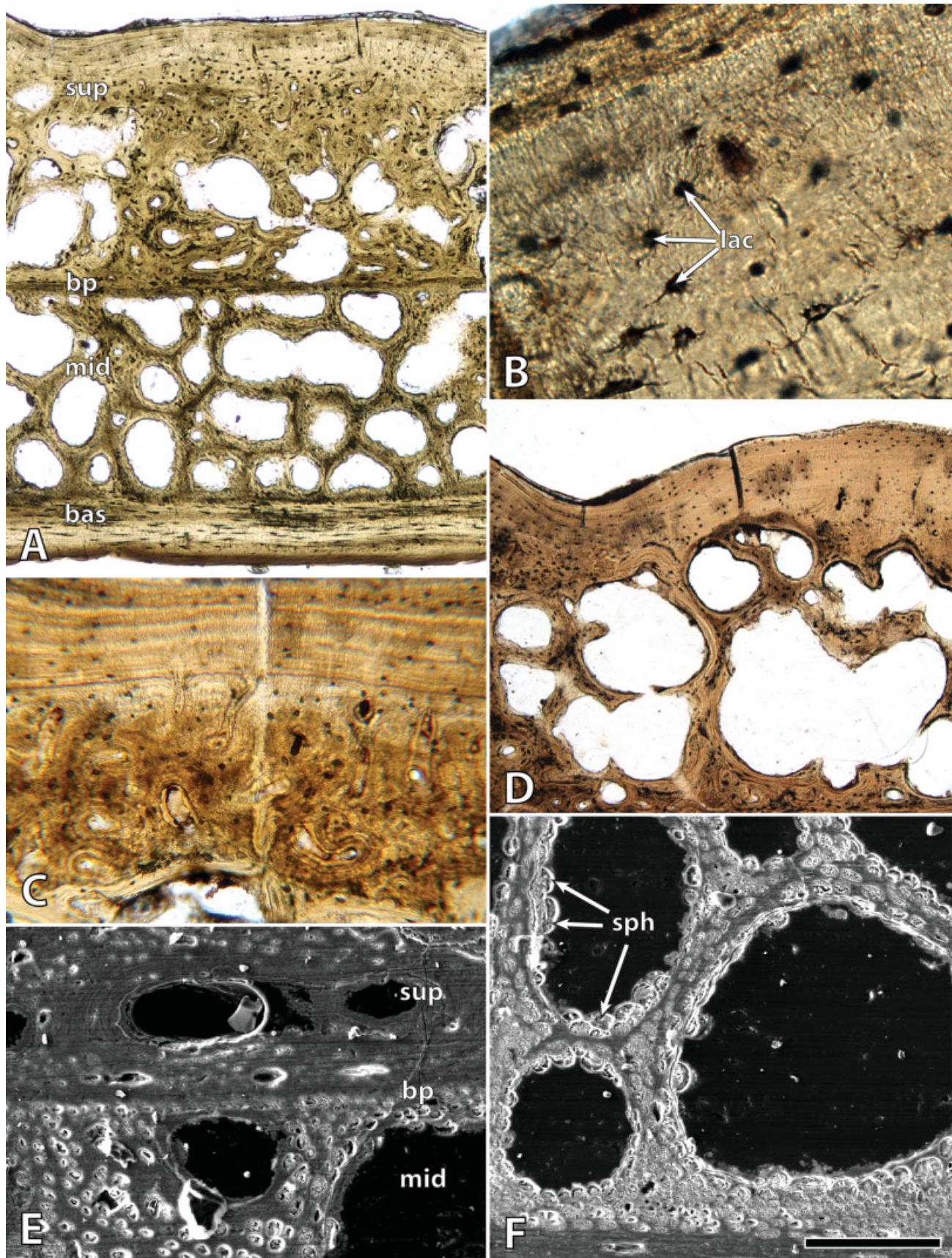


Figure 2.

ities that reside just beneath the most superficial lamination. Spherites are abundant throughout the external skeleton of *Bothriolepis canadensis* and, while present in this superficial tissue, are most abundant in the underlying middle cancellous zone (Fig. 2F).

The middle cancellous tissue is less compact than the superficial cancellous tissue. Round or star-shaped lacunae within the woven-fiber tissue appear randomly distributed. The globular appearance of the woven-fibered tissue results from a dense aggregation of mineral spherites. They are surrounded by a fibrous network that is revealed through crossed nicols (Fig. 3A). The mineral spherites average between 10 and 20 μm in diameter, smaller than those previously reported from the internal skeleton of the *Plourdosteus canadensis* pectoral appendage (Ørvig, 1951). The most superficial spherites are themselves comprised of spherical structures at multiple size scales; the smallest microspherites have a diameter on the order of 100 nm (Fig. 3B). These cluster into individual larger scale units (Fig. 3C) that themselves may cluster into even larger scale units (Fig. 3D). Even those units that we here refer to as "spherites" cluster together along the woven-fiber framework of this tissue zone. Concentric dark and light banding of the spherites, associated with compositional differences, is observable in backscatter electron images (Fig. 3C). In contrast to the more superficial spherites, many of the deeper spherites are hollow (Fig. 3D). The orientation, size, and shape of the microspherites in the *Bothriolepis canadensis* external skeleton are inconsistent with our understanding of spheritic mineralization in vertebrates.

The middle tissue zone is not entirely without lamination. As observed in the superficial tissue zone, there are open spaces that are lined with concentric lamellar tissue. However, this is a far less common phenomenon in this middle tissue zone. In the majority of instances, the open spaces are lined by dense aggregations of spherites with no evidence at all for tissue lamellae.

Bands of mineralized tissue run along the lateral edges of each element and are approximately parallel in orientation to the lateral edge of the element. Within these bands of tissue, dark striations run

parallel to the superficial and basal surfaces of the element (Fig. 3E). Similar dark striations run in a superficial to basal direction in lenses of lamellar tissue that appear superficial to the lamellae of the superficial tissue zone (Fig. 3F).

The lamellae in the compact basal lamellar tissue zone average 20 μm in thickness. Abundant lacunae lie within and between the lamellae. The majority of the lacunae are spindle-shaped with primary processes that issue parallel to the lamellae and additional processes that issue largely perpendicular to the lamellae (Fig. 3G). As in the superficial lamellar tissue, the degree to which these factors are developed increases with proximity to the surface of the element, (the basal surface, in this instance). In the basalmost lamellae, however, lacunae are extremely rare. Through crossed nicols, these most basal lamellae share an interference color with one another but one that is distinct from the single or multiple interference colors displayed by the overlying lamellae (Fig. 3H).

External head skeleton. Although the relative depths of the tissue zones are unlike those of the external thoracic skeleton, the elements of the head skeleton demonstrate a similar stratification. The superficial tissue is lamellar with open spaces crosscutting the lamellae and with concentric lamellar tissue in those spaces. The middle cancellous tissue zone exhibits a woven matrix and mineralized spherites. A compact lamellar tissue forms the basal tissue zone. Unlike the condition in the external thoracic skeleton, there is no sharp boundary plane between the superficial and middle tissue zones (Fig. 3I).

In the head skeleton, cross-cutting open spaces of the superficial lamellar tissue exhibit concentric lamellae (Fig. 4A) and, in some instances, mineralized spherites. Deep to the surface of the element, the superficial tissue zone exhibits waves of lamellae that exhibit the size and appearance of superficial tubercles (Fig. 4B). Between these "tubercles" are zones of spheritically-mineralized tissue dense with round osteocytes and canaliculi issuing in all direction. Waves of lamellar tissue lie superficial to these internal "tubercles" and the zones of spherites between them. These lamellae form the tuberculated ornament of the element. Like the phenomenon described for the external thoracic skele-

Fig. 2. MHNM 02-616, *Bothriolepis canadensis*, mixilateral in thin section, superficial surface always toward top of frame; (A) LM image, full depth of element illustrates the division into superficial compact and cancellous lamellar tissue zones, middle woven-fibered cancellous tissue zone, and basal compact lamellar tissue zone. The sharp boundary plane between superficial and middle zones is prominent; scale bar = 0.40 mm; (B) LM image, superficial compact lamellar tissue zone with lacunae and network of canaliculi; scale bar = 0.07 mm; (C) LM image, vascular canals of the superficial tissue zone. Primary canals are surrounded by simple reticulating canals; scale bar = 0.18 mm; (D) LM image, superficial tissue zone shows division into compact and cancellous components. Open spaces with concentric bands of mineralized tissue are secondary osteons; scale bar = 0.40 mm; (E) SEI image, boundary between superficial and middle tissue zones; scale bar = 0.08 mm; (F) SEI image, middle woven-fibered tissue showing extensive spheritic mineralization; scale bar = 0.10 mm. bas, basal lamellar tissue zone; bp, boundary plane between superficial and middle tissue zones; lac, lacuna; mid, middle cancellous tissue zone; sph, mineral spherite; sup, superficial lamellar tissue zone.

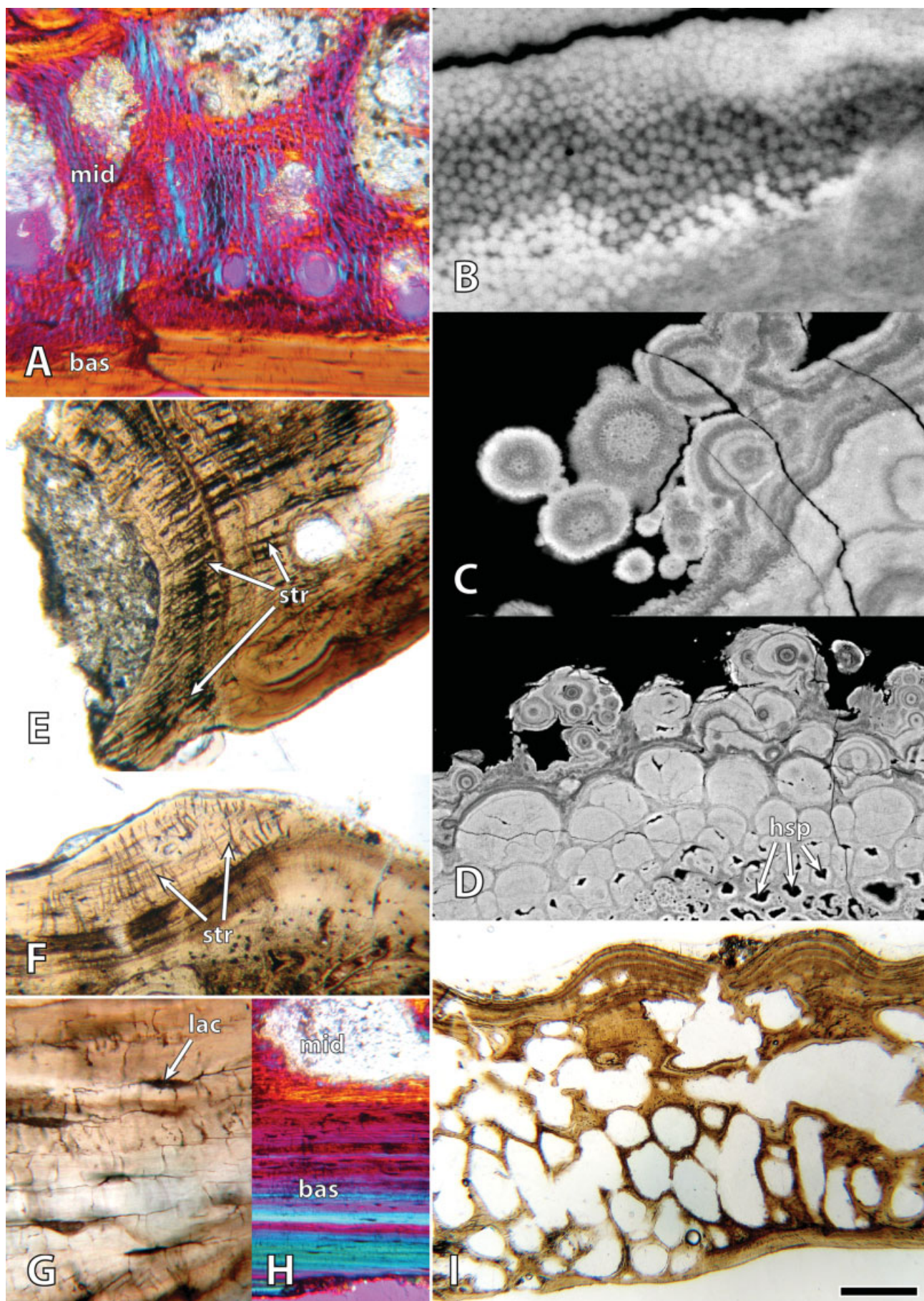


Figure 3.

ton, in positions where lamellar tissue lies superficial to internal "tubercles," the superficial tissue often exhibits dark striations that run perpendicular to the lamellation. Also similar to the condition in the external thoracic skeleton, the lateral margins exhibit banding of the tissue nearly parallel to the lateral edge of the element and dark striations generally parallel to the element's basal surface (Fig. 4C). The margins of the elements of the external head skeleton do not feature the overlap zones that characterize the external thoracic skeleton. Accordingly, the external head skeleton does not exhibit a clearly defined stratification into the superficial and basal units that make up the overlap zones in the thoracic skeleton.

The external head skeleton exhibits an extensive array of open spaces interrupting not only the lamellae of the superficial tissue zone but also the woven matrix of the middle cancellous zone; the postmarginal of MHNM 02-616 even exhibits these open spaces in the basal lamellar tissue. These open spaces sometimes feature concentric lamellae within them and sometimes feature dense aggregations of mineralized spherites (Fig. 4D). The degree of interruption imposed by these open spaces makes it nearly impossible to distinguish a boundary between the superficial lamellar tissue and the middle cancellous tissue. However, even in positions where we might expect to observe the boundary, the transition between the tissue zones is not sharply defined as it is in the thoracic skeleton. As a final note, compared to the condition observed in the thoracic skeleton, relative to the overall depth of the element, the basal lamellar tissue in the head skeleton is especially shallow.

External pectoral appendage skeleton. The proximal elements of the pectoral appendage skeleton (dorsal central 1 and ventral central 1) exhibit a superficial lamellar tissue zone, a middle woven-fiber cancellous tissue zone, and a basal lamellar tissue zone. The lamellar tissues, like the woven-fiber tissue, exhibit a dense presence of mineralized spherites. Like the conditions described in the head skeleton and thoracic skeleton, the lamellae of the superficial zone and the woven matrix of the cancellous zone exhibit cross-cutting open spaces.

These spaces feature concentric lamellae and spheritic mineralization (Fig. 4E). In the dorsal central 1, such spaces additionally appear in the basal lamellar tissue. The margins of these elements do not possess the overlap zones that are characteristic of the external thoracic skeleton. Relative to those of the corresponding tissue in the head and thoracic skeletons, the trabeculae of the middle cancellous zone are thicker and with a denser aggregation of spherites. In the distal pectoral appendage, the terminal plate fuses with members of the lateral marginal, medial marginal, dorsal central, and ventral central series. Both the dorsal central and the ventral central elements are composed entirely of superficial and basal lamellar tissues. Only a narrow, dense zone of mineralized spherites separates the two. Open spaces lined with concentric lamellae perforate the lamellar tissue (Fig. 4F). The only lamellae distinguishable in the medial and lateral marginal elements are along the superficial and basal surfaces. The middle of these elements is a mass of mineralized spherites without any obvious structure. Numerous open spaces perforate this mass of spherites. Many of these spaces have a vascular appearance (Fig. 5A). These open spaces exhibit both concentric lamellar tissue and mineralized spherites.

In the most distal pectoral appendage, fusion among the elements means that individual elements are discernible only in section. Stensiö (1948) described the element boundaries following acid preparation of the surface of the distal appendage. In transverse section, the boundaries are clearly observable. The dorsal and ventral central elements and the medial and lateral marginals between them feature bands of tissue at their lateral edges. In the zones of fusion, a mass of spheritically-mineralized tissue fills in the space between adjacent elements. Lamellae superficial and basal to the zone of fusion are continuous across the two elements. Between fused elements, open spaces crosscut not only the bands of tissue at the edges of the elements but also the spheritic tissue between the elements (Fig. 5B). These open spaces feature aggregations of spherites and concentric lamellar tissue.

Fig. 3. MHNM 02-616, *Bothriolepis canadensis* in thin section, superficial surface always toward top of frame; (A) LM image (crossed nicols), mixilateral, crossed nicols reveal the woven-fibered network of the middle tissue zone; scale bar = 0.14 mm; (B-D) BSE images, mixilateral middle cancellous tissue zone; (B) microspherites; scale bar = 0.91 μ m; (C) mineral spherites, dark and light bands signify compositional differences; scale bar = 3.77 μ m; (D) mineral spherites, black spots at bottom of frame are hollow spaces within spherites; scale bar = 14.46 μ m; (E) LM image, mixilateral, basal lamellar tissue zone, lateral edge shows dark striations parallel with tissue lamellae; scale bar = 0.14 mm; (F) LM image, mixilateral, superficial lamellar tissue zone showing dark striations perpendicular to tissue lamellae; scale bar = 0.14 mm; (G, H) LM images, mixilateral, basal lamellar tissue zone; (G) lacunae and canaliculi; scale bar = 0.04 mm; (H) crossed nicols show that basalmost lamellae share a single interference color and therefore alignment of their collagen fibers; scale bar = 0.15 mm; (I) LM image, postmarginal, full depth of element shows the division into superficial compact and cancellous lamellar tissue zones, middle cancellous tissue zone, and basal compact lamellar tissue zone. The external head skeleton does not exhibit a sharp plane of division between superficial and middle tissue zones; scale bar = 0.50 mm. bas, basal lamellar tissue zone; hsp, hollow spherite; lac, lacuna; mid, middle cancellous tissue zone; str, dark striation.

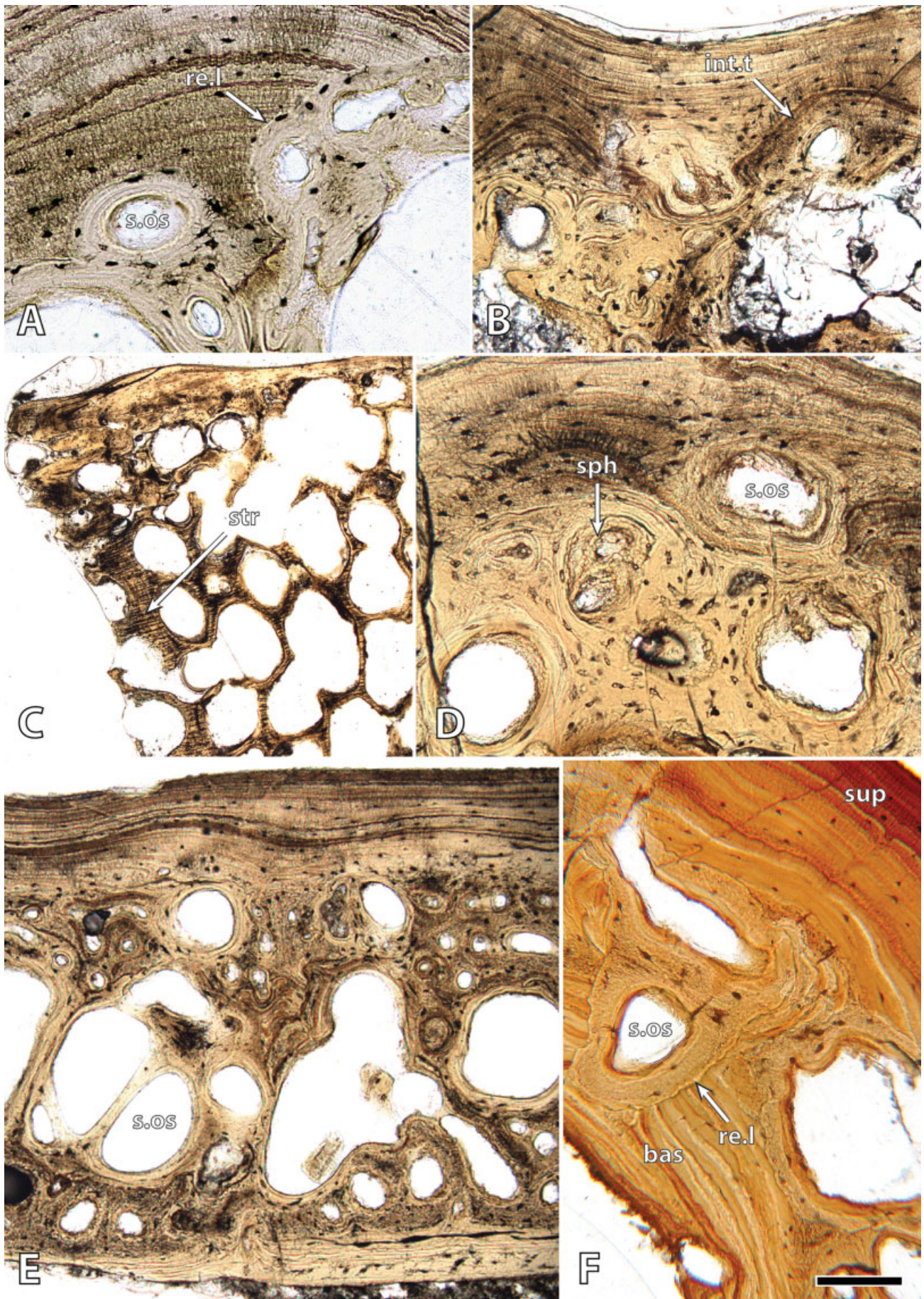


Figure 4.

DISCUSSION

Tissue Homologies

The bone classification scheme of de Ricqlès (1975) and de Ricqlès et al. (1991) is based on morphology and topology and therefore is readily applicable to bone in the fossil record. The majority of the tissues comprising the external skeleton *Bothriolepis canadensis* can readily be interpreted as cellular bone tissues; however, the spheritically mineralized tissue comprising the middle cancellous layer requires greater consideration (see below).

Inotropic mineralization. The most superficial zone of the external skeleton of *Bothriolepis canadensis*, including the tuberculated ornament, is comprised of compact, lamellar, dermal bone tissue with simple vascular canals. Several generations of osteons cross-cut earlier fabrics within the tissue and are evidence for multiple cycles of bone resorption and redeposition.

In the external thoracic skeletal elements, there is a sharp division between the superficial lamellar bone and the middle cancellous bone. This boundary is identified as a rest cementing line because it represents a discontinuity in bone deposition and does not occur at a resorption surface (de Ricqlès et al., 1991). This rest line, especially obvious in secondary electron images, is also the plane of overlap between articulating elements of the external thoracic skeleton. The middle tissue zone in the *B. canadensis* external skeletal elements is comprised of spheritically-mineralized primary cancellous bone tissue. This rare example of spheritic mineralization in a bone tissue makes it particularly difficult to classify, though it otherwise fits the diagnosis of spongiosa of dermal origin. Observable in even the smallest specimens of *Bothriolepis canadensis* (Fig. 5C), this primary tissue is identified according to the large open spaces in mineralized tissue, the lack of lamellation, and the delicate scaffolding of its trabeculae (Francillon-Vieillot et al., 1990).

The basal zone of the *Bothriolepis canadensis* external skeleton is comprised of a compact, lamellar, avascular, dermal bone tissue. This tissue is diagnosed by the combination of a topological position superficial to the organism and basal with respect to the element, a distinct lamellation, a lack of vasculature, and flattened cell spaces with

long axes parallel to the lamellation. Observed through crossed nicols, the most basal lamellae of this tissue exhibit a parallel-fiber bone matrix (Fig. 3H). The homogeneity of interference colors indicates that the mineral crystallites, and therefore the collagen fibers, in this series of lamellae are parallel to one another.

All three stratified tissue zones exhibit dark horizontal lines that are observable in the optical microscope images. Their topological positions suggest that they are attachment fiber spaces that run between the body of the skeletal element and zones of new tissue growth. The attachment fiber spaces occur along the lateral margins of the external skeletal elements in the bands of tissue that represent zones of marginal tissue growth. Elements of the head skeleton exhibit multiple generations of tubercles in the superficial zone of lamellar bone. The most superficial, latest generations of tubercles also exhibit these striations perpendicular to the lamellae.

Spheritic mineralization. Spheritic mineralization involves the radial deposition of mineral crystallites around a mineralization nucleus. Because it does not require the construction of a collagen fibril matrix, it is simple, quick, and energy efficient relative to inotropic mineralization (Ørving, 1951, 1968) or the deposition of mineral crystallites between the individual molecules that comprise collagen fibrils (Francillon-Vieillot et al., 1990). Evidence for spheritic mineralization occurs in all observed elements of the *Bothriolepis canadensis* external skeleton and it is exhibited in each of the three stratified tissue zones. Spheritic mineralization of the endoskeletal tissue, cartilage, is widely documented in the vertebrate endoskeleton. This fact, along with the sharp division between the superficial and basal tissues in the external thoracic skeleton, has led authors to interpret the basal tissues in the *B. canadensis* external skeleton as a contribution of the endoskeleton (Burrow, 2005).

To test this hypothesis, we use evidence of phylogenetic consistency, morphology, topology and comparative anatomy. First, the presence of mineralized endoskeletal tissues, including both calcified cartilage and perichondral bone, in placoderms, is consistent with present understanding of vertebrate phylogeny (Donoghue and Sansom, 2002;

Fig. 4. MNHM 02-616, *Bothriolepis canadensis* in thin section, superficial surface always toward top of frame; (A) LM image, postmarginal, secondary osteons of the superficial lamellar tissue zone; scale bar = 0.10 mm; (B) LM image, lateral, subsurface tubercles of the superficial lamellar tissue zone; scale bar = 0.14 mm; (C) LM image, lateral, lateral edge of element showing dark striations; scale bar = 0.56 mm; (D) LM image, lateral, secondary osteons of the superficial lamellar tissue zone showing evidence of spheritic mineralization; scale bar = 0.10 mm; (E) LM image, ventral central 1, full depth illustrates the division into superficial and basal lamellar tissue zones and middle woven-fibered zone. There is extensive evidence for resorption and redeposition of mineralized tissue; scale bar = 0.18 mm; (F) LM image, unidentifiable element of the distal dorsal/ventral central series, full depth shows lamellar tissue interrupted by secondary osteons; scale bar = 0.10 mm. bas, basal lamellar tissue zone; int.t, internal tubercle; re.l, resorption line; s.os, secondary osteon; sph, mineral spherite; str, dark striation; sup, superficial lamellar tissue zone.

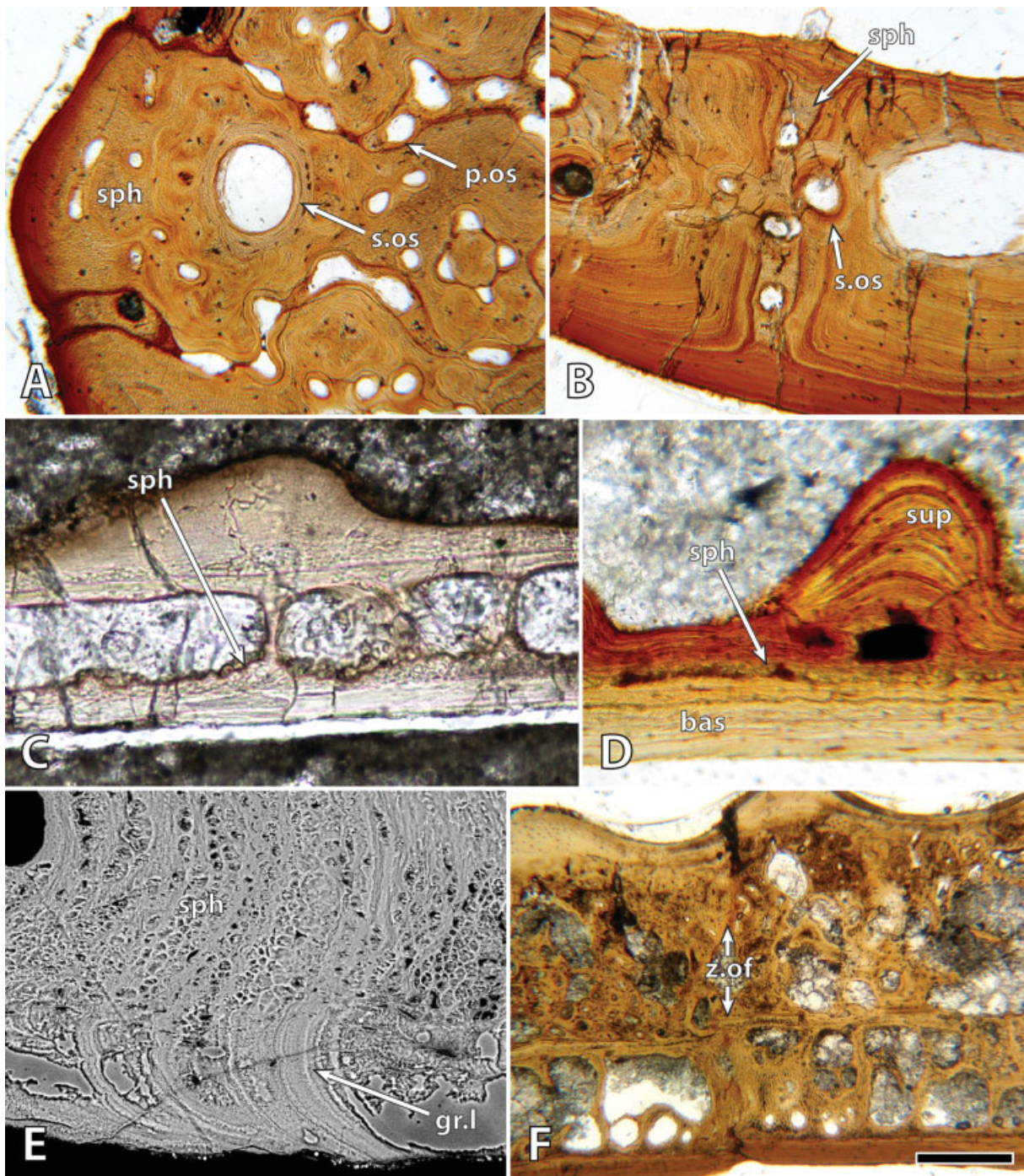


Fig. 5. *Bothriolepis canadensis* in thin section, superficial surface toward top of frame except where noted otherwise; (A) LM image, MHN 02-616, unidentifiable element of the distal medial/lateral marginal series, dense mass of spherites interrupted by secondary osteons, superficial surface toward left side of frame; scale bar = 0.15 mm; (B) LM image, MHN 02-616, zone of fusion between the dorsal/ventral central series and the medial/lateral marginal series, superficial surface toward bottom of frame. Note the spherites and secondary osteons in the zone of fusion; scale bar = 0.15 mm; (C) LM image, MHN 02-2355, juvenile, mixilateral, full depth shows superficial and basal lamellar tissue zones and middle woven-fibered tissue zone with mineral spherites; scale bar = 0.09 mm; (D) LM image, MHN 02-2617, juvenile, mixilateral, full depth without middle woven-fibered zone, superficial and basal lamellar tissue zones separated by zone of mineral spherites; scale bar = 0.09 mm; (E) BSE image, MHN 02-616, anterior median dorsal, bands of tissue growth at element's lateral edge. Note presence of mineral spherites; scale bar = 0.06 mm; (F) LM image, MHN 02-616, mixilateral, full depth, zone of offset shows evidence for repair of the mineralized tissue; scale bar = 0.45 mm; bas, basal lamellar tissue zone; gr.l, marginal growth lines; s.os, secondary osteon; sph, mineral spherites; str, dark striation; sup, superficial lamellar tissue zone; z.of, zone of offset.

Wang et al., 2005; Donoghue et al., 2006). Mineralized endoskeletal tissues have been reported in Petromyzontidae (Bardack and Zangerl, 1971; Langille and Hall, 1993), Astraspida (Ørvig, 1951; Denison, 1967; Sansom et al., 1997), Anaspida (Janvier and Arsenault, 2002), Arandaspida (Gagnier, 1993), Galeaspida (Janvier, 1984, 1990; Wang, 1991; Wang et al., 2005; Zhu and Janvier, 1998), Pituriaspida (Young, 1991), Osteostraci (Stensiö, 1927; Denison, 1947, 1951; Wängsjö, 1952; Ørvig, 1957a; Gross, 1961) and Placodermi (Ørvig, 1951). The fusion of endoskeletal tissues to the internal surface of external dermal skeletal elements has previously been reported in the stem gnathostome clades Osteostraci (Stensiö, 1927; Denison, 1947, 1951; Wängsjö, 1952; Ørvig, 1957a; Gross, 1961) and Galeaspida (Janvier, 1984, 1990; Wang, 1991; Wang et al., 2005; Zhu and Janvier, 1998). The external skeleton of the galeaspid head exhibits a basal zone of calcified cartilage that is separated from the dermal bone by a hypomineralized zone of spherites (Wang et al., 2005). In some osteostracans, the external head skeleton can exhibit a basal zone comprised entirely of cellular perichondral bone, or of perichondral bone bounding a core that is unmineralized or spheritically mineralized (Wang et al., 2005).

Second, the basic organization of a middle spheritically-mineralized tissue and a basal lamellar tissue approximates the condition described in osteostracans (Denison, 1947, 1951; Gross, 1961). However, the woven, cancellous tissue of *Bothriolepis canadensis* is structurally unlike the compact, densely spheritic tissue described in osteostracans (Ørvig, 1951), galeaspids (Wang et al., 2005) and, indeed, chondrichthyans (Dean and Summers, 2006), where spherites are bound by universal mineralization fronts.

Third, the tissue zones hypothesized to be endoskeletal in the *Bothriolepis canadensis* are distributed throughout the entire thoracic skeleton. In the galeaspid and osteostracan examples, endocranial tissues fuse to the basal surface of the dermal skull roof elements only (Janvier, 1984; Wang et al., 2005). In *B. canadensis*, the endoskeletal interpretation of these tissues imposes the suggestion that the entire thoracic region is encapsulated by an endoskeletal "armor." This interpretation is inconsistent with existing knowledge of the topology of the vertebrate endoskeleton and, indeed, knowledge of the topology of endoskeletal structures in *Bothriolepis*. Endoskeletal tissues that we would anticipate to be present in *Bothriolepis* are manifestly absent, for instance in the chondrocranium (Young, 1984), because they were not mineralized in the first instance. Finally, the tissues that are hypothesized to belong to the endoskeleton are sandwiched between tissue layers that are otherwise readily interpreted as superficial and basal lamellar dermal bone; there is certainly

no relationship between the lamellar tissue zones and the intervening cancellous zone that could support a perichondral interpretation for the lamellar tissues.

Thus, while the fabric of the spheritically mineralized tissues grossly resembles spheritically mineralized cartilage, the topology of these tissues requires that they are interpreted as another component of the dermal skeleton. Reaching this conclusion does not, however, address the questions about antiarch skeletal microstructure that originally motivated the endoskeletal interpretation. These ask first, why the middle and basal tissue zones are separated from the superficial by a sharp line of division, and second, why spheritic mineralization was extensively employed in the construction of the external skeleton, and third, is this tissue cartilage or bone? To resolve these issues, we offer interpretations that are informed by the anatomical and developmental context of our histological data.

The dividing line between the basal cancellous part of the superficial lamellar tissue zone and the middle cancellous zone is the plane of overlap between articulating elements. This line of division is observed in all the specimens of the growth series. Sections taken through the mixilateral of MHN 02-2355 (with an anterior median dorsal length of just 10 mm) demonstrate that, even at this early stage of ontogeny, the division into superficial and basal components is present. Even at certain positions in the plates (Fig. 5D) that are entirely lacking in cancellous tissue, the element is divided into a superficial and a basal compact lamellar tissue, separated by a shallow zone of mineralized spherites. Independent development of these two units enables the differential pacing of marginal growth in each, a condition that allows for overlapping articulations. Each element of the *Bothriolepis canadensis* external skeleton appears to have a single center of ossification (Graham-Smith, 1978) and growth out from this center of ossification is recorded in the marginal growth lines (Fig. 5E). As the organism grew, the centers of the elements in the thoracic shield migrated away from one another (Graham-Smith, 1978). *B. canadensis* and any organism with an external skeleton composed of large articulating plates, requires a mechanism of growth that does not require disarticulation of the component elements. Placoderms represent one of the most basal examples of overlapping or "scarf" jointing in vertebrates, a type of skeletal articulation that is observed in many more derived vertebrate clades. These unfused joints enable growth along the element margins while maximizing the surface area of the articulation.

The ontogenetic growth of *Bothriolepis canadensis* was extensive, even after the initial development of the dermal skeleton (Werdelin and Long,

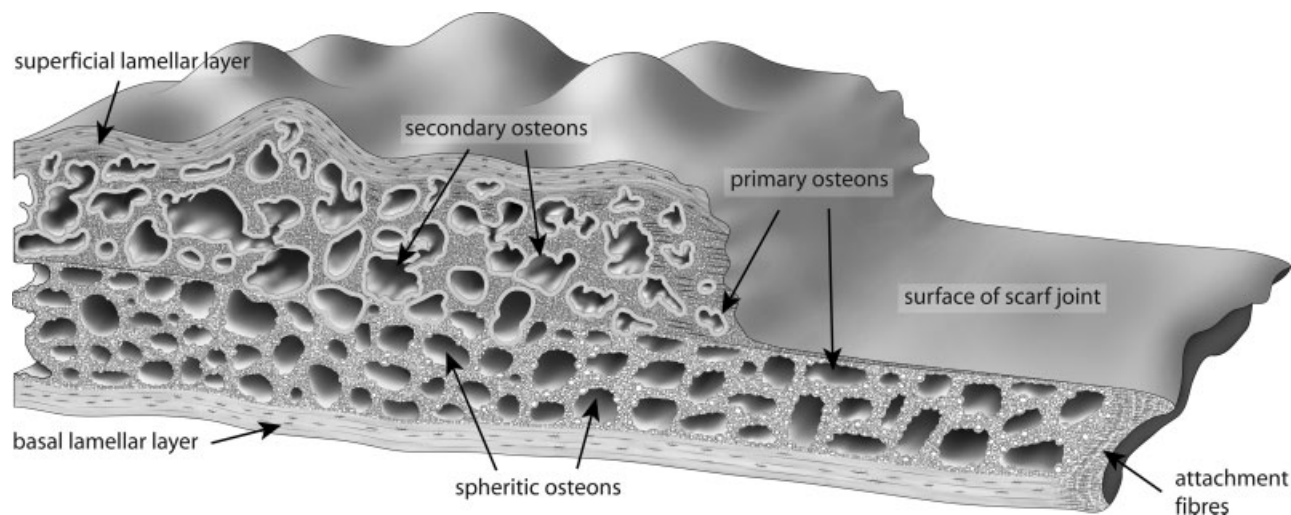


Fig. 6. Block diagram showing the internal structure of the dermal skeleton in *Bothriolepis canadensis*.

1986). Along with the evidence for resorption and secondary osteon development, spheritic mineralization points to the *B. canadensis* external skeleton being a dynamic system at least capable of rapid growth and reconstruction. Spheritic mineralization is often observed in secondarily deposited tissues in *B. canadensis*. In addition to the presence of spherites in resorption spaces, there is at least one example among our sections of a healed injury to the external skeleton. In the mixilateral element of MHN 02-616, a zone of offset runs all the way through the element from the superficial to the basal surface (Fig. 5F). This healed fracture exhibits a concentration of spherites along the plane of fracture. Through the action of resorption and secondary deposition of mineralized tissues, the external skeleton had the ability to continually strengthen itself, heal its injuries, and potentially sustain a high growth rate.

Ultimately, we must consider whether the spheritically mineralized tissue represents evidence of cartilage in the dermal skeleton. Although it has long been accepted that there is a fundamental dichotomy between the dermal skeleton and the endoskeleton (Patterson, 1977), and that cartilage has always been exclusive to the endoskeleton (Smith and Hall, 1990), there has been report of spheritically mineralized cartilage in association with the dermal skeleton of extinct placodonts (Scheyer, 2007). However, this interpretation relies entirely upon the presence of a mode of mineralization that transcends tissue type and germ layer (Ørvig, 1951; Donoghue et al., 2006). Ultimately, the distinction between tissue types can be exceedingly challenging, sometimes even more so when dealing with the tissues of living organisms where batteries of histochemical tests and molecular

markers often fail to clearly delineate one canonical tissue type from another. This has led to suggestions that the canonical tissue types are component elements of a spectrum of intermediates (Hall, 2005; Hall and Witten, 2007; Kawasaki and Weiss, 2008). However, in this discussion, it is important to distinguish between the question of whether there are distinct tissue types and the entirely separate question of whether we are able to distinguish between them. The topology of the tissues provides crucial, albeit inferential insight into the developmental origins of the tissues in question and these indicate that they are dermal. No compelling evidence has ever been presented for the presence of primary cartilage in the dermal skeleton (Smith and Hall, 1990).

Comparisons to Other Groups

Is *Bothriolepis canadensis* representative of antiarchs? Of placoderms? The skeletal histology of the dermal skeleton appears to be generally representative of antiarchs and other placoderms, at least within the context of what little is known currently. Like all placoderms, indeed, the majority of stem gnathostomes, *Bothriolepis canadensis* possesses a dermal skeleton with a basal compact, lamellar bone layer; a spongy middle bone layer; and an ornamented superficial layer. Where *B. canadensis* and other antiarchs (Gross, 1931; Ivanov et al., 1995) depart from other placoderms, is in the composition of this superficial layer, which is composed solely of compact bone in antiarchs (see Fig. 6). In the majority of other placoderms, the superficial layer is characterized by odontode-derived ornamental units composed of semidentine (a type of dentine identified by the en-

closure of unipolar odontocytes; Ørvig, 1967) and bone (Ørvig, 1957b; Denison, 1978). Semidentine has been considered a synapomorphy of Placodermi (Denison, 1978) and, under existing schemes of placoderm phylogeny (Goujet and Young, 1995, 2004), there have been multiple independent losses of the tissue throughout the clade (Ørvig, 1957b). If antiarchs are the sister group of all remaining placoderms plus crown gnathostomes as has recently been suggested (Johanson, 2002), the absence of semidentine in antiarchs would be a primitive character, and its later acquisition would represent a synapomorphy of the remaining clade of placoderms. Although the polarity of placoderm phylogeny is open to question, the lack of dental tissues (dentine, enameloid, bone of attachment) in antiarchs must represent a secondary loss since dental tissues are manifest in stem gnathostomes (Donoghue and Sansom, 2002).

The independent patterning of the dental and underlying skeletal tissues comprising the dermal skeleton is not unparalleled. Indeed, although both are primitive to the dermal skeleton (Donoghue and Sansom, 2002), they exhibit independent growth histories and in groups such as the galeaspid (skeletal only) and chondrichthyans (dental only), as well as the antiarchs, the dental and underlying skeletal tissues are mutually exclusive (Donoghue and Sansom, 2002). This may occur because, in the few groups that have been studied, the ectomesenchymal neural crest cells that contribute to the dermal skeleton are known to differentiate into distinct odontogenic and skeletogenic cell lineages with distinct potential (Smith and Hall, 1990; Donoghue and Sansom, 2002; Sire and Hysseune, 2003). The independent patterning of these cell lineages facilitates the common or mutually exclusive development of dental or skeletal derivatives.

Whether or not we consider the dermal skeleton of *Bothriolepis canadensis* to be representative of placoderms, its skeleton as a whole cannot be considered representative because, unlike a good deal of other placoderms, elements of its endoskeleton, such as the neurocranium (Young, 1984), were not mineralized. Little is known concerning the microstructure of this and other endoskeletal elements in placoderms (Ørvig, 1951).

Perhaps the most significant insight that this analysis of *Bothriolepis canadensis* has revealed is the extent of resorption and secondary bone deposition in its dermal skeleton (see Fig. 6). Bone resorption has been reported in clades that branch more basally within vertebrate phylogeny, specifically in heterostracans (Gross, 1935; Halstead Tarlo, 1964) and in osteostracans (Denison, 1952). However, in vertebrate phylogeny, placoderms provide the most basal evidence of secondary osteonal development (Johanson and Smith, 2005), and what clearly represents a concerted program of

skeletal tissue remodeling. This insight may highlight dermal skeletal responses to the lifestyle changes imposed by the advent of jaws. Jaws ultimately allowed vertebrates to access the ecological role of the active predator and representatives of Placodermi offer the most primitive examples of active vertebrate predation. Gans and Northcutt (1983) proposed that evolutionary changes in feeding habits correlate with evolutionary changes in growth rate. We may hypothesize then that the growth adaptations observed in the skeleton of *B. canadensis* are correlated to a shift in growth rate at the origin of jawed vertebrates. Certainly, such hypotheses will remain tenuous as long as placoderm systematics remain unresolved and until reaching a better understanding of the skeletal anatomy in other placoderms and in more basal stem gnathostomes.

Is the placoderm dermal skeleton representative of basal crown gnathostomes? It is clear from this description of *Bothriolepis canadensis* that the dermal skeleton of placoderms is more directly comparable to both the extinct "ostracoderms" (armored, jawless stem gnathostomes) and osteichthyans, than to chondrichthyans. Indeed, the placoderm and osteichthyan dermal skeletons are most closely comparable given their division into component cranial dermal plates (Graham-Smith, 1978), in comparison to the fused head capsule of the "ostracoderms." Given existing hypotheses on the evolutionary relationships of these groups (Janvier, 1996), the dermal skeleton of the last common ancestor of crown gnathostomes would have been more comparable to the condition seen in placoderms and osteichthyans, than to either stem or crown chondrichthyans. Thus, as Romer recognized of the skeleton in general and the dermal skeleton in particular, the condition in chondrichthyans is highly derived and should not serve as a model for understanding skeletal evolution (Romer, 1942). It is therefore unfortunate that chondrichthyans have been so influential in the synthesis of hypotheses of dermal skeletal developmental evolution among vertebrates more generally (Stensiö, 1961; Ørvig, 1951, 1968, 1977; Reif and Richter, 2001; Donoghue, 2002; Reif, 1980, 1982, 2002; Sire et al., 2009). The problem is that hypotheses such as the odontode regulation theory, and the lepidomorial theory from which it is derived, address only the odontogenically-derived component of the dermal skeleton, which is characteristic of the dermal skeleton of chondrichthyans, but represents only a relatively minor component of the dermal skeleton of the majority of early skeletonising vertebrates, and is otherwise only rarely manifest in living jawed vertebrates (Donoghue and Sansom, 2002). Future attempts to derive an holistic model for interpreting dermoskeletal developmental evolution must focus on integrating the skeletogenically-derived component.

This may be realized through molecular investigation of scale induction and patterning in teleosts (Sire and Akimenko, 2004), *Polypertus*, *Latimeria* and lungfishes.

CONCLUSIONS

In summary, tests of phylogenetic consistency, morphology, topology, and comparative anatomy support the conclusion that the external skeleton of *Bothriolepis canadensis* lacks dental tissues and is comprised exclusively of cellular dermal bone tissue. Despite variation in the organization of the bone tissues, the data support the identification of each according to the bone tissue classification scheme of de Ricqlès (1975) and de Ricqlès et al. (1991). The stratification of the external thoracic skeleton therefore does not suggest fusion between dermal and endoskeletal systems, but is rather associated with the overlapping nature of the articulations between individual elements. Discovery and confirmation of certain skeletal features in *B. canadensis*, once thought to be gnathostome innovations, demonstrate an earlier origin. Within vertebrate phylogeny, *B. canadensis* exhibits the most basal examples of secondary osteon development as well as a systematic program of skeletal remodeling through the resorption and secondary deposition of mineralized tissue. The presence of spheritic mineralization in the dermal skeleton and its association with zones of secondary mineral deposition indicate the potential for rapid growth and skeletal remodeling.

Whether placoderms represent the sister group of crown gnathostomes (Young, 1986; Goujet and Young, 1995; Janvier, 1996) or a paraphyly of stem gnathostomes (Johanson, 2002; Brazeau, 2009), the skeletal similarities between placoderms and osteichthyans suggest that it is these groups, and not chondrichthyans, that can best inform the primitive condition of the crown gnathostome skeleton.

The anatomical survey of histological microstructure that supports the interpretations of this work is the first for an antiarch placoderm. The evident histological similarities between the dermal skeleton of *Bothriolepis canadensis* and those of osteichthyans and basal stem gnathostomes highlight the problem with assuming a broad application for those hypotheses of dermoskeletal evolution that were based strictly on a chondrichthyan model. By addressing only the odontogenetic component of the dermal skeleton, hypotheses like the lepidomorial and odontode regulation theories do not account for the skeletogenetic component that comprises the majority or entirety of the dermal skeleton in most skeletonizing vertebrates.

The controversies that have long surrounded the interpretation of the antiarch dermal skeleton demonstrate that histological data require anatom-

ical and ontogenetic context if they are to support tissue interpretations. With this perspective, histological examinations of additional clades of basal vertebrates will generate considerable progress toward resolving our understanding of the phylogeny of vertebrate skeletal tissues.

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LITERATURE CITED

- Bardack D, Zangerl R. 1971. Lampreys in the fossil record. In: Hardisty MW, Potter IC, editors. *The Biology of Lampreys*. San Diego, CA: Academic Press. pp 67–84.
- Brazeau MD. 2009. The braincase and jaws of a Devonian "acanthodian" and modern gnathostome origins. *Nature* 457: 305–308.
- Burrow CJ. 2005. Histological structure of the cancellous bone layer in *Bothriolepis canadensis* (Antiarchi, Placodermi). *Lethaia* 38:205–210.
- Carr RK. 1995. Placoderm diversity and evolution. *Bulletin du Muséum national d'Histoire naturelle*, Paris 17:85–125.
- de Ricqlès A. 1975. Recherches paléohistologiques sur les os longs des tétrapodes. VII. Sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (première partie). *Annales de Paléontologie, Vertébrés* 61:51–129.
- de Ricqlès A, Meunier FJ, Castanet J, Francillon-Vieillot H. 1991. Comparative microstructure of bone. In: Hall BK, editor. *Bone*. Boca Raton: CRC Press. pp 1–78.
- Dean MN, Summers AP. 2006. Mineralized cartilage in the skeleton of chondrichthyan fishes. *Zoology* 109:164–168.
- Denison RH. 1947. The exoskeleton of *Tremataspis*. *Am J Sci* 245:337–365.
- Denison RH. 1951. The exoskeleton of early Osteostraci. *Fieldiana Geology* 11:199–218.
- Denison RH. 1952. Early Devonian fishes from Utah: Part I. Osteostraci. *Fieldiana Geology* 11:265–287.
- Denison RH. 1967. Ordovician vertebrates from Western United States. *Fieldiana Geology* 16:131–192.
- Denison RH. 1978. Placodermi. *Handbook of Paleichthyology* Volume 2. Stuttgart: Gustav Fisher. 128 p.
- Donoghue PCJ. 2002. Evolution of development of vertebrate teeth and scales: Unravelling concepts, regulatory theories and homologies. *Paleobiology* 28:474–507.
- Donoghue PCJ, Forey PL, Aldridge RJ. 2000. Conodont affinity and chordate phylogeny. *Biol Rev* 75:191–251.
- Donoghue PCJ, Sansom IJ. 2002. Origin and early evolution of vertebrate skeletonization. *Microsc Res Tech* 59:352–372.
- Donoghue PCJ, Sansom IJ, Downs JP. 2006. Early evolution of vertebrate skeletal tissues and cellular interactions, and the

- canalization of skeletal development. *J Exp Zool B Mol Dev Evol* 306B:278–294.
- Donoghue PCJ, Smith MP. 2001. The anatomy of *Turinia pagei* (Powrie) and the phylogenetic status of the Thelodonti. *Trans R Soc Edinb Earth Sci* 92:15–37.
- Francillon-Vieillot H, de Buffrénil V, Castanet J, Géraudie J, Meunier FJ, Sire J-Y, Zylberberg L, Ricqlès Ad. 1990. Microstructure and mineralization of vertebrate skeletal tissues. In: Carter JG, editor. *Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends*. New York: Van Nostrand Reinhold. pp 471–530.
- Gagnier P-Y. 1993. *Sacabambaspis janvieri*, vertébré Ordovicien de Bolivie: I: analyse morphologique. *Annales de Paléontologie* 79:19–69.
- Gans C, Northcutt RG. 1983. Neural crest and the origin of the vertebrates: A new head. *Science* 220:268–274.
- Goodrich ES. 1909. Vertebrata Craniata. In: Lankester ER, editor. *Treatise on Zoology Volume IX*. London: Adam and Charles Black. 518 p.
- Goujet D, Young GC. 1995. Interrelationships of placoderms revisited. *Geobios* 19:89–95.
- Goujet D, Young GC. 2004. Placoderm anatomy and phylogeny: New insights. In: Arratia G, Wilson MVH, Cloutier R, editors. *Recent Advances in the Origin and Early Radiation of Vertebrates*. München: Pfeil. pp 109–126.
- Graham-Smith W. 1978. On some variations in the latero-sensory lines of the placoderm fish *Bothriolepis*. *Philos Trans R Soc Lond B* 282:1–39.
- Gross W. 1931. *Asterolepis ornata* Eichwald und das Antiarchi-Problem. *Palaeontographica Abt A* 75.
- Gross W. 1935. Histologische Studien am Aussenskelett fossiler Agnathen und Fische. *Palaeontographica Abt A* 83:1–60.
- Gross W. 1961. Aufbau des Panzers übersilurischer Heterostraci und Osteostraci Norddeutschlands (Geschlechte) und Oesels. *Acta Zoologica (Stockholm)* 42:73–150.
- Hall BK. 2005. Bones and Cartilage: Developmental Skeletal Biology. London: Elsevier. 792 p.
- Hall BK, Witten PE. 2007. Plasticity of and transitions between skeletal tissues in vertebrate evolution and development. In: Anderson JS, Sues H-D, editors. *Major Transitions in Vertebrate Evolution*. Bloomington: Indiana University Press. pp 13–56.
- Halstead Tarlo LB. 1964. The origin of bone. In: Blackwood HJJ, editor. *Bone and Tooth*. Oxford: Pergamon Press. pp 3–17.
- Heintz A. 1929. Die Downtonischen u. Devonischen Vertebraten von Spitsbergen II. Acanthaspida. *Skrifter om Svalbard og Ishavet* 22:1–81.
- Hennig W. 1981. *Insect Phylogeny*. New York: John Wiley. 514 p.
- Hesse R, Sawh H. 1992. Geology and sedimentology of the Upper Devonian Escuminac Formation. Quebec, and evaluation of its paleoenvironment: Lacustrine versus estuarine turbidite sequence. *Atlantic Geol* 28:257–275.
- Ivanov A, Cherepanov G, Luksevics E. 1995. Ontogenetic development of antiarch dermal ossifications. *Geobios, Memoire Speciale* 19:97–102.
- Janvier P. 1981. The phylogeny of the Craniata, with particular reference to the significance of fossil “agnathans”. *J Vertebrate Paleontol* 1:121–159.
- Janvier P. 1984. The relationships of the Osteostraci and the Galeaspida. *J Vertebrate Paleontol* 4:344–358.
- Janvier P. 1990. La structure de l'exosquelette des Galeaspida (Vertebrata). *Compte Rendu de l'Academie des Sciences de Paris* 310:655–659.
- Janvier P. 1996. *Early Vertebrates*. Oxford: Oxford University Press. 393 p.
- Janvier P, Arsenault M. 2002. Palaeobiology—Calcification of early vertebrate cartilage. *Nature* 417:609–609.
- Jeffries RPS. 1979. The origin of chordates: a methodological essay. In: House MR, editor. *The Origin of Major Invertebrate Groups: Systematics Association*. London: Academic Press. pp 443–447.
- Jeppsson L, Anehus R, Fredholm D. 1999. The optimal acetate buffered acetic acid technique for extracting phosphatic fossils. *J Paleontol* 73:964–972.
- Johanson Z. 2002. Vascularization of the osteostracan and antiarch (Placodermi) pectoral fin: Similarities, and implications for placoderm relationships. *Lethaia* 35:169–186.
- Johanson Z, Smith MM. 2005. Origin and evolution of gnathostome dentitions: a question of teeth and pharyngeal denticles in placoderms. *Biol Rev* 80:303–345.
- Kawasaki K, Weiss KM. 2008. SCPP gene evolution and the dental mineralization continuum. *J Dent Res* 87:520–531.
- Langille RM, Hall BK. 1993. Calcification of cartilage from the lamprey *Petromyzon marinus* (L.) in vitro. *Acta Zoologica* 74: 31–41.
- Ørving T. 1951. Histologic studies of ostracoderms, placoderms and fossil elasmobranchs 1. The endoskeleton, with remarks on the hard tissues of lower vertebrates in general. *Arkiv för Zoologi* 2:321–454.
- Ørving T. 1957a. Notes on some Paleozoic lower vertebrates from Spitzbergen and North America. *Norsk Geologisk Tidsskrift* 37:285–353.
- Ørving T. 1957b. Remarks on the vertebrate fauna of the lower upper Devonian of Escuminac Bay. P. Q., Canada, with special reference to the Porolepiform Crossopterygians. *Arkiv för Zoologi* 10:367–427.
- Ørving T. 1967. Phylogeny of tooth tissues: Evolution of some calcified tissues in early vertebrates. In: Miles, AEW, editor. *Structural and Chemical Organization of Teeth*. New York and London: Academic Press. pp 45–110.
- Ørving T. 1968. The dermal skeleton: General considerations. In: Ørving T, editor. *Current Problems of Lower Vertebrate Phylogeny*. Stockholm: Almquist and Wiksell. pp 374–397.
- Ørving T. 1977. A survey of odontodes (dermal teeth) from developmental, structural, functional, and phyletic points of view. In: Andrews SM, Miles RS, Walker AD, editors. *Problems in Vertebrate Evolution: Linnean Society Symposium Series 4*. London: Academic Press. pp 53–75.
- Patterson C. 1977. Cartilage bones, dermal bones, and membrane bones, or the exoskeleton versus the endoskeleton. In: Mahala Andrews S, Miles RS, Walker AD, editors. *Problems in Vertebrate Evolution*. London: Academic Press. pp 77–121.
- Prichonnet G, Di Vergilio M, Chidiac Y. 1996. Stratigraphical, sedimentological and paleontological context of the Escuminac Formation: paleoenvironmental hypotheses. In: Schultze H-P, editor. *Devonian Fishes and Plants of Miguasha, Quebec, Canada*. München: Pfeil. pp 23–36.
- Reif W-E. 1980. A model of morphogenetic processes in the dermal skeleton of elasmobranchs. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 159:339–359.
- Reif W-E. 1982. Evolution of dermal skeleton and dentition in vertebrates: The odontode regulation theory. *Evol Biol* 15: 287–368.
- Reif W-E. 2002. Evolution of the dermal skeleton of vertebrates: concepts and methods. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 223:53–78.
- Reif W-E, Richter M. 2001. Revisiting the lepidomorph and odontode regulation theories of dermo-skeletal morphogenesis. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 219:285–304.
- Romer AS. 1942. Cartilage and embryonic adaptation. *Am Nat* 76:394–404.
- Sansom IJ, Smith MP, Smith MM, Turner P. 1997. Astraspis—The anatomy and histology of an Ordovician fish. *Palaeontology* 40:625–643.
- Scheyer TM. 2007. Skeletal histology of the dermal armor of Placodontia: The occurrence of ‘postcranial fibro-cartilaginous bone’ and its developmental implications. *J Anat* 211:737–753.
- Sire J-Y, Akimenko M-A. 2004. Scale development in fish: a review, with description of sonic hedgehog (shh) expression in the zebrafish (*Danio rerio*). *Int J Dev Biol* 48:233–247.

- Sire J-Y, Huysseune A. 2003. Formation of dermal skeletal and dental tissues in fish: A comparative and evolutionary approach. *Biol Rev* 78:219–249.
- Sire J-Y, Donoghue PCJ, Vickaryous MK. 2009. Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *J Anat* 214:409–440.
- Smith MM, Hall BK. 1990. Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. *Biol Rev* 65:277–373.
- Stensiö EA. 1927. The Downtonian and Devonian vertebrates of Spitsbergen. Part 1. Family Cephalaspididae. *Skrifter om Svalbard og Nordishavet* 12:1–391.
- Stensiö EA. 1931. Upper Devonian vertebrates from East Greenland collected by the Danish Greenland expeditions in 1929 and 1930. *Meddelelser om Grønland* 86:1–212.
- Stensiö EA. 1948. On the placodermi of the upper Devonian of East Greenland II. *Meddelelser om Grønland* 139:1–622.
- Stensiö EA. 1961. Permian vertebrates. In: Raasch GO, editor. *Geology of the Arctic*. Toronto: University of Toronto. pp 231–247.
- Sundström B. 1968. Histological decalcification using aqueous solutions of basic chromium (III) sulphate. *Odontologisk Revy* 19:1–19.
- Wang N-Z. 1991. Two new galeaspids (jawless craniates) from Zhejiang Province, China, with a discussion of galeaspid-gnathostome relationships. In: Chang M-M, Liu Y-H, Zhang G-R, editors. *Early Vertebrates and Related Problems in Evolutionary Biology*. Beijing: Science Press. pp 41–65.
- Wang NZ, Donoghue PCJ, Smith MM, Sansom IJ. 2005. Histology of the galeaspid dermoskeleton and endoskeleton, and the origin and early evolution of the vertebrate cranial endoskeleton. *J Vertebrate Paleontol* 25:745–756.
- Wängsjö G. 1952. The Downtonian and Devonian vertebrates of Spitsbergen. IX. Morphologic and systematic studies of the Spitsbergen cephalaspids. *Norsk Polarinstitutts Skrifter* 97:1–615.
- Werdelin L, Long JA. 1986. Allometry in the placoderm *Bothriolepis canadensis* and its significance to antiarch evolution. *Lethaia* 19:161–169.
- Whiteaves JF. 1880. On a new species of Pterichthys from the Devonian rocks of the Baie des Chaleurs. *Am J Sci* 20:132–136.
- Young GC. 1984. Reconstruction of the jaws and braincase in the Devonian placoderm fish *Bothriolepis*. *Palaeontology* 27: 635–661.
- Young GC. 1986. The relationships of placoderm fishes. *Zoo J Linnean Soc* 88:1–57.
- Young GC. 1991. The first armoured agnathan vertebrates from the Devonian of Australia. In: Chang MM, Liu YH, Zhang GR, editors. *Early Vertebrates and Related Problems in Evolutionary Biology*. Beijing: Science Press. pp 67–85.
- Zhu M, Janvier P. 1998. The histological structure of the endoskeleton in galeaspids (Galeaspida. Vertebrata). *J Vertebrate Paleontol* 18:650–654.