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HISTOLOGY OF THE GALEASPID DERMOSKELETON AND ENDOSKELETON, AND THE ORIGIN AND EARLY EVOLUTION OF THE VERTEBRATE CRANIAL ENDOSKELETON

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ABSTRACT—The histological composition of the galeaspid cephalothoracic skeleton has been much debated: here we attempt to resolve this through the analysis of well-preserved remains of galeaspids from Yunnan Province, and Tarim Basin, Xinjiang Uygur Autonomous Region, China. Our results indicate that the galeaspid dermoskeleton is dominantly composed from an acellular laminar bone in which the mineral is organised into cylindrical crystal bundles that are arranged into three orthogonal sets with associated extrinsic fiber spaces, a unique histology for which the term galeaspedin is coined. This is permeated by a coarse vascular plexus that divides the dermoskeleton into upper and lower zones, and the upper zone into distinct tesserae which, like the bounding vascular network, are polygonal in outline.

The outer surface of the dermoskeleton is ornamented by a series of tubercles centered on tesserae, the latter composed partly from galeaspedin, and partly from a capping layer of microspherulitic, acellular bone, similar to the limiting layer of bone of elasmoid scales. Neither dentine nor enameloid is present, nor do the tissue compositions or their arrangement indicate an odontogenic origin.

The endoskeleton is composed of an outer zone of globular calcified cartilage in contact with the dermoskeleton through a poorly mineralized intermediate zone. The inner zone is finely laminated, resulting from progressive zones of calcification embracing the calcospherites in a direction away from the dermoskeleton. There is no persuasive histological evidence for the presence of appositional perichondral bone. As in osteostracans, the galeaspid endoskeleton is interpreted as an expanded neurocranium. However, the presence of a calcified cartilaginous neurocranium in galeaspids in the absence of a perichondral bone layer indicates that these two histogenic components have distinct evolutionary origins. The presence of perichondral bone is a synapomorphy of osteostracans and jawed vertebrates, while the presence of a mineralized neurocranium unites galeaspids to this clade (possibly also including pituriaspids).

INTRODUCTION

Galeaspids constitute an extinct clade (Silurian-Devonian) of jawless vertebrates endemic to China (P'an and Dineley, 1988), Tarim (Wang, Wang, and Zhu, 1996) and northern Vietnam (Thanh and Janvier, 1987). Phylogenetic analyses of early vertebrates consistently resolve the group, with the osteostracans and pituriaspids, as immediate sister taxa to the placoderms and crown-group gnathostomes (Janvier, 1981a, 1996a; Forey and Janvier, 1993; Forey, 1995; Donoghue, Forey, and Aldridge, 2000; Donoghue and Smith, 2001), mainly on the basis of hard tissue histological characteristics. As such, the galeaspids occupy a critical position in our understanding of the assembly of the body plan of jawed vertebrates, and of gnathostomes in general. However, this understanding rests in large part upon a correct interpretation of the homologies of the tissues constituting the galeaspid skeleton and, unfortunately, there is no clear consensus on this issue.

The structure and histological composition of the galeaspid skeleton has been interpreted in many different ways, largely because early studies were based on inferences of the presence, composition, and organisation of various tissue layers from macroscopic observations of unprepared fractured surfaces. Thus, Janvier (1981a) and P'an (1984) interpreted small polygonal impressions as the basal layer of the dermoskeleton, derived from an original honeycomb sub-structure, comparable with the condition in heterostracans and possibly betraying close affinity between these two groups. Janvier (1984) later interpreted the polygonal fabric as reflecting a dermoskeleton composed from many small, discrete units fused side-to-side, the component units compared to the scales of thelodonts and, by implication, composed predominantly of dentine, surrounding a pulp cavity, and united by a continuous basal layer in some taxa (e.g., *Polybranchiaspis*, as opposed to *Hangyangaspis*), overlying perichondral bone of the endoskeleton. The first microscopic analyses, based on indeterminate isolated fragments, were undertaken by Thanh and Janvier (1987), who described a base of spongy bone, but no trace of a basal lamellar layer. Although no cell spaces or dentine tubules were apparent, they considered this an artefact of the effects of diagenetic recrystallization, justified by the histology of the skeletal remains of osteichthyans and placoderms from the same locality.

Janvier (1990) later provided a more complete reinterpretation of galeaspid histological microstructure on the basis of '*Polybranchiaspis*,' emphasising that the dermoskeleton was not composed of scales, but from minute, polygonal blocks of enameloid-capped acellular bone with horizontal stratification, penetrated by a fabric of vertically aligned fine canals, interpreted as Sharpey's fibers. The laminated acellular histology was considered comparable to the dermoskeleton in anaspids. The endoskeleton was not well preserved, but patches of perichondral bone were observed underlying the dermoskeleton and ossifying the plexus of subaponeurotic vascular canals at the junction between the endoskeleton and dermoskeleton. Although Wang (1991) documented the occurrence of cellular bone in *Polybranchiaspis*, it was subsequently disputed by Zhu and Janvier (1998).

Prior to this, Janvier, Thanh, and Phuong (1993) observed that each of the polygonal units in *Bannhuanaspis* bore a single tubercle, which Thanh *et al*. (1995) later compared to the polygonal units in *Polybranchiaspis*, which bear star-shaped tubercle complexes, in a framework of homology reminiscent of the lepidomorial theory (e.g., Ørvig, 1975). These authors concluded, nevertheless, that the dermoskeleton of *Polybranchiaspis* was, like that of *Bannhuanaspis* and *Xiushiaspis*, composed of an acellular, aspidin-like structure constituting the whole of the polygonal units, including the tubercles, and completely lacking in dentine. This interpretation was echoed by Janvier (1996a) who considered that the dermoskeletons of galeaspids and *Astraspis* were comparable, based on the presence of enameloid tubercle caps. However, by contrast, the endoskeleton of galeaspids was considered more akin to the condition in osteostracans. Finally, Zhu and Janvier (1998) provided a complete redescription of galeaspid hard tissue histology, reaching conclusions common to those of Janvier (1990) and Thanh *et al*. (1995), though emphasising the presence of an acellular perichondral bone-lined core of calcified cartilage comprising the galeaspid endoskeleton.

Thus, debate over the composition of the galeaspid cephalothoracic skeleton has narrowed to a consensus where it is viewed as being composed of horizontally (or perhaps more accurately, circumferentially) laminated acellular bone permeated by vertical canals left by Sharpey's fibers, divided into polygonal units in which the upper surface is developed into a tubercular ornament and capped by enameloid. The dermoskeleton is underlain by a layer of acellular perichondral bone lining a core of calcified cartilage, and also lining the extensive subaponeurotic vascular plexus that occurs at the interface between the dermoskeleton and endoskeleton, as well as lining some of the deep lateral-line grooves and canals that lie suspended from, but below the level of the dermoskeleton.

Despite the consensus, this interpretation suggests a very unusual histology, most cogently articulated by Janvier (1996b: 277) "fragments of galeaspids . . . would never have been referred to the vertebrates if not found on complete skulls (they would probably have been referred to an arthropod)." Most curious is the arrangement of enameloid directly overlying bone, a derived condition only otherwise met with in osteichthyans (teeth of *Neoceratodus*, Smith, 1989; Satchell, Shuler, and Diekwisch, 2000; scales of *Lepisosteus*, Sire, 1994), but the nature and, indeed, presence of this tissue has never been adequately documented in galeaspids. Nevertheless, this apparent arrangement results from the absence of dentine, a condition apparently unique to galeaspids amongst stem-gnathostomes, and possibly unique among total-group Gnathostomata as a whole. The acellular condition of the perichondral bone is also peculiar, not least because it indicates parallelism in the phylogenetic precedence of acellular over cellular bone between the endoskeleton and dermoskeleton. However, the very presence of perichondral bone has long been assumed solely on the presence of a mineralized cranial endoskeleton. The microstructural evidence for perichondral bone presented by Zhu and Janvier (1998) is unconvincing, their argumentation turning on the statement (Zhu and Janvier, 1998:650) "the external perichondral layer . . . has a laminar structure which is in many ways similar to that of the basal layer of exoskeleton, but it can be regarded as perichondral bone."

These apparent idiosyncrasies impact not only upon our understanding of the evolution of vertebrate skeletal systems, but also our understanding of the structure of the gnathostome stem group and, as a result, our understanding of the assembly of the gnathostome bauplan. Perichondral bone, for instance, currently represents one of only two unequivocal synapomorphies uniting Galeaspida, Pituriaspida, Osteostraci, Placodermi, and crown Gnathostomata, to the exclusion of all other vertebrates (e.g., Janvier, 1984, 1996a, b; Donoghue, Forey, and Aldridge, 2000; Donoghue and Smith, 2001). Thus, with the aim of resolving these uncertainties we have undertaken a complete reappraisal of the histological composition of the galeaspid cephalothoracic skeleton based on new material, and employing techniques that facilitate a more complete characterization of the component tissues.

MATERIAL AND METHODS

This study was based on material from two localities. Fragments of polybranchiaspid skeleton were recovered from the Xishancun and Xitun Formations which comprise the lower half of the Cuifengshan Group, at the type section in the Cuifengshan Mountains, 13 km NW of Qujing City, eastern Yunnan, P. R. China. The Cuifengshan Group is 2100 metres in total thickness, and composed of red sandstone and interbedded shaly mudstones, with interbedded gray-blue marls and limestones in its lower part. The entire sequence has been interpreted as marine on the occurrence of brachiopods, chitinozoans, acritarchs, and chondrichthyans (Wang, 1995a), and has been considered to be of Late Silurian (Pridolian) age by Wang (1995b, 1997), but of Lochkovian age (at the most) by Wang and McKenzie (2000). Specimens referred to Hanyangaspida come from the Tataaiertage Formation at Tielikewatie, a small village situated 25 km north of Kalpin county, in the northwest part of the Tarim Basin, Xinjiang (Wang, Wang, and Zhu, 1996). The Tataaiertage Formation consists of a sequence of purplish-red and pale gray siltstones interbedded with purplish-red shales and mudstones. Invertebrate fossils are rare, consisting of a small number of gastropods and brachiopods, but vertebrate fossils, including galeaspids, *Sinacanthus* and mongolepid scales, are common in the calcareous siltstones. Stratigraphically, the Tataaiertage Formation is considered to be Early Silurian (Llandovery) by Wang *et al.* (1996) and Zhu and Wang (2000), and thus these represent the oldest galeaspids recorded to date.

Representative fragments of the dorsal cephalothoracic skeleton, pectoral cornuae, individual tesserae, and body scales were embedded in a cold-curing transparent polyester resin and sectioned using a Buehler Isomet low speed saw. The cut faces were impregnated with Buehler Epothin, a low viscosity, cold-curing resin, which was allowed to cure before grinding and polishing. Polished blocks were then either bonded to a frosted glass slide and cut into polished thin sections for light microscopy, or else etched with dilute (1%) HCl for 1 minute, and coated with gold for SEM analysis. Sections were made at the Insititute of Vertebrate Palaeontology and Palaeonanthropology (IVPP), Beijing, China (N-ZW), by Tony Wighton in the Department of Mineralogy, Natural History Museum, London, UK, and also in the School of Geography, Earth and Environmental Sciences, University of Birmingham, UK (IJS). Thin sections were examined and photomicrographed using transmitted and polarised light, a Zeiss Photomicroscope III with Nomarski Differential Interference Contrast (DIC) (MMS, N-ZW, PD), and a Zeiss Axioskop (IJS, PD); some specimens were also examined in a confocal TSM at the Dental Institute, King's College London (MMS). SEM analysis was undertaken at the IVPP, The University of Birmingham, and King' College London. Galeaspid material is deposited in the collections of IVPP. Comparative histological sections of osteostracans are deposited at the Field Museum of Natural History (FM).

DERMAL ORNAMENT

The galeaspid dermoskeleton is characterised by a cephalothoracic head capsule succeeded caudally by a scale-bearing trunk and tail, of which little is known because of a dearth of articulated remains. Despite this distinction, the surface ornamentation between the cephalothoracic (Fig. 1A–C) and trunk (Fig. 1D–G) regions are readily comparable and intergrade, both

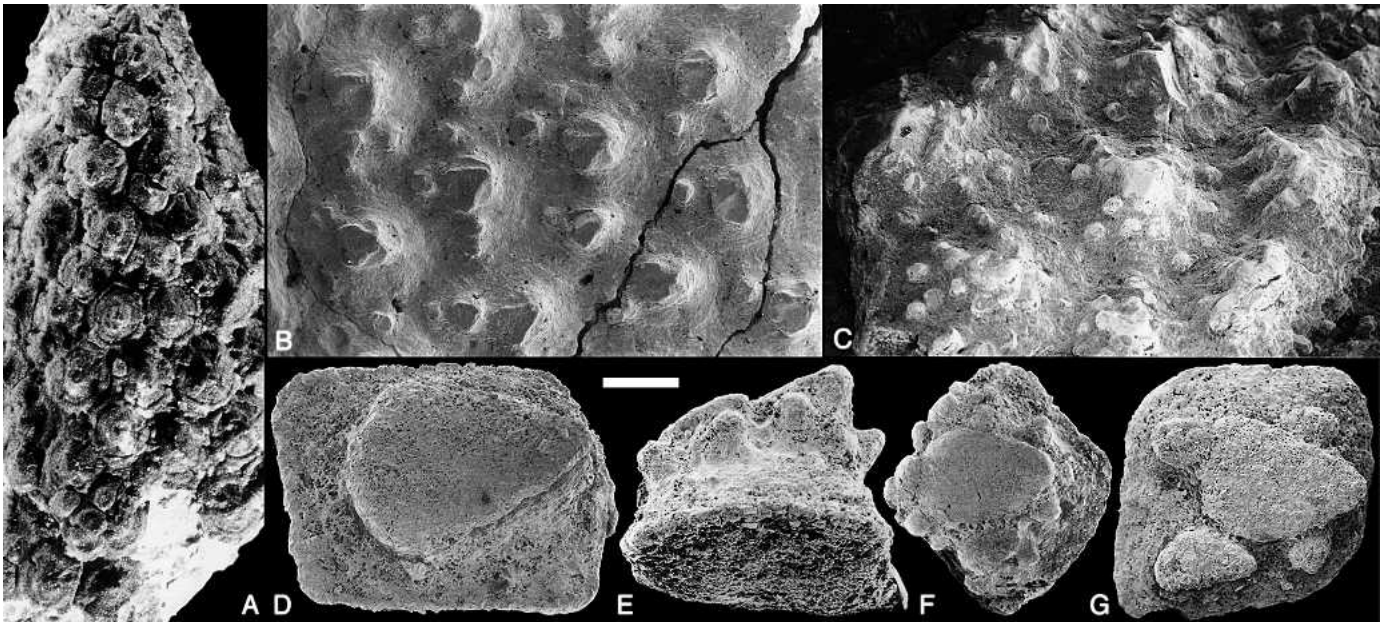


FIGURE 1. Surface morphology of the dermoskeleton of galeaspids representative of the material on which the histological study is based. **A**, pectoral cornua of a hanyangaspid indet. (IVPP V12599). **B**, **C**, dorsal surface of the cranial region of polybranchiaspid indet. (IVPP V12601; V12600). **D**–**G**, isolated scales of polybranchiaspids indet. (IVPP V12602.3; V12602.3; V12602.1; V12602.2). Relative scale bar equals: **A**, 1.8 mm; **B**, 338 μm; **C**, 500 μm; **D**, 104 μm, **E**, 143 μm; **F**, 113 μm; **G**, 142 μm.

exhibiting a similar, albeit highly variable ornamentation. The cephalothoracic shield is divided more (Fig. 1A) or less (Fig. 1B, C) clearly into a series of polygonal units, each similar to the individual scales of the squamation (Fig. 1D–G), and characterised by a gently domed surface ornamented with a central, large tubercle surrounded by a series of smaller tubercles arranged, broadly radially, in diminishing size order (Fig. 1B, F). The partial overlap between tubercles suggests an irregular pattern of sequential addition, though not in a strictly cyclomorial growth pattern as has been suggested by Thanh *et al.* (1995).

HISTOLOGY

Overall Features

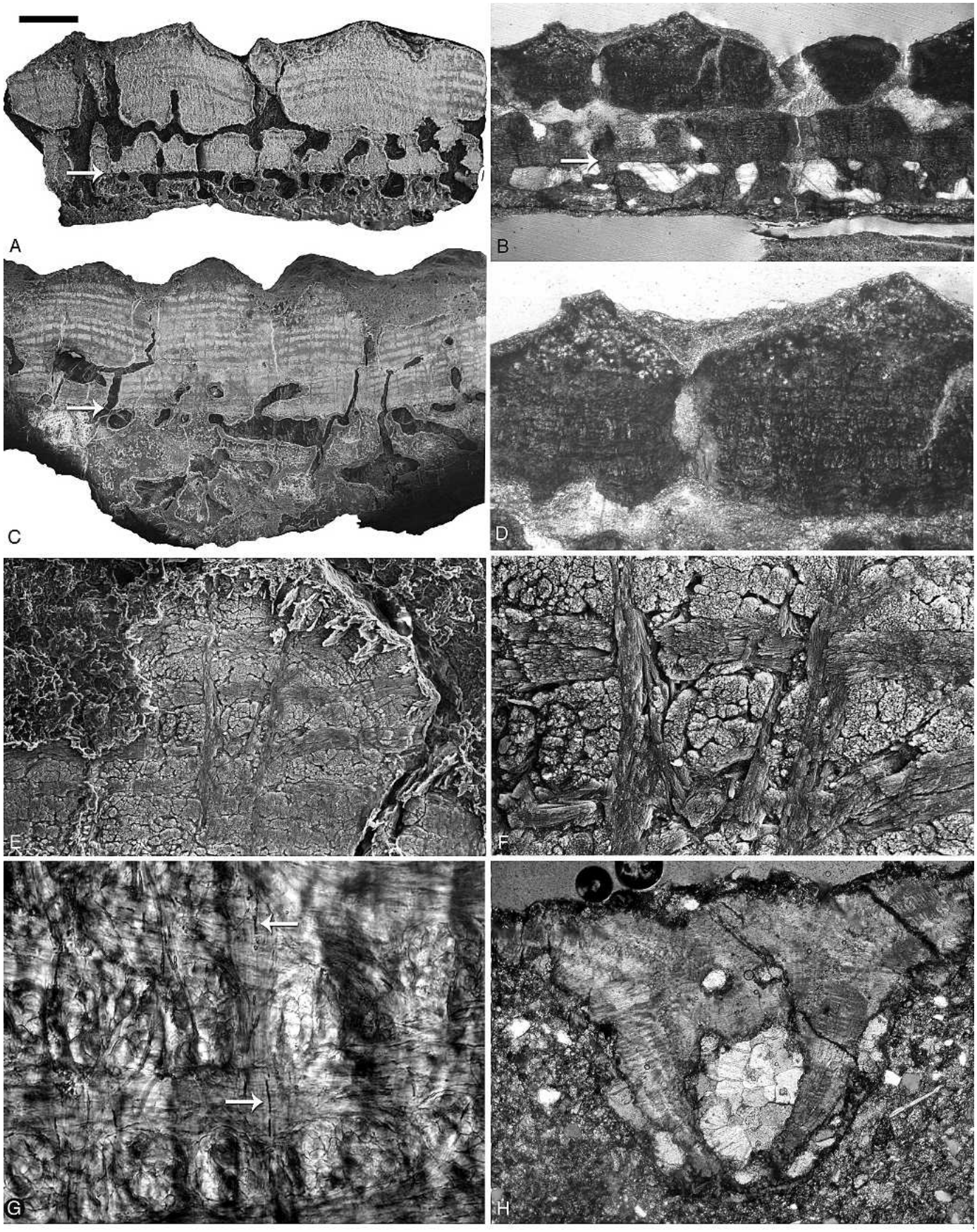
The mineralized tissues that constitute the galeaspid cephalothoracic skeleton can be readily divided into histologically distinct inner endoskeletal and outer dermoskeletal layers, the boundary between which is permeated by a circumferentially-aligned network of coarse canals (>100 μm diameter) (Figs. 2A–D; endo-dermoskeletal boundary arrowed). All parts of the dermoskeleton are composed of an outer, tuberculated compact layer without vascular spaces.

Dermoskeleton

Description—The dermoskeleton is more (Fig. 2A–B), or less (Fig. 2C), distinctly divided into more or less regular tesserae, about which each tubercle, or complex of tubercles, is centered. The tesserae are separated from an underlying, more continuously laminated layer by a network of canals connected to a second, parallel, underlying network of canals, and by vertical canals to the surface, therein also defining the boundaries between many of the tesserae. Both SEM analysis of etched section and light microscopic analysis of polished thin sections indicate that the bulk of the dermoskeleton is composed of an acellular tissue with a broadly laminated fabric (Figs. 2A–F, 4A–B). The laminations are configured in a sub-parallel alignment with the outer surface (Fig. 2A, C), the latter domed, with a tubercular ornament. The laminated fabric is defined by alternating layers (approximately 20 μm in thickness) of densely packed crystal bundles aligned parallel to the outer surface, and approximately perpendicular to the preceding and proceeding layers (Figs. 2E–F, 4A–B). The crystal bundles are oval to polygonal in cross-sectional profile and broadly cylindrical (Fig. 2F). A third suite of crystal bundles is oriented radially, perpendicular to the other two, completing an orthogonal arrangement (Fig. 2E–F, 3A–B). This last suite is aligned approximately perpendicular to the outer surface, although the precise arrangement is focused about the center of the tubercles such that they are vertical at the center of each tubercle, and inclined at a progressively shallower angle as distance increases away from the tubercle center (Figs. 3A, 4A–B). Fine-caliber canals (circa 1 μm diameter) occur aligned to, or even associated with each of the three sets of crystal bundles (Fig. 2G).

Each tubercle is composed, either in total (Figs. 2C–D, 4A, C) or in part (Figs. 2B, D, 3A), of a less etch-resistant upper layer with an irregular lower boundary separating it from the underlying laminated tissue. The upper tissue component may constitute as little as a capping layer, thickest at the tubercle's center (e.g., Figs. 2D, 3A), and diminishing laterally to cover only approximately half of the surface area. In optical sections the outer layer can be seen to intergrade with the underlying tissue with its characteristic dense crystal bundles, but is distinguished by a fabric dominated by individual spheres, approximately 10 μm in diameter, with a radial crystallite orientation (Fig. 3C–D). Fine calibre tubules, otherwise found in association with the perpendicular crystal bundles (Fig. 3D), can be seen to permeate the lateral margins of each tubercle, and also pass through the spherulitic superficial layer (Fig. 3E–H). Although larger tubercles intergrade with the underlying dermoskeleton, they are superimposed by smaller marginal tubercles (Fig. 3C, D), indicating a pattern of sequential formation.

Although lateral line canals often occur below the level of the surrounding dermoskeleton, their composition is histologically indistinguishable from that constituting the dermoskeleton and,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indeed, the laminar fabric in the adjacent dermoskeleton is continuous with canal walls (Fig. 2H).

Interpretation—The fabric of the tissue comprising the bulk of the dermoskeleton is compatible with the conventional interpretation as acellular bone. Operationally, acellular bone and aspidin are often used interchangeably in the literature, but aspidin was originally described from heterostracans (Gross, 1935) where it is characterised by trabeculae consolidated by osteons, and these are not present in the galeaspid dermoskeleton. Rather, the tissue comprising the galeaspid dermoskeleton is more comparable to isopedin, conventionally characterised by layers of acellular bone that exhibit controlled but varying crystallographic orientation between successive layers (e.g., Meunier, 1981). However, the condition in galeaspids differs in that a third arrangement of crystal bundles is present, orientated perpendicular to the two sets of bundles that are aligned parallel to the surface. This overall tissue fabric is unique to, and general among galeaspids. Therefore, the term 'galeaspedin' is coined for this tissue to convey both its distinctiveness from other dermoskeletal tissues and for its exclusivity to galeaspids. By comparison to the genesis of isopedin we assume galeaspedin to be derived by calcification of a fibrous matrix.

The third, perpendicular suite of calcified fiber bundles appears to coincide with the features that Janvier (1990) identified as canals for Sharpey's fibers (Janvier, 1990). Although they are clearly mineralized, and not open canals, we concur with their identification as Sharpey's fibers (which are usually extrinsic to the intrinsic mineralized fiber matrix—see e.g., Francillon-Vieillot et al., 1990) and there is evidence for their extension from the dermoskeleton to the subdermis. The fine-caliber canals that occur in association with each of the three fiber bundles sets may be spaces for the cell processes of fibroblasts, but the most likely interpretation is that they represent unmineralized cores to the Sharpey's fiber bundles (extrinsic fiber spaces).

The tissue comprising each tubercle, or the capping layer to each tubercle, is characteristic of the spheritic mode of mineralization (as defined by Schmidt, 1955; see also Ørvig, 1967), which is a characteristic of cartilage, dentine, and bone. The topology of this tissue, comprising the superficial layer of the dermoskeleton, is incompatible with an interpretation as cartilage, which is an exclusively endodermal component (e.g., Patterson, 1977; Smith and Hall, 1990). Dentine is intuitively the most likely interpretation on the basis of the topology and because spheritic dentines are particularly prevalent among stem-gnathostomes (see Smith and Sansom, 2000; Dong et al., 2005), but the permeation of this tissue by extrinsic fiber spaces (Fig. 3D, F–H) would be extremely unusual. Bone is also known to mineralize through focally accreted layers of mineral (corpuscles of Mandl), especially in association with isopedin (Mandl 1839). However, despite their globular appearance, corpuscles of Mandl are known to be inotropic (Schönbörner, Boivin, and Baud, 1979), that is, in crystallographic alignment with the surrounding organic matrix, as opposed to the radially arranged crystallites comprising the spheres in the dermoskeleton of galeaspids. Nevertheless, truly spheritic mineralization does occur in bone, and particularly in the superficial limiting layer of scales that lack dentine or enamel, in association with the fibrils of anchoring fibers (Sire, 1988); both these conditions are met in the galeaspid dermoskeleton. Thus, the spheritic capping layer in galeaspids is best interpreted as bone, with the permeating extrinsic fiber spaces representing points of attachment for the overlying dermis and epidermis to the dermoskeleton.

We found no evidence for a capping layer of enamel or enameloid, as suggested by previous authors, although it is possible that earlier interpretations refer to the superficial spheritic bone layer. However, given the published figures (e.g., Zhu and Janvier, 1998:fig. 1), it is likely that the layer identified as enameloid is an artifact, possibly resulting from recrystallization of the superficial layer or else representing a polishing artefact.

There is no histological distinction between the tubercular ornament and the underlying tesserae in the dermoskeleton of the cephalothorax. However, where the dermoskeleton is divided into a number of discrete units, such as in the isolated scales that appear to represent components of trunk squamation (Fig. 3C, D, G, H), the tubercular ornament shows clear evidence of sequential addition. Although the pattern of sequential addition is reminiscent of odontodes, there is never any evidence that the tissues formed within a dental papilla (e.g., dentine, enamel, a pulp cavity). Rather, the tubercles are compositionally indistinguishable from those intergrading with the tesserae of the cephalothorax, and appear to have a common morphogenetic origin with the tesserae. Thus, there is never evidence for the presence of odontodes in galeaspids, but of tubercles composed solely of bone.

Endoskeleton

Description—Although the distinction between the dermoskeleton and endoskeleton is clear in etched sections (Fig. 2A, C), nothing more detailed than the position of vascular canals can be discerned concerning the endoskeleton from these sections. Optical thin sections, nevertheless, reveal the endoskeleton to be composed of a zone of dominantly spheritic fabric immediately below the dermoskeleton (Fig. 4A–C, E), intergrading with an underlying zone of dominantly spheritic calcification with concentric growth, linking with incremental zones of linear calcification (Fig. 4A–E). Both zones vary in thickness. The spheritic zone is incompletely mineralized, including spaces between adjacent spherites that superficially resemble cell lacunae (e.g., Fig. 4B). The relationship between incremental layers in the endoskeleton indicates the inward spread of calcification from the junction with the dermoskeleton, incorporating and linking the fused calcospherites (Fig. 4D, F).

A number of sections from one specimen that we examined included another tissue layer contributing to the endoskeleton, directly underlying and sharply separated from the dermoskeleton, and overlying the endoskeleton as otherwise described above (Fig. 4F, H). This intermediate layer is acellular, shows evidence of hypocalcification (Fig. 4F), a ghost fabric of spheritic mineralization (Fig. 4H), and is sparsely permeated by crystal

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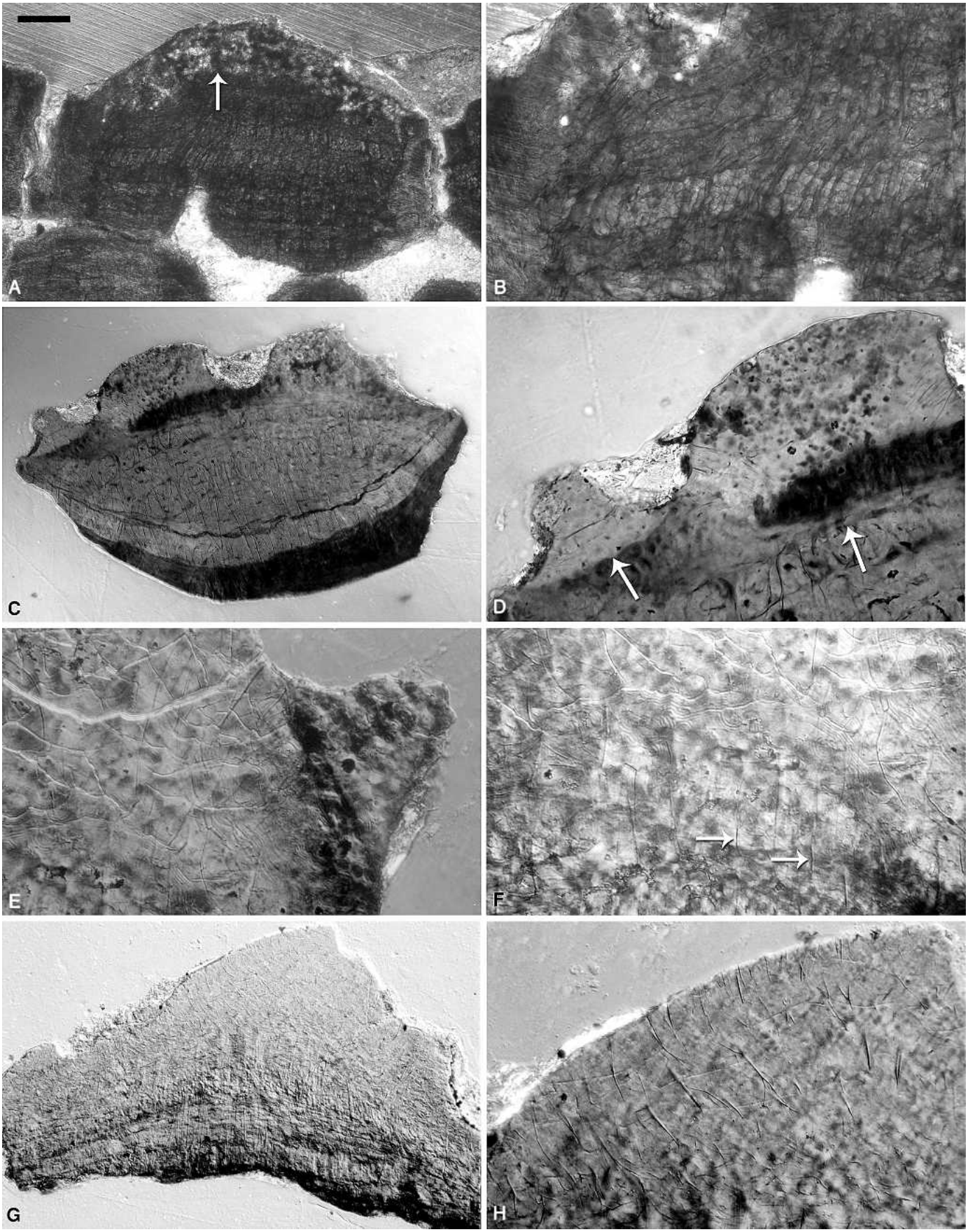
FIGURE 2. **A**, **C**, etched ground sections through the cephalothoracic skeleton of a pectoral cornual process of polybranchiaspid indet., the arrow indicating the junction between the dermoskeleton (upper) and endoskeleton (lower) (IVPP V12599.2). **B**, **D**, dermoskeleton of polybranchiaspid indet. showing well-developed vascular network separating upper and lower levels, and dividing adjacent tesserae, the arrow indicating the junction between the dermoskeleton (upper) and endoskeleton (lower) (IVPP V12603). **E**, etched ground section of a pectoral cornual process of polybranchiaspid indet. showing the orthogonal arrangement of crystal fiber bundles (IVPP V12599.5). **F**, detail of **E** showing the cross-section through one set of fiber bundles, and the relative arrangement of the other two sets. **G**, polished thin section of a pectoral cornual process of polybranchiaspid indet. showing the orthogonal arrangement of crystal fiber bundles and fine-caliber canals that lie at their core (arrowed) (IVPP V12599.6). **H**, section through the dorsal part of the cephalothorax skeleton of polybranchiaspid indet. showing a lateral line canal deep within the corium but composed of galeaspedin, nevertheless; note the continuity of fabric layers within the galeaspedin continuing down and around the canal from the surrounding, superficial dermoskeleton (IVPP V14613). Relative scale bar equals: **A**, 399 μm; **B**, 334 μm; **C**, 380 μm, **D**, 136 μm; **E**, 47 μm; **F**, 15 μm; **G**, 15 μm; **H**, 187 μm.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fiber bundles extending down from the overlying dermoskeleton (Fig. 4H). Fine-calibre tubules (ca. 1 μm diameter) occur diffusely throughout this layer but show particular concentration around vascular spaces.

Interpretation—In the vast majority of cases, the endoskeleton is composed wholly of calcified cartilage and there is no evidence for perichondral bone. The layer interpreted as perichondral bone by Zhu and Janvier (1998) compares favorably to the hypocalcified zone of dominantly spheritic mineralization immediately underlying the dermoskeleton (Fig. 4B, E), a junctional tissue fused to both dermal and endochondral skeleton and functionally unable to grow as perichondral bone by surface apposition.

The additional, superficial, endoskeletal tissue layer described above is histologically distinct from the underlying calcified cartilage. Given its topology, surrounding a core of calcified cartilage, it could be interpreted as perichondral bone. However, given that in other specimens the cartilage of the endoskeleton lies juxtaposed to the dermoskeleton, it is difficult to conceive of a perichondrium developing around it, if for no other reason than because of space constraints. Although histological detail in the dermoskeleton is well preserved, the calcified cartilage sometimes lacks structural detail and it is likely that the superficial layer of endoskeleton is diagenetically altered, obliterating histological detail. However, it is consistent with a less well-calcified biological tissue as the intermediate layer of mixed skeletal origin, at the junction of the two skeletons. Thus, on the basis of this material there is no evidence for the presence of perichondral bone.

Comparison to Other Vertebrates

Dermoskeleton—The dermoskeleton of galeaspids shows no evidence of three-layered division typical of other stem-gnathostomes such as heterostracans and the vast majority of osteostracans, where there is a superficial layer composed of dentine or of dentine tubercles, a middle layer of spongy, invariably osteonal bone, and a basal layer of laminated bone (isopedin sensu Gross, 1956). It is tempting to recognize the tubercles of the galeaspid dermoskeleton as equivalent to the superficial layer, and the remainder of the dermoskeleton, composed as it is of galeaspedin, as equivalent to the basal layer (isopedin) in the dermoskeleton of other stem gnathostomes. However, such a distinction has significance beyond mere topological description as experimental studies have shown that the superficial layer, which is characterised by elements that develop within a dental papilla (see e.g., Donoghue, 2002), has a cell lineage source (odontogenic) that is distinct from the underling (skeletogenic) dermoskeletal layers (Smith and Hall, 1990; Sire and Huysseune, 2003). As we have argued above, there is evidence against, and no evidence for, the interpretation of the surface sculpture of galeaspids as composed of odontodes. Thus, it can be concluded that there is no odontogenic contribution to the galeaspid dermoskeleton. Such a condition is also met with in the antiarch placoderms (cf. Stensiö, 1934), although on the basis of existing phylogenies (Goujet and Young, 1995) this is presumably a derived condition (but see Johanson, 2002). The anaspid dermoskeleton has also been interpreted as lacking odontogenic derivatives (Gross, 1938, 1958) where it must be primitive for the clade.

Division of the dermoskeleton into a series of tesserae appears to be a passive response to the patterning of the pervading vascular network, as there is no evidence of growth of the component tesserae concordant with their boundaries, as is the case in many other primitive vertebrates with tesserate dermoskeleton (e.g., Westoll, 1967; Ørvig, 1968). A similar condition is met with in the dermoskeleton of thyestidian osteostracans (e.g., Gross, 1961; Donoghue and Sansom, 2002; Fig. 4G). Most osteostracans also possess a superficial network of vascular canals that permeate the dermoskeleton (inter-areal canals, Stensiö 1927, 1932; circum-areal canals, Gross 1935) and, depending on its depth, it may (e.g., Wängsjö, 1946, 1952) or may not (Denison, 1947) divide the dermoskeleton into a series of polygonal tesserae.

The structure of galeaspedin is very closely comparable to the isopedin in osteostracans, a variety of basal sarcopterygians, and actinopterygians (Gross, 1956, 1961; Meunier and Castanet, 1982). In teleosts, the successive laminae are extremely thin, composed of just a few sheets of crystal fibers, based upon a collagen fabric of common orientation (Meunier, 1981, 1984; Meunier and Castanet, 1982). However, in the isopedin of osteostracans, lungfish and stem tetrapods, as in galeaspedin, the crystal fibers are arranged into cylindrical bundles (Gross, 1956, 1968; Fig. 3G). In galeaspedin (and the isopedin of some osteostracans), successive laminae are composed of a number of crystal bundles with common crystallographic orientation, successive laminae alternating in orientation by approximately 90°. By comparison, in osteostracans, lungfish, and stem tetrapods, successive laminae are composed of only a single layer of crystal bundles (Gross, 1956, 1961).

The three main components of isopedin, intrinsic collagen fibrils, cell spaces, extrinsic fiber spaces and mineral, are not always present in all combinations, and Francillon-Vieillot et al. (1990) have suggested a histological classification according to these various manifestations. Meunier (1987) has discussed the phylogenetic polarity of these states although he considered only actinopterygians. In particular, the cellular condition of the isopedin of lungfish, stem-tetrapods, amphibians, and osteostracans, as described by Gross (1956), is open to interpretation. In the isopedin of these groups (e.g., Fig. 4G), a number of star-shaped spaces occur that elongate along three orthogonal axes, and these have been interpreted as cell spaces (Gross, 1956). Given the tenuous comparison to cell spaces preserved elsewhere in the same skeletons and their occurrence at the intersection between successive laminae of cylindrical fiber bundles, these structures are, more likely, spaces remaining after close packing of the fiber bundles. Thus, plesiomorphically, it appears that isopedin is acellular, at least with regard to the mineralized product.

The condition of the isopedin in galeaspids differs from all others in including a vertical (radial) array of fiber bundles that penetrate almost the entire thickness of the dermoskeleton, and distinguish the tissue as galeaspedin. They are incomparable to the Sharpey's fibers present in the dermoskeleton of other stem gnathostomes, both in terms of size and because they are most often unmineralized in these other groups. They could be interpreted as derived by mineralization of thick Sharpey's fiber

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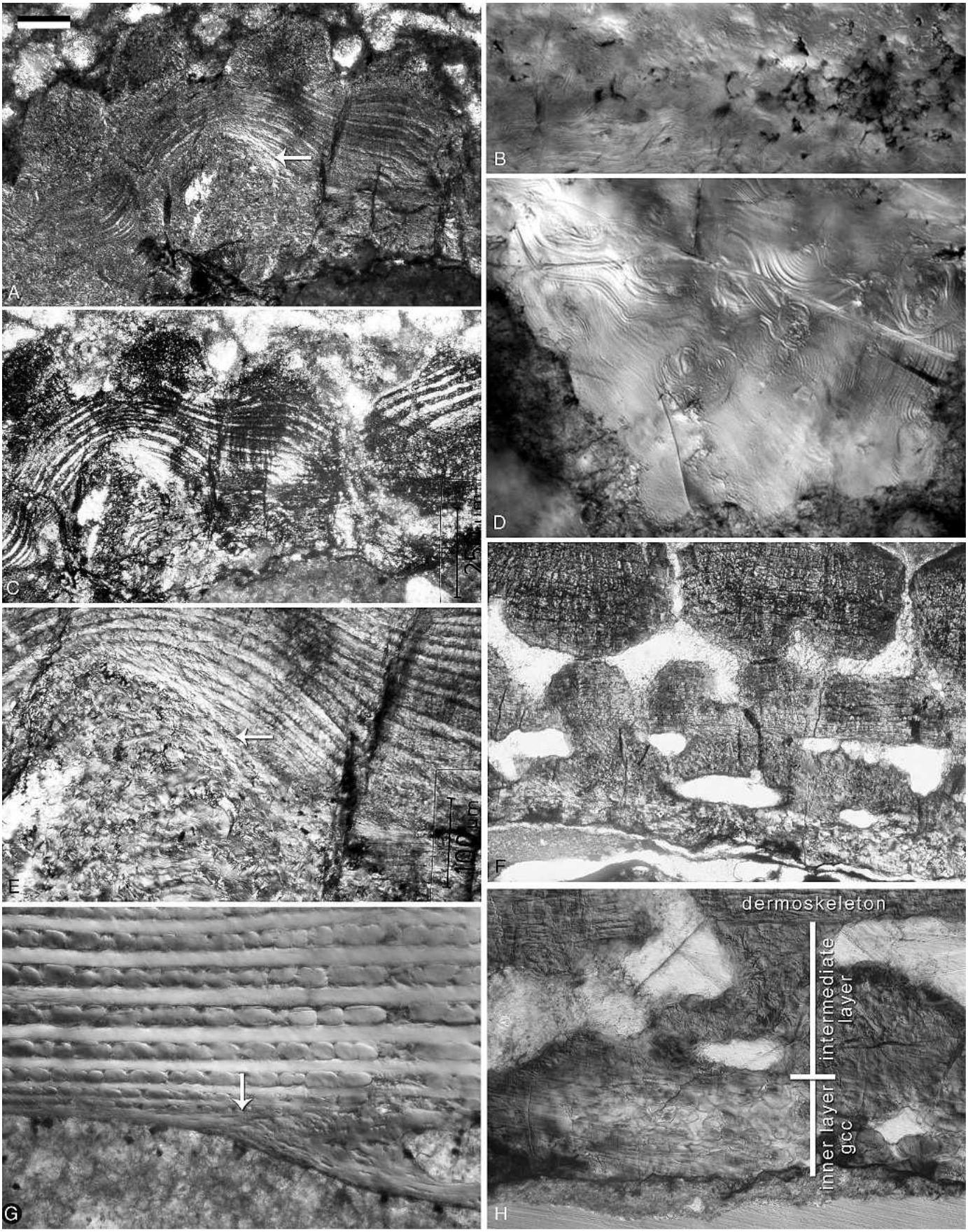
FIGURE 3. **A**, **B**, individual tessera in transmitted light showing the upper spherulitic layer (arrowed) and lower, galeaspedin layer (IVPP V12603); **B**, detail of A showing weakly developed vertical fiber bundles. **C**, **D**, tessera of *Polybranchiaspis* sp. showing the spherulitic composition of the tubercles; arrows indicate the point of attachment of these tubercles to the underlying dermoskeleton and, thus, of their relative superposition (IVPP V12604). **E**, **F**, detail of the tubercular ornament on a tessera of *Polybranchiaspis* sp. showing the spherulitic and convoluted lamination of a fabric penetrated by fine calibre canal spaces (IVPP V12605). **G**, **H**, individual tessera of *Polybranchiaspis* sp. showing the fine calibre canal spaces that permeate the tubercular ornament (IVPP V12606). Relative scale bar equals: **A**, 136 μm; **B**, 49 μm; **C**, 52 μm; **D**, 22 μm; **E**, 26 μm; **F**, 22 μm; **G**, 55 μm; **H**, 24 μm.


A
B
C
D
E
F
G
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dermoskeleton
intermediate layer
inner layer
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bundles, in places leaving thin canals, or tubules occupied in life by unmineralized parts, either cell processes or collagen fibers (e.g., Francillon-Vieillot et al., 1990).

Although spheritic mineralization is prevalent in the dermoskeleton of a number of plesiomorphic skeletonizing vertebrates, where it is usually manifest in dentine (e.g., Karatajute-Talimaa et al., 1990; Sansom, 1996; Sansom, Aldridge, and Smith, 2000; Smith and Sansom, 2000; Dong et al., 2005), the structure of the bone constituting the capping layer of tubercles is unusual from a perspective based on extant vertebrates. Nevertheless, spheritic mineralization occurs in the superficial layer of scales in a number of extant groups including teleosts and caecilians (Bereiter-Hahn and Zylberberg, 1993; Zylberberg and Wake, 1990; Sire, 1988) in association with scales that are otherwise composed solely from bone (isopedin). Here, as in galeaspids, the ornament exhibits some morphogenetic independence from the underlying dermoskeleton, and the units are capable of sequential addition (Sire, 1988); despite their pattern of morphogenesis they are not comparable to odontodes in terms of process. Rather, tubercle growth in the superficial layer is centered on fiber bundles, some of which serve ultimately for the attachment of the overlying dermis and epidermis. We draw comparison between these and the fine caliber canals that permeate the superficial layers of the galeaspid dermoskeleton, including the spherulitic layers capping the tubercles (Fig. 3H). Indeed, these examples may even provide appropriate models for interpreting the galeaspid dermoskeleton given that this condition must have been achieved independently in these disparate lineages.

Endoskeleton—The extent of the mineralized endoskeleton in the galeaspid cephalothorax is comparable to the condition in osteostracans where the precise interface with the overlying dermoskeleton is similarly difficult to discern using simple light microscopy (e.g., Fig. 4G). However, where present, the endoskeleton of osteostracans is composed either of cellular perichondral bone in its entirety or else a layer of perichondral bone surrounding an unmineralized core, or core of calcified cartilage (e.g., Stensiö, 1927, 1932; Denison, 1947; Wängsjö, 1952; Janvier, 1985). However, it should be noted that in many instances, osteostracans completely lack a mineralized endoskeleton (Stensiö, 1927, 1932; Wängsjö, 1952). Indeed, specimens with and without a mineralized endoskeleton have been encountered side by side (Wängsjö, 1952), and so it is more likely that this variation results not from postmortem diagenetic processes, but from variation in the degree of mineralization of the endoskeleton in vivo. Thus, the presence of perichondral bone can be considered polymorphic for osteostracans.

The presence of a mineralized endoskeleton has long been taken to indicate the presence of perichondral ossification, despite observations of solely globular calcified cartilage in the endoskeleton of the late Ordovician jawless vertebrate *Eriptychius* (see e.g., Denison, 1967; Smith and Hall, 1990). This assumption has underpinned not only early interpretations of galeaspid endoskeletal histology, but also the composition of the endoskeleton of osteostracans (Wängsjö, 1952), and pituriaspids (Young, 1991; Janvier, 1996a; Donoghue et et al., 2000), the latter known only from demineralized moulds of the dermoskeleton and endoskeleton. However, the assumption is directly falsified by the presence of a mineralized endoskeleton in galeaspids composed solely of calcified cartilage, and corroborates observations in *Eriptychius.* Consequently, both taxa demonstrate the phylogenetic primacy of calcified cartilage in the endoskeleton over perichondral bone just as in developmental time perichondral ossification is preceded by calcification of the cartilage.

Homology of the Galeaspid Cranial Endoskeleton

Despite controversy surrounding the histological interpretation of the galeaspid endoskeleton, there has been little consideration of its homology. Like the cephalic endoskeleton of osteostracans, which was interpreted as an expanded neurocranium by Janvier (1981b, 1984, 1996a), even though it encompasses the endoskeletal pectoral girdle and the pericardial chamber, as well as the brain and sensory capsules, there are no macroscopic or microscopic sutures in the galeaspid cranial endoskeleton and so it appears to represent a single mineralization. Thus, there is no evidence to support contributions from distinct mesenchymal condensations, as is typical of crown gnathostomes (e.g., Donoghue and Sansom, 2002), and it is possible that the stereotypical regionalization of craniofacial mesenchyme (neural crest and/or mesodermally derived) was not a feature of the embryology of stem gnathostomes. Indeed, the migration paths of craniofacial ectomesenchyme in lamprey and mice are distinct, suggesting wholescale repatterning during vertebrate phylogeny (Kuratani et al., 2001). This event has been constrained to the origin of jawed vertebrates (Kuratani et al., 2001) but, given the condition of the neurocranium in at least some placoderms, it might more appropriately be interpreted to have occurred after their divergence from the lineage leading to crown gnathostomes.

Alternatively is it possible that the absence of distinct calcifications comprising the galeaspid neurocranium is an artifact of disassociation between compartmentalization of mesenchymal condensations (that are known to contribute to the braincase and pericardium of lampreys and the braincase, pericardium and scapulacoracoid of crown gnathostomes) from mineralization of their cartilaginous derivatives. This would meet with the observation that the distinct ossifications comprising the neurocranium of osteichthyans are a manifestation of the perichondrium, while the neurocranium of galeaspids is composed solely of calcified cartilage in which the incremental layers represent arrest lines of a mineralizing front rather than incremental growth layers. Nevertheless, absence of evidence of distinct ossifications in the perichondrally-lined neurocranium of osteostracans supports the view that the osteichthyan condition is derived. The extensive development of the neurocranium may be associated with the absence of perichondral bone in galeaspids and the limited development of perichondral bone in some (but not all) osteostracans. Recent studies have demonstrated that both the perichondrium and periosteum exert negative control on cartilage development in the long bones of chick and mouse (Long and Linsenmayer, 1998; Di Nino et al., 2002; Colnot et al., 2004). The relevance of appendicular long-bone development as a

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FIGURE 4. **A**–**C**, **E**, section through the cornual process of hangyangaspid indet. (IVPP V12607.1) revealing the upper layer of laminated galeaspedin surmounted by tubercles composed entirely from a spherulitic acellular bone, and underlain (junction arrowed) by an endoskeleton composed of an outer spherulitic zone and inner laminated zone; **A**, Nomarski transmitted light; **B**, detail of the spaces amongst the spherulitic zone, **C**, cross-polarized light; **E**, detail of A, C showing the junction between the dermoskeleton (upper) and endoskeleton (lower). **D**, detail demonstrating the progressive infilling of undulations on the inner surface of the endoskeleton with laminations of cartilage (IVPP V12607.1). **F**, **H**, section through the dermoskeleton and underlying endoskeleton of polybranchiaspid indet. showing a distinct layer intermediate between the base of the dermoskeleton and the spherulitic basal layer of the endoskeleton, exhibiting a diffuse fabric of fiber spaces adjacent to vascular canals (IVPP V12603). **G**, polished thin section through the dermoskeleton (upper) and endoskeleton (lower) of *Tremataspis mammilata* (boundary arrowed) demonstrating that a precise distinction between the two can be difficult on histological grounds (FM 4109). Relative scale bar equals: **A**, 133 μm; **B**, 65 μm; **C**, 133 μm; **D**, 35 μm; **E**, 54 μm; **F**, 187 μm; **G,** 37 μm; **H**, 71 μm.

model for neurocranial development in stem-gnathostomes might be questioned but the available evidence indicates that a caudal portion of the galeaspid and osteostracan neurocranium is a transformational homologue of the scapulacoracoid of crown gnathostomes, i.e. that they are derived from a common mesenchymal source.

The absence of distinct calcifications associated with the otic and olfactory regions in osteostracans and galeaspids is potentially of further significance because the otic and olfactory capsules of crown gnathostomes develop from neural crest-derived mesenchyme. Rather, their incorporation into an endoskeleton that is otherwise associated with mesodermally-derived mesenchyme suggests that in stem gnathostomes the neurocranium may have been entirely mesodermally derived (cf. Janvier, 2001). Thus, the chimaeric composition of the neurocranium in crown gnathostomes may be a derived adaptation of a skeletal system that was entirely mesodermally derived plesiomorphically; the distinct ossification of the olfactory capsules in many placoderms (Stensiö, 1964) may indicate that ontogenetic repatterning of ectomesenchymal migration may, after all, have begun before the divergence of the placoderms from the lineage leading to crown gnathostomes. Either way, the presence of a broadly developed, homogenous cephalic endoskeleton in galeaspids, in the absence of pectoral fins, is compatible with its interpretation as an expanded neurocranium.

CONCLUDING SUMMARY AND DISCUSSION

Acellularity has been achieved convergently in a variety of bone tissue types. Aspidin is but one of these as typified by heterostracans, and that present in galeaspids is another type, here termed galeaspedin, and considered a diagnostic synapomorphy of galeaspids. The whole dermoskeleton of galeaspids, including the superficial ornament, is composed of this tissue, which is characterized by its three orthogonal sets of crystal-fiber bundles and associated extrinsic fiber spaces. The dermoskeleton is divided into a series of more-or-less distinct polygonal tesserae that are delineated by a permeating network of vascular canals. The tesserae, though distinct, are joined together with regions of strong attachment, typical of Sharpey's fibers in many squamous fish. Parts of the head shield and pectoral processes are formed from tesserae with soft tissue junctions between them; in other regions the head shield is continuous and the tesserae have fused junctions. The laminae of the galeaspedin are continuous across all the units and may represent an underlying dermal fabric that has become mineralized. The spheritic capping layer of the tubercular ornamentation may be more comparable with the superficial layer of the elasmoid scales of teleosts, formed from mineralized spherules, than to the superficial layer of more closely related groups such as Osteostraci or Heterostraci. Here it provides a strong base for attachment of the epithelium. No odontogenic derivatives, such as enamel, enameloid, dentine, pulp cavity, or even pulp canals, are present.

The galeaspid endoskeleton is composed of calcified cartilage and there is no evidence for perichondral bone. Hitherto, perichondral bone has been one of the few recognised characters to unequivocally support the affinity of galeaspids and osteostracans with jawed vertebrates. However, inferences concerning the presence or absence of this character have not been based on

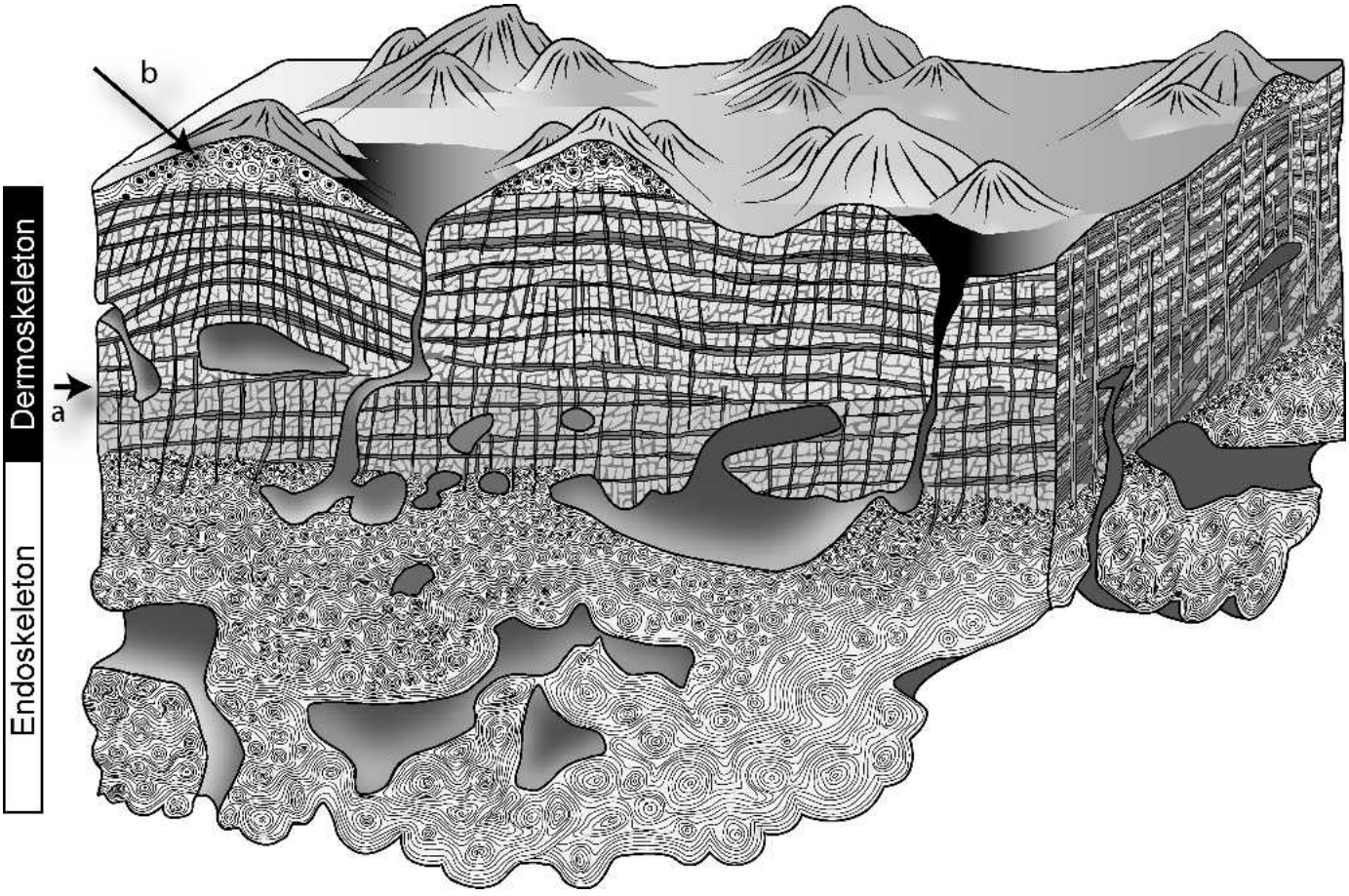

FIGURE 5. Diagrammatic reconstruction of the skeletal histology of the galeaspid dermoskeleton. Note the discordance in alignment of the lower and upper layers of the dermoskeleton. The boundary between the dermoskeleton and neurocranial endoskeleton is irregular. Note also that the mineralization fronts preserved in the neurocranium are not growth lines.

histology, but merely on the presence of a mineralized endoskeleton. We conclude not only that perichondral bone is absent from galeaspids, but that the presence of a mineralized endoskeleton need not coincide with the presence of perichondral bone. A graphical summary of galeaspid skeletal histology is presented in Figure 5.

Perichondral bone is not only absent from galeaspids, it is polymorphic in osteostracans. Given the significance of this character for resolving the affinity of galeaspids, osteostracans, and jawed vertebrates, our understanding of the interrelationships of early vertebrates is diminished. Despite this, and excepting the enigmatic *Eriptychius* (see Donoghue, Forey, and Aldridge, 2000), a mineralized cranial endoskeleton can now be considered a distinct synapomorphy of galeaspids, osteostracans and jawed vertebrates (and, possibly, pituriaspids), and the presence of perichondral bone reserved as a synapomorphy of osteostracans and jawed vertebrates.

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LITERATURE CITED

Bereiter-Hahn, J. and L. Zylberberg. 1993. Regeneration of teleost fish scale. Comparative Biochemistry and Physiology 105A:625–642.

Colnot, C., C. Lu, D. Hu, and J. A. Helms. 2004. Distinguishing the contributions of the perichondrium, cartilage, and vascular endothelium to skeletal development. Developmental Biology 269:55–69.

Denison, R. H. 1947. The exoskeleton of *Trematapsis*. American Journal of Science 245:337–365.

Denison, R. H. 1967. Ordovician vertebrates from Western United States. Fieldiana Geology 16:131–192.

Di Nino, D. L., M. L. Crochiere, and T. F. Linsenmeyer. 2002. Multiple mechanisms of perichondral regulation of cartilage growth. Developmental Dynamics 225:250–259.

Dong, X-P., P. C. J. Donoghue, and J. E. Repetski. 2005. Basal tissue structure in the earliest euconodonts: testing hypotheses of developmental plasticity in euconodont phylogeny. Palaeontology 48:411-421.

Donoghue, P. C. J. 2002. Evolution of development of vertebrate teeth and scales: unravelling concepts, regulatory theories and homologies. Paleobiology 28:474–507.

Donoghue, P. C. J., and I. J. Sansom. 2002. Origin and early evolution of vertebrate skeletonization. Microscopy Research & Technique 59: 352–372.

Donoghue, P. C. J., and M. P. Smith. 2001. The anatomy of *Turinia pagei* (Powrie) and the phylogenetic status of the Thelodonti. Transactions of the Royal Society of Edinburgh (Earth Sciences) 92:15–37.

Donoghue, P. C. J., P. L. Forey, and R. J. Aldridge. 2000. Conodont affinity and chordate phylogeny. Biological Reviews 75:191–251.

Forey, P. L. 1995. Agnathans Recent and fossil, and the origin of jawed vertebrates. Reviews in Fish Biology and Fisheries 5:267–303.

Forey, P. L., and P. Janvier. 1993. Agnathans and the origin of jawed vertebrates. Nature 361:129–134.

Francillon-Vieillot, H., V. de Buffrénil, J. Castanet, J. Géraudie, F. J. Meunier, J.-Y. Sire, L. Zylberberg, and A. d. Ricqlès. 1990. Microstructure and mineralization of vertebrate skeletal tissues; pp. 471–530 in J. G. Carter (ed.), Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends. Van Nostrand Reinhold, New York.

Goujet, D., and G. C. Young. 1995. Interrelationships of placoderms revisited. Geobios 19:89–95.

Gross, W. 1935. Histologische Studien am Aussenskelett fossiler Agnathen und Fische. Palaeontographica (Abt. A) 83:1–60.

Gross, W. 1938. Der histologische aufbau der Anaspiden-schuppen. Norsk Geologisk Tiddskrift 17:191–195.

Gross, W. 1956. Über Crossopterygier und Dipnoer aus dem baltischen Oberdevon im Zusammenhang einer vergleichenden Untersuchung des Porenkanalsystems paläozoischer Agnathen und Fische. Kungliga Svenska Vetenskapsakademiens Handlingar 5:1–140.

Gross, W. 1958. Anaspidenschuppen aus dem Ludlow des Ostseegebiets. Paläontologische Zeitschrift 32:24–37.

Gross, W. 1961. Aufbau des Panzers obersilurischer Heterostraci und Osteostraci Norddeutschlands (Geschiebe) und Oesels. Acta Zoologica (Stockholm) 42:73–150.

Gross, W. 1968. Beobachtungen mit dem Elektronenraster-Auflichtmikroskop an den Siebplatten und dem Isopedin von *Dartmuthia* (Osteostraci). Paläontologische Zeitschrift 42:73–82.

Janvier, P. 1981a. The phylogeny of the Craniata, with particular reference to the significance of fossil "agnathans." Journal of Vertebrate Paleontology 1:121–159.

Janvier, P. 1981b. *Norselaspis glacialis* n.g., n.sp. et les relations phylogénétiques entre les Kiaeraspidiens (Osteostraci) du Dévonien Inférieur du Spitsberg. Palaeovertebrata 11:19–131.

Janvier, P. 1984. The relationships of the Osteostraci and the Galeaspida. Journal of Vertebrate Paleontology 4:344–358.

Janvier, P. 1985. Les Céphalaspides du Spitsberg: anatomie, phylogénie et systématique des Ostéostracés siluro-devoniens; révisions des Ostéostracés de la Formation de Wood Bay (Dévonien inférieur du Spitsberg). Cahiers de Paléontologie, Centre national de la Recherche scientifique, Paris. Édition CNRS, Paris, 244 pp.

Janvier, P. 1990. La structure de l'exosquelette des Galeaspida (Vertebrata). Compte Rendu Académie des Sciences, Paris 310:655–659.

Janvier, P. 1996a. Early Vertebrates. Oxford University Press, Oxford, 393 pp.

Janvier, P. 1996b. The dawn of the vertebrates: characters versus common ascent in the rise of current vertebrate phylogenies. Palaeontology 39:259–287.

Janvier, P. 2001. Ostracoderms and the shaping of the gnathostome characters; pp. 172–186 in P. E. Ahlberg (ed.), Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development. Taylor and Francis, London.

Janvier, P., T.-D. Thanh, and T.-H. Phuong. 1993. A new Early Devonian Galeaspid from Bac Thai Province, Vietnam. Palaeontology 36: 297–309.

Johanson, Z. 2002. Vascularization of the osteostracan and antiarch (Placodermi) pectoral fin: similarities, and implications for placoderm relationships. Lethaia 35:169–186.

Karatajuté-Talimaa, V. N., L. I. Novitskaya, K. S. Rozman and Z. Sodov. 1990. *Mongolepis*—a new lower Silurian genus of elasmobranchs from Mongolia. Paleontologicheskii Zhurnal 1990:76–86.

Kuratani, S., Y. Nobusada, N. Horigome, and Y. Shigetani. 2001. Embryology of the lamprey and evolution of the vertebrate jaw: insights from molecular and developmental perspectives. Philosophical Transactions of the Royal Society of London, Series B 356: 1615–1632.

Long, F. X., and T. F. Linsenmayer. 1998. Regulation of growth region cartilage proliferation and differentiation by perichondrium. Development 125:1067–1073.

Mandl, L. 1839. Recherches sur la structure intime des écailles des poissons. Annales des Sciences Naturelles 2:337–371.

Meunier, F. J. 1981. 'Twisted plywood' structure and mineralization in the scales of a primitive living fish, *Amia calva*. Tissue & Cell 13: 165–171.

Meunier, F. J. 1984. Structure et minéralisation des écailles de quelques Osteoglossidae (Ostéichthyens, Téléostéens). Annales des Sciences Naturelles, Zoologie, Paris 6:111–124.

Meunier, F. J. 1987. Os cellulaire, os acellulaire et tissus dérivés chez les Ostéichthyens: les phénomènes de l'acellularisation et de la perte de minéralisation. L'Année biologique 26:201–233.

Meunier, F. J. and J. Castanet. 1982. Organisation spatiale des fibres de collagène de la plaque basale des écailles des Téléostéens. Zoologica Scripta 11:141–153.

Ørvig, T. 1967. Phylogeny of tooth tissues: evolution of some calcified tissues in early vertebrates; pp. 45–110 in A. E. W. Miles (ed.),

Structural and Chemical Organisation of Teeth. Academic Press, New York and London.

Ørvig, T. 1968. The dermal skeleton: general considerations; pp. 374–397 in T. Ørvig (ed.), Current Problems of Lower Vertebrate Phylogeny. Almquist & Wiksell, Stockholm.

Ørvig, T. 1975. Description, with special reference to the dermal skeleton, of a new radotinid arthrodire from the Gedinnian of Arctic Canada; pp. 41–71 in Problèmes Actuels de Paléontologie—Évolution des Vertébrés. Colloque international CNRS 218.

Pan, J. 1984. The phylogenetic position of the Eugaleaspida in China. Proceedings of the Linnean Society of New South Wales 107: 309–319.

P'an, J., and D. L. Dineley. 1988. A review of early (Silurian and Devonian) vertebrate biogeography and biostratigraphy of China. Proceedings of the Royal Society of London, Series B 235:29–61.

Patterson, C. 1977. Cartilage bones, dermal bones, and membrane bones, or the exoskeleton versus the endoskeleton; pp. 77–121 in S. Mahala Andrews, R. S. Miles, and A. D. Walker (eds.), Problems in Vertebrate Evolution. Academic Press, London.

Sansom, I. J. 1996. *Pseudooneotodus*: a histological study of an Ordovician to Devonian vertebrate lineage. Zoological Journal of the Linnean Society 118:47–57.

Sansom, I. J., R. J. Aldridge, and M. M. Smith. 2000. A microvertebrate fauna from the Llandovery of South China. Transactions of the Royal Society of Edinburgh (Earth Sciences) 90:255–272.

Satchell, P. G., C. F. Shuler, and T. G. H. Diekwisch. 2000. True enamel covering in teeth of the Australian lungfish *Neoceratodus forsteri*. Cell and Tissue Research 299:27–37.

Schmidt, W. J. 1955. Die Erforschung molekularer Texturen tierischer Zellen und Gewebe an Hand der Polarisationsoptik. Nova Acta Leopoldina 17:497–516.

Schönbörner, A. A., G. Boivin, and C. A. Baud. 1979. The mineralization process in teleost fish scales. Cell Tissue Research 202:203–212.

Sire, J.-Y. 1988. Evidence that mineralized spherules are involved in the formation of the superficial layer of the elasmoid scale in cichlids *Cichlasoma octofasciatum* and *Hemichromis bimaculatus* (Pisces, Teleostei): an epidermal active participation? Cell Tissue Research 253:165–172.

Sire, J.-Y. 1994. Light and TEM study of nonregenerated and experimentally regenerated scales of *Lepisosteus oculatus* (Holostei) with particular attention to ganoine formation. The Anatomical Record 240:189–207.

Sire, J.-Y., and A. Huysseune. 2003. Formation of dermal skeletal and dental tissues in fish: a comparative and evolutionary approach. Biological Reviews 78:219–249.

Smith, M. M. 1989. Distribution and variation in enamel structure in the oral teeth of sarcopterygians: its significance for the evolution of a protoprismatic enamel. Historical Biology 3:97–126.

Smith, M. M., and B. K. Hall. 1990. Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. Biological Reviews 65:277–373.

Smith, M. M., and I. J. Sansom. 2000. Evolutionary origins of dentine in the fossil record of early vertebrates: diversity, development and function; pp. 65–81 in M. F. Teaford, M. M. Smith, and M. W. J. Ferguson (eds.), Development, Function and Evolution of Teeth. Cambridge University Press, Cambridge.

Stensiö, E. A. 1927. The Downtonian and Devonian vertebrates of Spitsbergen. Part 1. Family Cephalaspidae. Skrifter om Svalbard og Nordishavet 12:1–391.

Stensiö, E. A. 1932. The Cephalaspids of Great Britain. British Museum (Natural History), London, 220 pp.

Stensiö, E. A. 1934. On the Placodermi of the Upper Devonian of East Greenland. Meddelelser om Grønland 97:1–58.

Stensiö, E. A. 1964. Les cyclostomes fossiles ou ostracodermes; pp. 96–382 in J. Piveteau (ed.), Traité de Paléontologie. Masson & Cie, Paris.

Thanh, T.-D., and P. Janvier. 1987. Les vertébrés Dévoniens du Viêtnam. Annales de Paléontologie 73:165–194.

Thanh, T.-D., P. Janvier, T. H. Phuong, and D. N. Truong. 1995. Lower Devonian biostratigraphy and vertebrates of the Tong Vai Valley, Vietnam. Palaeontology 38:169–186.

Wang, J.-Q., N.-Z. Wang, and M. Zhu. 1996. Middle Paleozoic vertebrate fossils from the north-western margin of the Tarim Basin, and the related stratigraphy; pp. 8–16 in X.-G. Tong, D.-G. Liang, and C.-Z. Jia (eds.), New Advances on the Study of Petrolic Geology in Tarim Basin. Science Press, Beijing.

Wang, N.-Z. 1991. Two new galeaspids (jawless craniates) from Zhejiang Province, China, with a discussion of galeaspid-gnathostome relationships; pp. 41–65 in M.-M. Chang, Y.-H. Liu and G.-R. Zhang (eds.), Early Vertebrates and Related Problems in Evolutionary Biology. Science Press, Beijing.

Wang, N.-Z. 1995a. Silurian and Devonian jawless craniates (Galeaspida, Thelodonti) and their habitats in China. Bulletin du Muséum national d'Histoire naturelle, Paris 17:57–84.

Wang, N.-Z. 1995b. Thelodonts from the Cuifengshan Group of East Yunnan, China and its biochronological significance. Geobios, Mémoire Spécial 19:403–409.

Wang, N.-Z. 1997. Restudy of thelodont microfossils from the lower part of the Cuifengshan Group of Qujing, eastern Yunnan, China. Vertebrata PalAsiatica 35:1–17.

Wang, S. Q., and K. McKenzie. 2000. Sinoleperditini (Ostracoda) from the Devonian of South China. Senckenbergiana Lethaea 79: 589–601.

Wängsjö, G. 1946. On the genus *Dartmuthia* Patten, with special reference to the minute structure of the exoskeleton. Uppsala University Bulletin of Geology 31:349–362.

Wängsjö, G. 1952. The Downtonian and Devonian vertebrates of Spitsbergen. IX. Morphologic and systematic studies of the Spitsbergen cephalaspids. Norsk Polarinstitutt Skrifter 97:1–615.

Westoll, T. S. 1967. *Radotina* and other tesserate fishes. Zoological Journal of the Linnean Society 47:83–98.

Young, G. C. 1991. The first armoured agnathan vertebrates from the Devonian of Australia; pp. 67–85 in M. M. Chang, Y. H. Liu, and G. R. Zhang (eds.), Early Vertebrates and Related Problems in Evolutionary Biology. Science Press, Beijing.

Zhu, M., and P. Janvier. 1998. The histological structure of the endoskeleton in galeaspids (Galeaspida, Vertebrata). Journal of Vertebrate Paleontology 18:650–654.

Zhu, M., and J. Q. Wang. 2000. Silurian vertebrate assemblages of China. Courier Forschungsinstitut Senckenberg 223:161–168.

Zylberberg, L., and M. Wake. 1990. Structure of the scales of *Dermophis* and *Microcaecilia* (Amphibia: Gymnophiona), and comparison to dermal ossification of other vertebrates. Journal of Morphology 206: 25–43.

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