



The microstructural variability of the intercentra among temnospondyl amphibians

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The histology of the vertebral centrum of the morphologically diverse Temnospondyli is poorly known. In this study, the variability of the histological framework of various taxa from several Permian and Triassic localities was investigated for the first time. Twelve intercentra, forming the anterior part of the diplospondylous centra of temnospondyls, were examined histologically. The intercentra of all studied taxa share a highly vascularized cortex on the ventro-lateral side and primary and secondary trabecular, endochondral bone on the dorsal side. A high variability is present, among others, within the arrangement of the vascular cavities, the density of the trabeculae and the distribution of calcified cartilage. The Stereospondyli possess a high amount of calcified cartilage between the trabeculae, in all other taxa the calcified cartilage covers only the dorsal surface of the intercentrum. Among the plagiosaurids, despite morphological similarities, the intercentra show a different development. In *Gerrothorax* and *Plagiosaurus*, periosteal bone is also present on the dorsal side around the neural canal, tentatively indicating a fusion of pleurocentrum and intercentrum. The different histological framework of the investigated intercentra may indicate the phylogenetic value of intercentra microstructure, however further studies are necessary. The preservation of calcified cartilage between the trabeculae seems to be a paedomorphic character typical for all Stereospondyli. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **112**, 747–764.

ADDITIONAL KEYWORDS: calcified cartilage – intercentrum – palaeohistology – periosteal bone – Temnospondyli – vertebrae.

INTRODUCTION

Temnospondyli (Zittel, 1888) are a large and diverse group of early tetrapods with a cosmopolitan distribution. Their known stratigraphic range spans from Early Carboniferous (Holmes & Carroll, 1977) to Early Cretaceous (Warren, Rich & Vickers-Rich, 1997). Originally, they were classified according to the morphology of their vertebrae. Romer (1945), for instance, used the architecture of the vertebrae in naming the suborders Rhachitomi and Stereospondyli. Over the intervening years, different computer based phylogenetic analyses have been published and have

proposed various interrelationships. Some researchers have analysed the more basal temnospondyls (Sequeira & Milner, 1993; Holmes, Carroll & Reisz, 1998; Ruta, Coates & Quicke, 2003; Ruta *et al.*, 2007), others the more derived ones (Yates & Warren, 2000; Damiani, 2001; Damiani & Yates, 2003; Witzmann & Schoch, 2006; Schoch *et al.*, 2007; Schoch, 2008; Schoch & Witzmann, 2009a, b). The study of Yates & Warren (2000) divided the Temnospondyli into two major clades: Euskelia and Limnarchia. Euskelia includes the temnospondyls that were once called rhachitomes and two groups, the Dissorophoidea and the Eryopoidea. Limnarchia includes the newly created clade Dvinosauria, the Archegosauroida, and the most derived temnospondyls, i.e., the stereospondyls. Within Stereospondyli, Yates & Warren (2000) erected two major clades: Capitosauria and Trematosauria.

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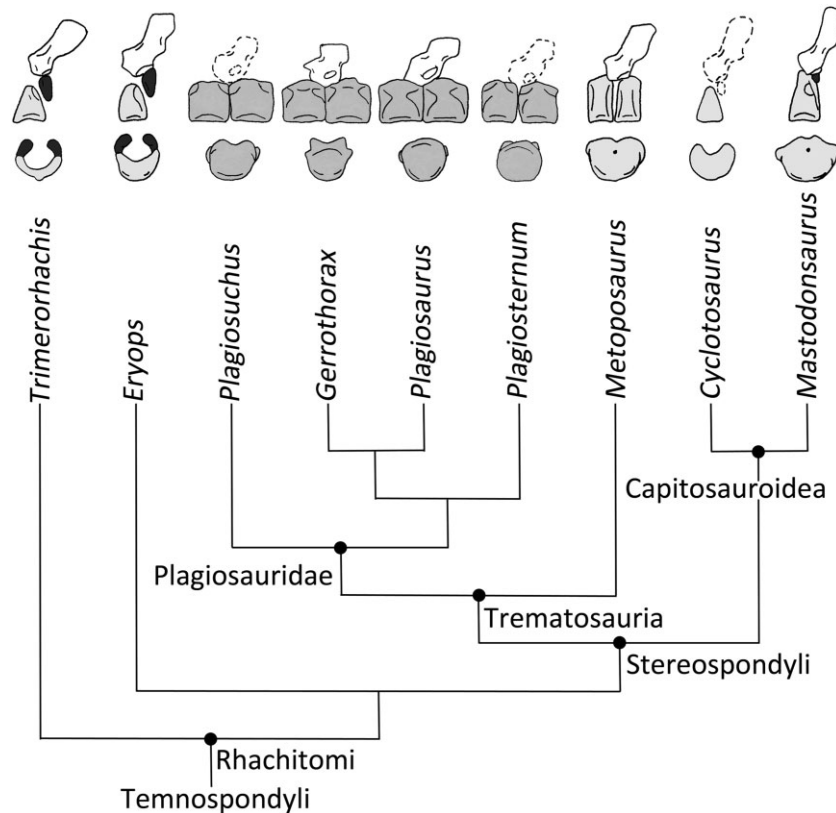


Figure 1. Phylogenetic relationships of the taxa investigated in this study (after Hellrung, 2003; Schoch, 2013 and Schoch *et al.*, 2014). The schematic drawings are not to scale and have been redrawn from Huene (1922), Moulton (1974), Warren (1985), Shishkin (1989), Warren & Snell (1991), Schoch (1999), Pawley (2007) and Sulej (2007). Light grey: intercentrum; dark grey: pleurocentrum; grey: doubtful origin of the vertebral body for the plagiosaurids (for details see text). Dashed lines show the reconstructed parts of the vertebrae.

Trematosauria includes the trematosauroids, plagiosaurids, brachyopoids, and metoposauroids.

Based on morphological data, a new phylogeny of temnospondyls was published by Schoch in 2013. This study is of particular interest as it includes various Paleozoic and Mesozoic specimens of all major clades. Schoch proposed to use the name Rhachitomi for an inclusive group of largely Permian-Triassic temnospondyls (Fig. 1). The clade contains all temnospondyls except for the edopoids and dendrerpetontids. This is close to Romer's (1945) concept. Eryopids were found to be more closely related to stereospondyls than to dissorophoids, which were grouped with the dvinosaurs. We follow herein the phylogenetic definitions of Schoch (2013).

VERTEBRAL CHARACTERISTICS OF THE TEMNOSPONDYLI

Among basal tetrapods, the vertebral morphology is diverse; both multipartite as well as monospondylous

vertebral centra occur (Fig. 2). Two types of diplospondylous (with both intercentrum and pleurocentrum) centra exist: the rhachitomous and embolomerous types. In the typical embolomerous structure (Fig. 2A), both intercentrum and pleurocentrum form complete discs (Holmes, 1989). The rhachitomous type (Fig. 2B) consists of a crescent-shaped, ventral intercentrum and paired, postero-dorsally placed pleurocentra (Williams, 1959; Andrews & Westoll, 1970; Panchen, 1977). This vertebral type is the most common one among Temnospondyli and has long been considered the ancestral condition for tetrapods (Romer, 1945, 1964; Panchen, 1967; Arratia, Schultze & Casciotta, 2001). Pierce *et al.* (2013) morphological study confirmed that the rhachitomous type is the ancestral condition for limbed vertebrates. However, they followed the idea emitted by Shishkin (1989) that the rhachitomous condition is reversed (Fig. 2C). This idea implies that a vertebral unit is composed of anteriorly located paired pleurocentra and a subsequent intercentrum.

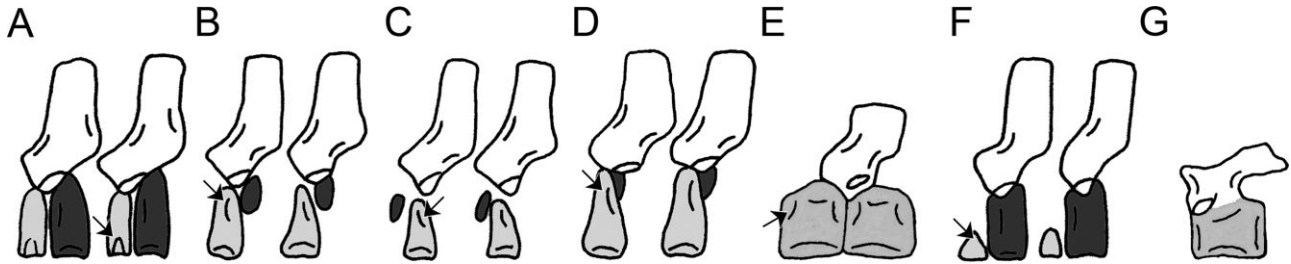


Figure 2. Schematic drawings of the different vertebra types (after Romer, 1956 and Clack, 2012). The drawings are not to scale. (A), Embolomerous condition. (B), Rhachitinous condition. (C), Reverse rhachitinous condition. (D), Stereospondylous condition. (E), Plagiosaurid condition, note the intervertebral position of the neural arch. (F), Gastrocentrous condition. (G), Holo-spondylous condition. Light grey: intercentrum; dark grey: pleurocentrum; grey: doubtful origin of the vertebral body. The position of the parapophyses is indicated by an arrow.

In the stereospondylous type (Fig. 2D, E), the pleurocentrum is highly reduced, but still present, and the intercentrum is considerably larger and usually forms a full disk (Warren & Snell, 1991). Intercentra are identifiable by the presence of laterally located parapophyses for the articulation with the rib tuberculum. This morphology can be found in several groups of Palaeozoic and Mesozoic temnospondyls (Warren & Snell, 1991; Schoch, 1999; Schoch & Milner, 2000; Sulej, 2007).

The monospondylous vertebral type of amniotes and modern amphibians is achieved either by the fusion of the two central elements or by the reduction of either one of the two. In stem amniotes, this reduction or absence of the intercentrum leads to the gastrocentrous (Fig. 2F) vertebral type (Romer, 1956; Clack, 2012). Schmalhausen (1968) regarded the holo-spondylous vertebral centrum in lissamphibians (and lepospondyls) as fused inter- and pleurocentrum (Fig. 2G). However, more recent studies rather suggest a reduction of the intercentrum, as it is partly preserved in some taxa; the pleurocentrum is the dominant element (Carroll, 1989).

THE MORPHOLOGICAL CHARACTERISTICS OF THE INTERCENTRA

All taxa investigated (Table 1) belong, according to Schoch (2013), to the Rhachitomi clade (Fig. 1). In *Trimerorhachis* (Cope, 1878), the intercentrum forms a thin ring with a large notochordal space. The parapophyses are located on the dorsolateral posterior edge. The lateral and ventral surfaces of the larger intercentra have pronounced carinae (Pawley, 2007).

Eryops (Cope, 1877) has typical rhachitinous vertebrae. The crescent-shaped intercentrum with parapophyses on the postero-lateral surface on the dorsal side is a median ventral element encircling the persistent notochord. Usually it becomes the major

element in the construction of the centrum. Some centra, however, may be formed by the fusion of the intercentrum and the pleurocentrum (Moulton, 1974).

The clade Trematosauria, sister group of the Capitosauroida (Schoch, 2013), consists of Plagiosauridae and Trematosauroida, with *Metoposaurus* (Lydekker, 1890) belonging to the latter group (Fig. 1). In plagiosaurids (*Gerrothorax* Nilsson, 1934, *Plagiosuchus* Nilsson, 1934 *Plagiosaurs depressus* Jaekel, 1913 and *Plagiosternum* Fraas, 1896), vertebral centra are essentially platycoelous with only a shallow central notochordal depression. The centrum is a single unit, and the rib has a triple articulation between adjacent centra and the neural arch above them (Fig. 2E). Towards the cranium, ribs and neural arches shift from an intervertebral to a single vertebral articulation (Hellrung, 2003; Jenkins *et al.*, 2008).

Based on morphological data, the homology of the plagiosaur centrum has been debated. Panchen (1959) suggested a fully pleurocentral origin based on morphological characters. Other studies indicated that the intercentrum forms the whole centrum and that the pleurocentrum is reduced (Warren & Snell, 1991; Warren, 1998). Shishkin however (1987: figs 34, 35) suggested the fusion between pleurocentra and intercentrum to form a single central unit. Hellrung (2003) proposed that the *Gerrothorax* centra consisted of the intercentrum and that the pleurocentra were reduced or fused to the neural arch.

The vertebral centrum of *Metoposaurus* represents the typical stereospondylous condition, with a large disk-shaped intercentrum (Fig. 1). For the *Metoposaurus diagnosticus krasiejowensis* Sulej, 2002 described hereinafter, the pleurocentra are not known (Sulej, 2007), in contrast to the well known, closely related *Dutuitosaurus ouazzoui* (Dutuit, 1976) in which a few pleurocentra are preserved (Dutuit, 1976). This sporadic occurrence suggests their

Table 1. The list of sectioned intercentra of Temnospondyli

Taxon	Catalog number	Locality/age	Position in the vertebral column	Wide (mm)*	Thickness (mm)*	High (mm)*
<i>Trimerorhachis</i> sp. (Dvinosauria)	StIPB A 164	Nocona-Formation, Archer County, Texas/Lower Permian	indet.	16	6	10,3
<i>Eryops</i> sp. (Eryopidae)	IPBSH 63	Briar Creek Bone Bed, Archer County, Texas/Lower Permian	indet.	30	24	21,5
<i>Plagiosuchus</i> sp. (Plagiosauridae)	MHI 1078/17	Wolpertshausen-Heidehöfelate, Germany/Middle Triassic	indet.	27,5	24	18
<i>Gerrothorax</i> sp. (Plagiosauridae)	MGUH 30534	Fleming Fjord Formation, Greenland/Upper Triassic	indet.	20	21	18
<i>Gerrothorax</i> sp. (Plagiosauridae)	MHI 1046/18	Michelbach an der Bilz, Germany/Middle Triassic	indet.	13	14	12
<i>Plagiosaurus depressus</i> (Plagiosauridae)	MB-Am.621	Halberstadt, Sachsen-Anhalt, Germany/Upper Keuper, Upper Triassic	indet.	15	20	18
<i>Plagiosternum</i> sp. (Plagiosauridae)	MHI 609	Schwäbisch Hall-Steinbach, Germany/Middle Triassic	indet.	31	22	22
<i>Metoposaurus diagnosticus</i> (Metoposauridae)	UOPB 00118	Krasiejów, Poland/Upper Triassic	presacral	59	21	41
<i>Cyclotosaurus intermedius</i> (Capitosauroidae)	UOPB 00107	Krasiejów, Poland/Upper Triassic	indet.	59	32	46
<i>Cyclotosaurus</i> sp. (Capitosauroidae)	MB-Am.1475.8	Tongrube Klapproth, Erfurt Mittelhausen, Germany/Middle Keuper, Upper Triassic	indet.	35	20	13
<i>Mastodontosaurus</i> sp. (Capitosauroidae)	MHI 2082	Tiefenbach, Germany/Middle Triassic	thoracal	118		66
<i>Mastodontosaurus</i> sp. (Capitosauroidae)	MHI 1046/20	Michelbach an der Bilz, Germany/Middle Triassic	anterior caudal	54	28	42

*The position of the measurement marked on Figure 3.

cartilaginous condition, preventing fossilization. Their ossification was probably pathological (Sulej, 2007).

Vertebrae of metoposaurs and plagiosaurids are quite distinct compared with other temnospondyls in terms of the position of the neural arch. In these taxa, it is placed between two adjacent centra (Nilsson, 1946; Panchen, 1959; Dutuit, 1976; Warren, 1985; Shishkin, 1987; Sulej, 2007). This condition, in metoposaurs and plagiosaurids, is derived relative to other stereospondyls (Warren & Snell, 1991).

Cyclotosaurus (Fraas, 1889) and *Mastodonsaurus giganteus* (Jaeger, 1828) belong to the Capitosauroida (Fig. 1). In *Mastodonsaurus* a considerable variation in centrum morphology along the vertebral column is observable (Schoch, 1999). The thoracic intercentra form a nearly complete disk with a slightly concave anterior and posterior surface. The sacral intercentrum is typically dorsally open, with a broad and deep ventrally reaching incisure for the chorda. Toward the caudal end of the tail, the shape of the intercentra becomes successively more crescent shaped (Schoch, 1999). In *Cyclotosaurus*, there is almost nothing known about the variability of the vertebrae construction along the vertebral column. However, at the Late Triassic locality Krasiejów, from where the *Cyclotosaurus intermedius* Sulej & Majer, 2005 material comes, regardless of the size of the material only the crescent-shaped intercentra, are known (D.K.M., pers. obs.). This may suggest a poor morphological variability of the intercentra.

TEMNOSPONDYL VERTEBRAL HISTOLOGY

Histological descriptions of temnospondyl vertebrae are extremely rare (Enlow & Brown, 1956; Mukherjee, Ray & Sengupta, 2010; Konietzko-Meier, Bodzioch & Sander, 2013), although the vertebral column forms an important part of the entire skeleton within tetrapods, and vertebrae are one of the most numerous and common remains among fossils (de Buffrénil, Sire & Schoevaert, 1986).

Enlow & Brown (1956) provided a picture of a histological section of an intercentrum of *Eryops*. However, a detailed description is lacking. Mukherjee *et al.* (2010) described transverse vertebral sections of two stereospondyl taxa. They show a narrow cortex composed of avascular or poorly vascularized lamellar bone tissue. Sometimes lines of arrested growth (LAGs) are preserved. Abundant Sharpey's fibres are seen throughout the cortical region. Both thin sections preserve a large medullary region completely filled with cancellous bone, but no information is provided about the presence and distribution of calcified cartilage. The first, and so far only, paper with a comprehensive description of the histological variability of

the intercentral inner structure along the vertebral column and during ontogeny in a temnospondyl was provided by Konietzko-Meier *et al.* (2013). The detailed description of numerous intercentra of *Metoposaurus diagnosticus krasiejowensis* shows that the general histological architecture is quite uniform along the entire vertebral column, differences being restricted to the thickness of the cortex or the degree of remodelling. The highly cancellous structure of endochondral and periosteal domains, the parallel-fibred primary bone tissue, the organization of the vascular canals, the occurrence of calcified cartilage remains and the numerous Sharpey's fibres were similar in all specimens investigated. To date, no pleurocentrum of a temnospondyl has been investigated histologically.

AIM OF THE STUDY

The intercentrum histology of different temnospondyls showing the various characteristic morphotypes is investigated, addressing the following questions: (1) Do intercentra, which are morphologically diverse among basal tetrapods, also differ in histological regards? (2) Is there any information on growth pattern seen in vertebral microstructure in temnospondyls? (3) Are possible ecological adaptations visible? and (4) Does the histological framework match established phylogenetic relationships?

MATERIAL AND METHODS

MATERIALS

In this paper, 12 intercentra of nine temnospondyl taxa were studied (Table 1).

The rhachitomous intercentra of the Lower Permian species, *Trimerorhachis* sp. (StIPB A 164) from the Nocona-Formation (Archer County, Texas) and *Eryops megacephalus* Cope, 1877 (IPBSH 63) from the Briar Creek Bone Bed (Archer County, Texas) are completely preserved. The *Plagiosuchus* intercentrum (MHI 1078/17) from the Middle Triassic of Germany is diagenetically deformed; it is flattened and the neural canal is moved to the right side. Two completely preserved vertebrae of *Gerrothorax* sp. come from the Middle Triassic of Germany (MHI 1046/18) and Upper Triassic of Greenland (MGUH 30534). The intercentrum of *Plagiosaurus depressus* (MB.Am. 621) is completely preserved and comes from the Upper Triassic of Germany. In the intercentrum of *Plagiosternum* (MHI 609) from the Middle Triassic of Germany, the right anterior-lateral and dorsal parts are missing. The fully ossified, presacral intercentrum of *Metoposaurus diagnosticus krasiejowensis* (UOPB 00118) and the completely preserved intercentrum of *Cyclotosaurus intermedius*

(UOPB 00107) are from the Upper Triassic of Poland. The intercentrum of the Upper Triassic *Cyclotosaurus* sp. (MB.Am. 1475.8) from Germany is fully preserved. The anterior caudal intercentrum of *Mastodonsaurus* (MHI 1046720) is completely preserved, whereas the thoracic intercentrum (MHI 2082) is preserved only partially as a transversely sectioned fragment with preserved dorsal, ventral and one lateral surfaces.

INSTITUTIONAL ABBREVIATIONS

IPBSH – Paleohistology Collection, Steinmann Institute, Paleontology, University of Bonn, Germany; MB – Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Germany; MGUH – The Natural History Museum of Denmark; MHI – Muschelkalkmuseum Hagdorn, Ingelfingen, Germany; StIPB – Goldfuss Museum, Steinmann Institute, Paleontology, University of Bonn, Germany; UOPB – Department of Biosystematics, University of Opole, Poland.

METHODS

All specimens were measured in three dimensions and sectioned in the transverse plane (Table 1; Fig. 2). Additionally, sagittal cuts were made in both *Plagiosaurus depressus* and *Cyclotosaurus* sp. to illustrate details of the dorsal periosteal bone, and the unusual tissue compactness, respectively (Fig. 3). The thin sections were prepared using the classical methodology (Stein & Sander, 2009). The thin sections were studied under a LEICA DMLP light microscope in normal and polarized light in magnification ranges from $\times 25$ to $\times 400$. The photos were taken by a Leica DFC 420 camera associated with the microscope. Cross sections were scanned with an Epson V740

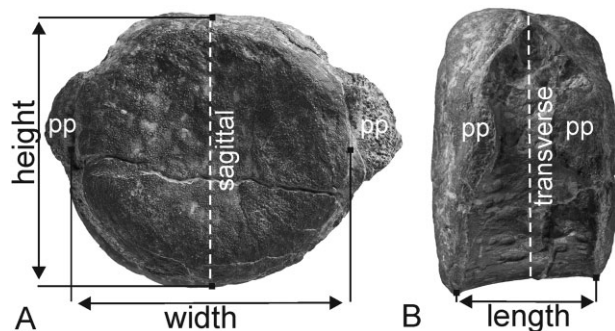


Figure 3. Measurement positions and cutting planes of the vertebrae. Presacral intercentrum of *Metoposaurus diagnosticus* in (A) posterior and (B) left lateral views. Abbreviation: pp, parapophyses.

PRO high resolution scanner. The histological terminology follows Francillon-Vieillot *et al.* (1990).

RESULTS

MICROSTRUCTURE

In *Eryops*, the shape of the section is triangular (Fig. 4A). The ventral and lateral sides of the intercentrum are made of periosteal bone. The external part of this cortex forms a thin, poorly vascularized layer (Fig. 4B). It progresses into a highly eroded region with remains of primary tissue and large erosion cavities often bordered by secondary bone deposits (Fig. 4C). Dorsally, the cortex structure becomes more trabecular and only consists of secondary bone. Growth marks are not present. The cortex become gradually more cancellous towards the endochondral territory (Fig. 4A), however the limit between the periosteal bone and endochondral area is not visible.

The thin, half-ring shaped intercentrum of *Trimerorhachis* has visible ventral and lateral carinas (Fig. 4D). A thin and compact layer of cortex is present on the ventral and lateral sides (Fig. 4E). The microanatomical organization is similar to *Eryops*'s.

The thin-section of the *Plagiosuchus* vertebra shows that the whole intercentrum is deformed; the bottom of the neural canal is moved to the right direction (Fig. 5A). The cortex is present only below the parapophyses. The vascular canals form very regular circumferential rows around the section. The size of vascular canals decreases towards the external surface and resorption increase toward the core. Growth marks are represented by five zones and four LAGs (Fig. 5B).

The two sections of *Gerrothorax* intercentra are U-shaped with the ventral and lateral part of the section made of periosteal bone (Fig. 5C, F). In the German specimen, additionally two triangular patches are observable on the dorsal side (Fig. 5D). Between these two patches, remains of endochondral bone are present. In the Greenland specimen, the entire bottom of the neural canal is covered by periosteal bone (Fig. 5F). Except for this, the microstructure in both specimens is similar. The vascular canals in the periosteal region have mostly a longitudinal orientation. Based on the morphology of the vascular canals, three kinds of layers can be distinguished from the outer to the inner cortex: a first layer with small and irregularly arranged canals, a second one with very small but regularly arranged canals, and a third layer, located next to the endochondral bone, with large erosion cavities and secondary deposits (Fig. 5E, G). The erosion is more advanced in the Greenland specimen. The periosteal

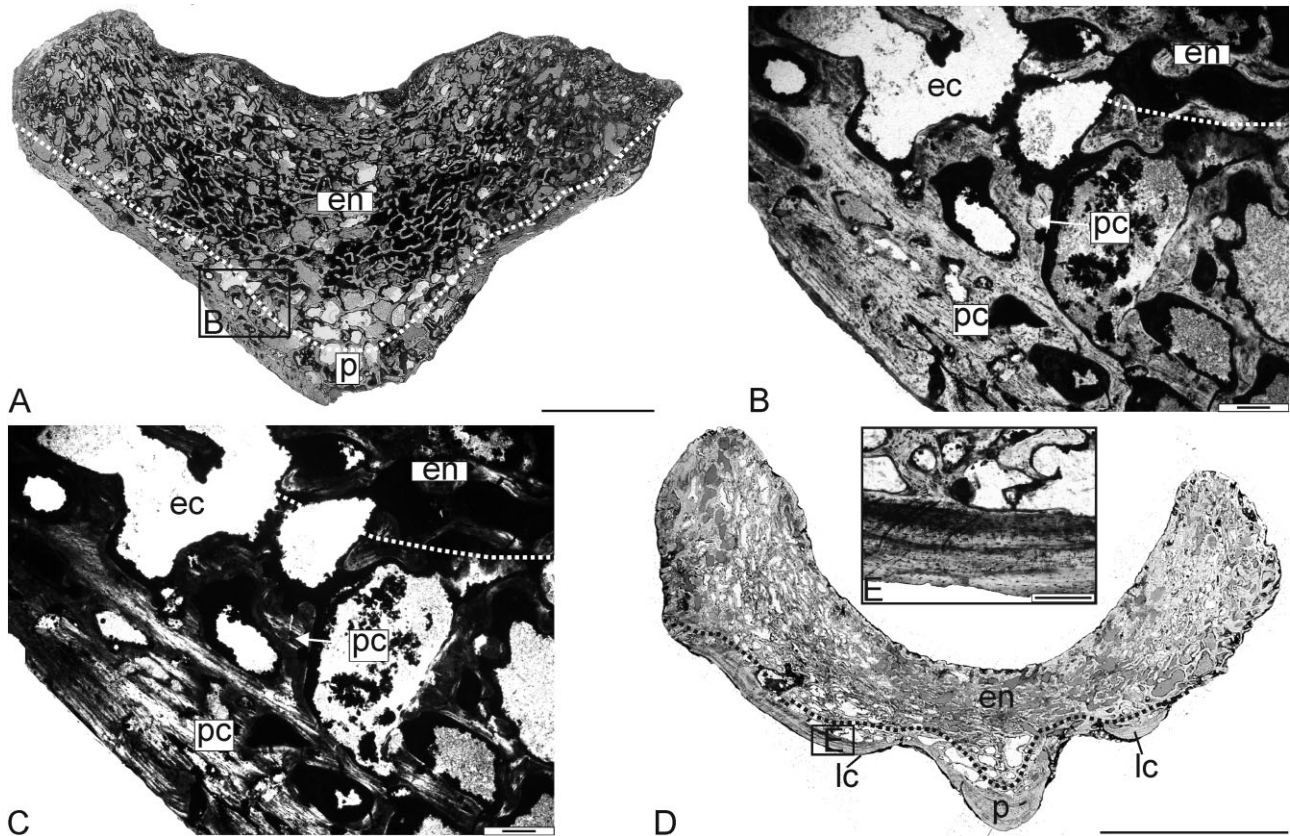


Figure 4. Microanatomy of the vertebrae of *Eryops megacephalus* (A–C) and *Trimerorhachis* sp. (D, E). A, transverse section of the intercentrum of *Eryops megacephalus* (IPBSH 63). B and C, close-up of (A) with the compact layer of primary cortex and the trabecular part with large erosion cavities and remains of primary bone, in (B) normal and (C) polarized light. D, transverse section of the intercentrum of *Trimerorhachis* sp. (StIPB A 164). E, close-up of (D), in normal light. The dotted lines on all images indicate the supposed limit between periosteal and endochondral parts. Scale bars for A and D equal 10 mm, for close-ups 250 μ m. Abbreviations: ec, erosion cavity; en, endochondral bone; lc, lateral carina; p, periosteal territory with primary and secondary bone; pc, primary cortex bone.

bone gradually transits into endochondral bone, however the limit between two domains is visible. The endochondral bone fills the central part of the section and forms the latero-dorsal edges of the section where the parapophyses are present (Fig. 5C, F). The trabeculae in the endochondral region are thick, irregularly arranged with large intertrabecular spaces.

The shape of the transverse section of *Plagiosaurus depressus* can be described as U-shaped (Fig. 5H). Ventrally, periosteal bone occurs. Vascular cavities are arranged in circular layers around the centre of the vertebra (Fig. 5H). Around the neural canal, a small area of periosteal bone is also visible (Fig. 5I). The endochondral bone composed of a dense trabecular system fills the central part of the section and forms the latero-dorsal edges of the section (Fig. 5H). The sagittal section of *Plagiosaurus depressus* is more rectangular in outline (Fig. 5J).

Dorsally and ventrally, the vertebra consists of two areas of periosteal bone with regularly arranged longitudinal canals (Fig. 5J). No growth marks have been identified. The two regions of periosteal bone are completely separated by triangular-shaped areas of endochondral bone (Fig. 5J).

The outline of the *Plagiosternum* sp. intercentrum thin-section is oval (Fig. 5K). Periosteal bone is present only below the parapophyses. The entire intercentrum has a cancellous structure, with irregular, large cavities and thick trabeculae in the endochondral area and numerous vascular canals in the cortex. The limit between these two parts is not distinct. In the cortex, six canals run transversally from the outer surface to the centre and, between them, longitudinal canals dominate (Fig. 5K). Growth marks are observed in the succession of three annuli and three zones (Fig. 5L). The innermost zone spans up to the large, empty cavity visible in the centre of

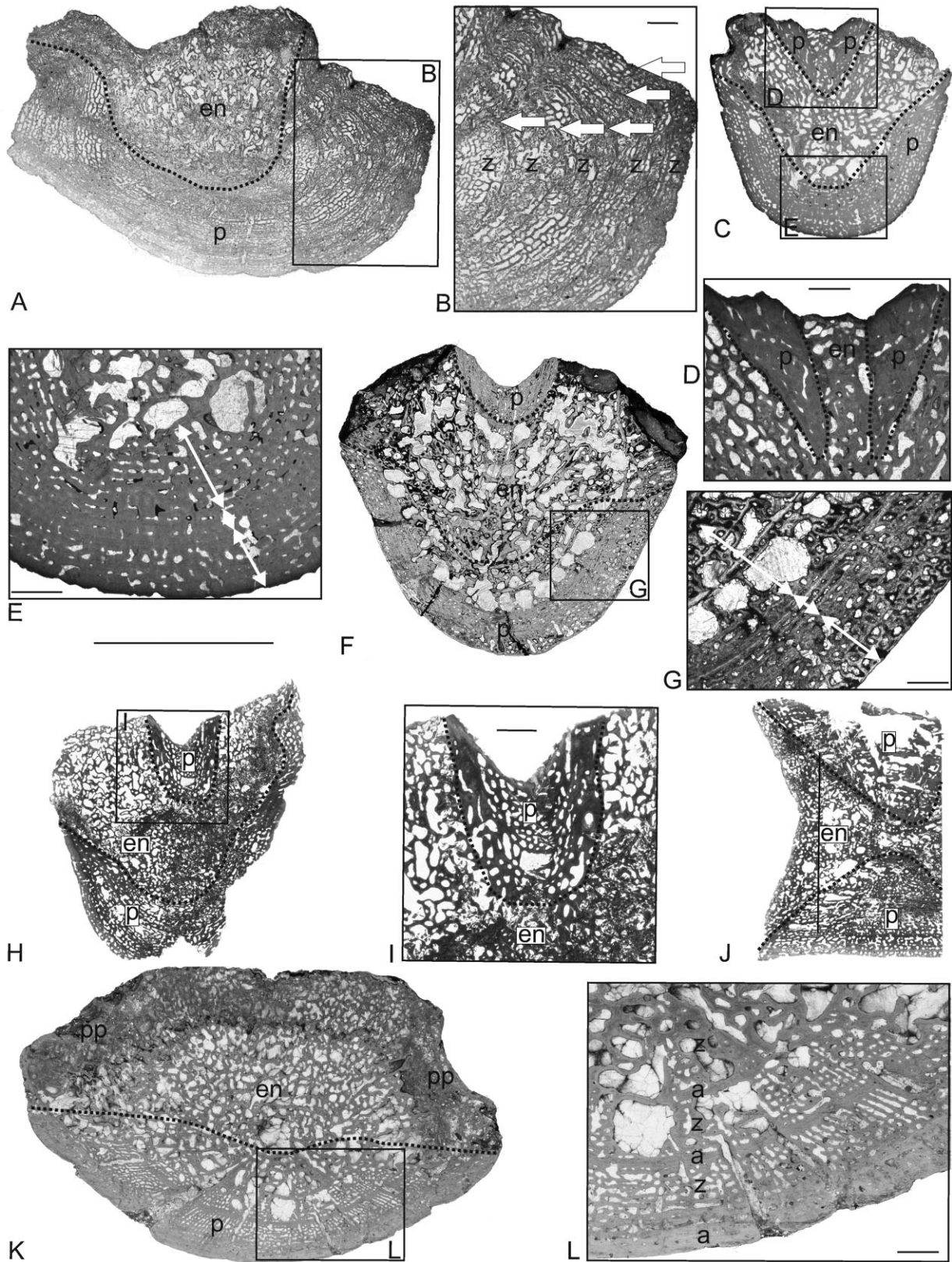


Figure 5. Microanatomy of the vertebrae of plgiosaurids. A, transverse section of the intercentrum of *Plagiosuchus* (MHI 1078/17); note the deformation with the neural canal moved to the right side. B, close-up of (A) with visible zones (z) and LAGs (white arrows). C, transverse section of the centrum of *Gerrothorax* sp. (MHI 1046/18). Note the two triangular patches of periosteal bone visible on the dorsal side. D, close-up of the dorsal fragment of (C) with dorsal located periosteal bone. E, close-up of the ventral part of the cortex from (C). The arrows mark three layers with the different orientation of vascular canals. F, transverse section of the *Gerrothorax* sp. (MGUH 30534) centrum. The periosteal bone on the dorsal side covers the whole bottom of the neural canal. G, close-up of (F). The arrows mark three layers with the different orientation of vascular canals, note that the innermost layer is considerable more remodelled than in previous specimen (E). H, transverse section of the centrum of *Plagiosaurus depressus* (MB.Am. 621). Note the periosteal bone on the ventral and dorsal sides. J, close-up of H, periosteal bone covers the whole bottom of the neural canal. J, sagittal section of the same centrum of *Plagiosaurus depressus* (MB.Am. 621). Note that the image shows only the anterior half of the whole centrum. The periosteal bone is visible as a not-full triangular-shape deposition on the dorsal and ventral sides. K, transverse section of the intercentrum of *Plagiosternum* (MHI 609). The right antero-lateral and dorsal parts are missing. F, close-up of (K) with visible zones and annuli. In all sections the dotted lines mark the border between periosteal and endochondral areas. The large scale bar equals 10 mm, the scale bars for close-ups equal 1 mm. All images were taken in normal light. Abbreviations: a, annulus; en, endochondral bone; p, periosteal territory with primary and secondary bone; pp, parapophyses, z, zone.

the section at the level of the parapophyses. The thickness of zones stays constant, but the thickness of annuli successively increases toward the surface (Fig. 5L).

The *Metoposaurus* intercentrum is a fully ossified disk (Fig. 6A). The ventro-lateral cortex is thin relative to the vertebral size, as compared with the other taxa. Large cavities on the external surface are visible; some of them reach the endochondral region (Fig. 6A). The vascular system forms a dense network of longitudinal canals in the cortex. In the more internal part of the cortex, large erosion cavities are present and only sparse remains of primary bone trabeculae are visible (Fig. 6B). In contrast, the endochondral part is relatively dense; the trabeculae are numerous and thin with reduced intertrabecular spaces (Fig. 6A).

In the section of *Cyclotosaurus intermedius*, the ventral and lateral sides are composed of periosteal bone; the dorsal side and the centre of the vertebra are filled in by endochondral bone. On the ventral surface, large, externally open cavities are visible (Fig. 6C). The mostly longitudinal vascular canals are arranged in irregular rows (Fig. 6D). The long axis of most of the canals is perpendicular to the long axis of the vertebral body; however, the arrangement of canals is not regular. The density of trabeculae in the endochondral domain is similar to that in *Mastodonsaurus*, but a few, large erosion cavities are also visible (Fig. 6D).

The intercentrum of *Cyclotosaurus* sp. is only fragmentarily preserved, from the centre to one lateral tip (Fig. 6E, F). In addition, the matrix is distorted due to diagenesis, but the general shape of the histological structures (e.g. osteons) is unchanged. The bone matrix is dense, intertrabecular spaces are almost absent (Fig. 6E, F).

In both *Mastodonsaurus* sections, a thick and very well vascularized cortex is visible on the ventral side below the parapophyses (Fig. 7A, E). The thoracic intercentrum (MHI 2082) possesses longitudinal and circular vascular canals, arranged in a laminar vascularization pattern. In the innermost and outermost area of the cortex, the size of vascular canals is smaller than in the middle (Fig. 7B, C, D). In the innermost region, there are thick, secondary depositions inside the canals. The dorsal edge consists only of endochondral bone (Fig. 7A). The trabeculae in both domains are thin and numerous, but irregularly orientated in the endochondral part. The remodelling degree is significantly lower in the smaller specimen (MHI 1046/20) and the size of the vascular canals is uniform in the entire periosteal cortex (Fig. 7E). Only an increase in thickness of the trabeculae toward the ventral side can be observed.

BONE TISSUE DESCRIPTION

The bone tissue of the intercentra is not as variable as their microstructure is. The cortex of almost all sectioned intercentra is composed of well vascularized parallel-fibred bone with some amounts of lamellar bone and both primary and secondary osteons (Fig. 8A). The exceptions are the intercentra of *Eryops* and *Trimerorhachis* in which a thin external part of the cortex is highly organized as lamellar bone. In *Cyclotosaurus* sp., the compactness of the bone is very high. Extensive remodelling has led to a dense Haversian tissue in the centre of the bone (Fig. 8B, C).

In all taxa, the following graduation can be recognized: primary bone, present in the periosteal area, changes internally into secondary bone with intensive remodelling. The periosteal bone transits into the

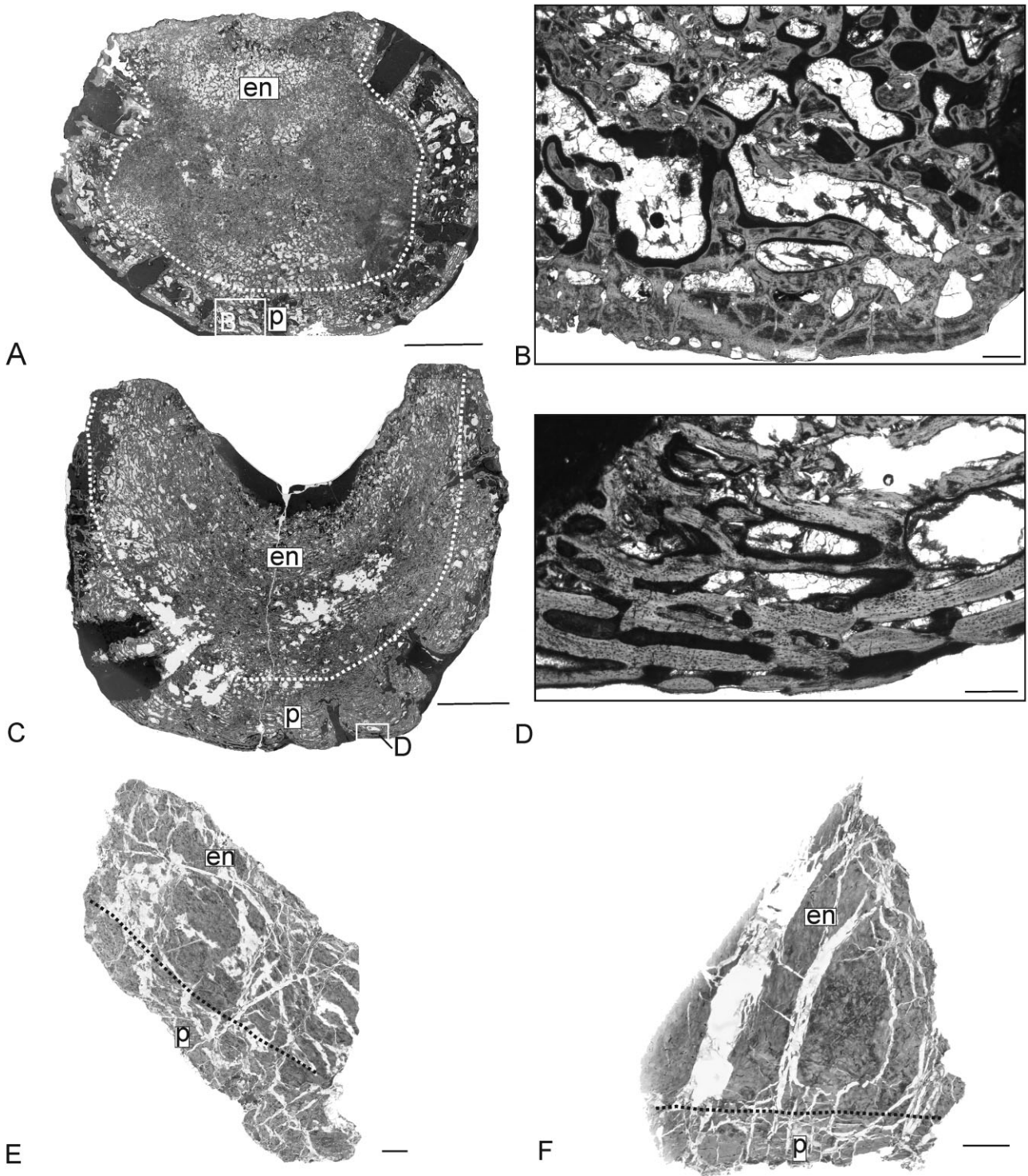


Figure 6. Microanatomy of the vertebrae of *Metoposaurus* (A, B) and cyclotosaurids (C–F). A, transverse section of the presacral intercentrum of *Metoposaurus diagnosticus krasiejowensis* (UOPB 00118). B, close-up of (A), note the irregular arrangement of trabeculae in cortex. C, transverse section of the intercentrum of *Cyclotosaurus intermedius* (UOPB 00107). D, close-up of (C) with circular orientation of canals. E, transverse section of the intercentrum of *Cyclotosaurus* sp. (MB.Am. 1475.8). Only half of the original intercentrum is preserved. F, sagittal section of the same specimen. Note, in both (E and F) images the extremely compact cortex. The dotted lines in all sections indicate the limit between the endochondral and periosteal areas. The scale bars for A and C equal 10 mm, for E and F 1 mm and for close-ups 500 μ m. All images were taken in normal light. Abbreviations: en, endochondral bone; p, periosteal bone.

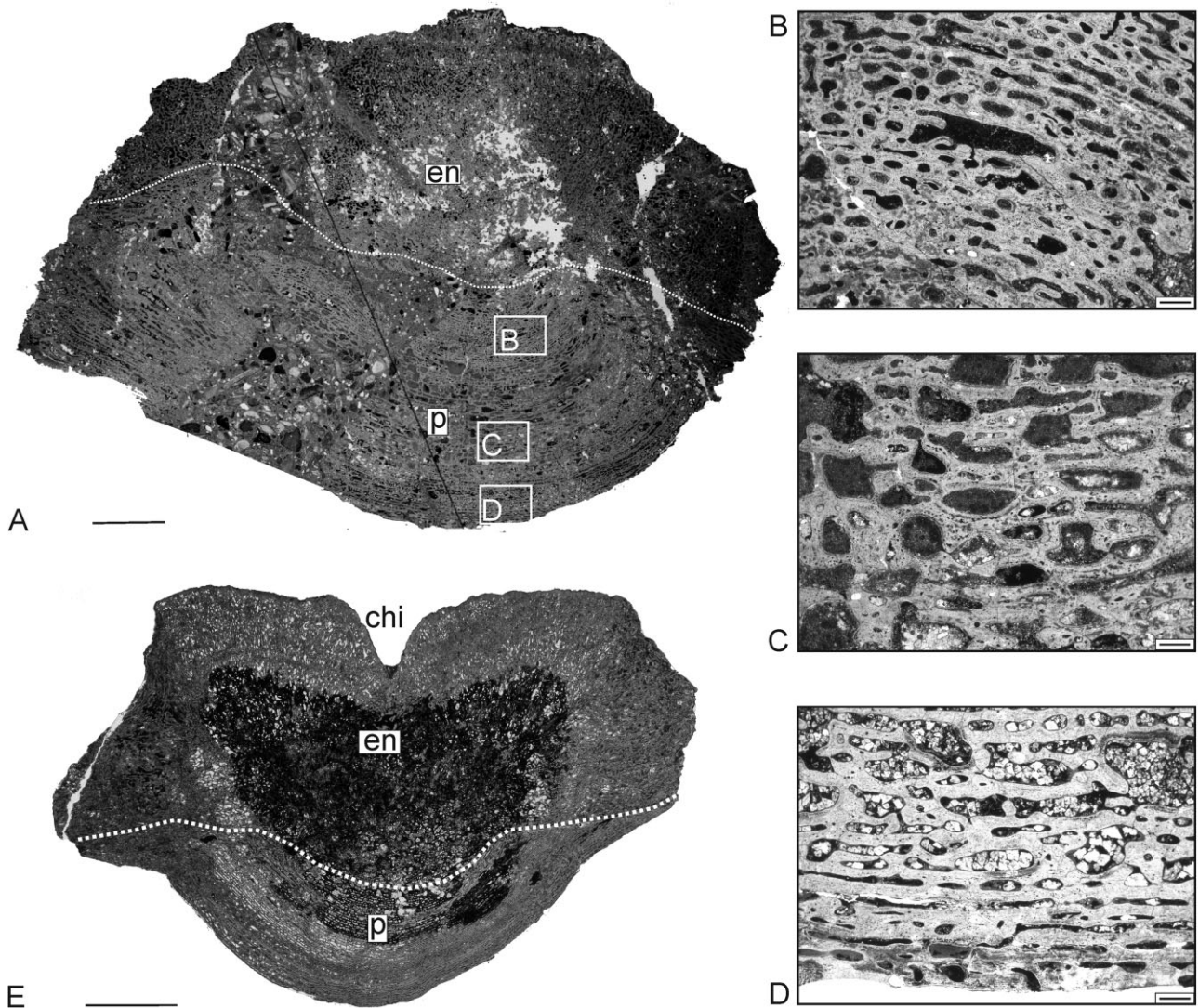


Figure 7. Microanatomy of the vertebrae of *Mastodonsaurus*. A, transverse section of the thoracic intercentrum of *Mastodonsaurus* sp. (MHI 2082). B, close-up from innermost layer of cortex with small, regularly arranged vascular canals with secondary deposition inside canals. C, the close-up of middle part of cortex with larger vascular canals. D, close-up of the eternal part of cortex with small, not remodelled vascular canals. E, transverse section of the anterior caudal intercentrum of *Mastodonsaurus* (MHI 1046720). The chorda incisura is visible on the dorsal side. The dashed lines in all sections indicate the limit between the endochondral and periosteal areas. The scale bars for A and E equal 10 mm, for close-ups 1 mm. All images were taken in normal light. Abbreviations: chi, chorda incisura; en, endochondral bone; p, periosteal bone.

area of endochondral bone, which is composed of primary and secondary trabeculae. The lateral and ventral cortex is usually penetrated by long Sharpey's fibres (Fig. 8D). In all sections, the osteocyte lacunae are numerous. In parallel-fibred bone, they are larger and rounded, in lamellar bone, smaller and flattened. Large remains of cartilage cover the whole anterior and posterior articular surface of the intercentra and the floor of the neural canal (Fig. 8E), except for *Gerrothorax* and *Plagiosaurus*. The endochondral

region of all Stereospondyli sections is rich in remains of calcified cartilage preserved between the trabeculae (Fig. 8F). This is especially visible in the mastodonsaurid intercentra (Fig. 8F).

DISCUSSION

Temnospondyls form a large and diverse group of basal tetrapods. This diversity is also reflected in the microanatomy of their intercentra. Each

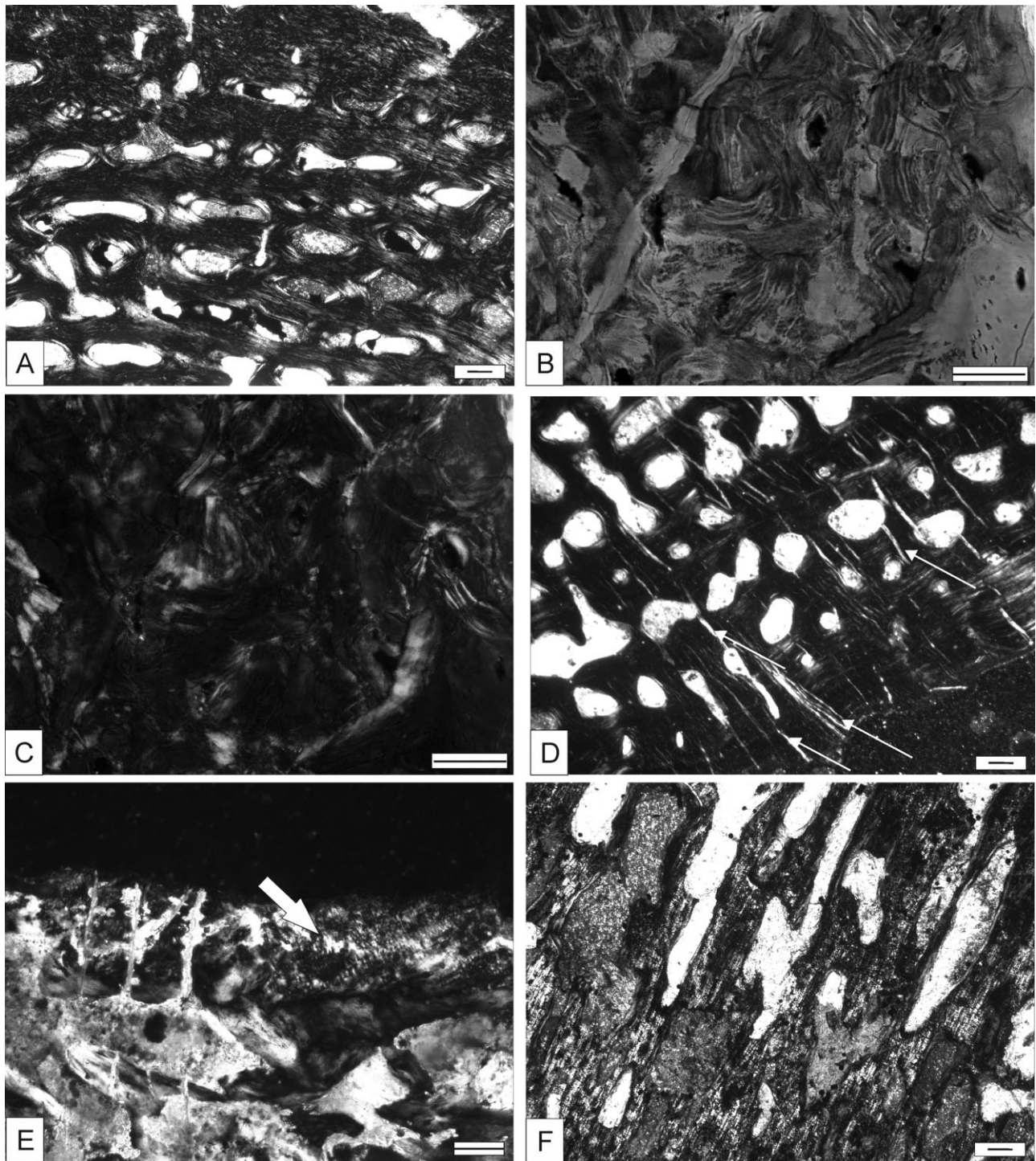


Figure 8. Histology of the temnospondyl vertebrae. A, Parallel-fibred bone typical in the cortex illustrated with the example of the *Gerrothorax* sp. (MHI 1046/18) intercentrum, image in polarized light. B and C, Fragment of the cortex of the *Cyclotosaurus* sp. intercentrum (MB.Am. 1475.8) in (B) normal and (C) polarized light. Note the extremely compact tissue with visible secondary osteons. D, Sharpey's fibres (white arrows) visible on the ventro-lateral surface of the intercentrum of *Plagiosternum* (MHI 609), image in polarized light. E, Calcified cartilage remains (white arrow) covering the dorsal surface of the intercentrum of *Trimerorhachis* sp. (StIPB A 164). F, Calcified cartilage visible in the *Mastodonsaurus* (MHI 1046720) intercentrum. Note that the tissue is almost exclusively built from cartilage. Scale bars equal 100 μ m.

intercentrum represents a different, for each specimen characteristic pattern of trabeculae organization, in both, periosteal and endochondral domains. According to Konietzko-Meier *et al.* (2013), the vertebral inner structure is rather stable along the vertebral column and during ontogeny. Differences can be seen in the thickness of the cortex or the degree of remodelling. The arrangement of the vascular canals, the type of primary tissue or the preservation of calcified cartilage between the trabeculae and their organization, do not change along the vertebral column or ontogenetically. Therefore, even based on only one vertebra per taxon, general conclusions concerning vertebral histology of different taxa can be made.

However, because the knowledge about the bone histology of temnospondyls, and especially about their vertebrae, is still very scanty, the palaeobiological interpretation of the histological framework remains doubtful. Furthermore, conclusions based on comparisons with amniotes may be erroneous, because the Temnospondyli are well outside of the Amniota. Contrary to the basal tetrapods, a vertebral segment in amniotes consists of a small to reduced intercentrum and a large pleurocentrum (Romer, 1956). The histology of the vertebral centra in various amniotes has been well described (e.g., Dumont *et al.*, 2013; Hayashi *et al.*, 2013; Houssaye *et al.*, 2014). In temnospondyls, the intercentrum is mostly the major central element. For a better understanding of the microanatomical organization in temnospondyl vertebral centra, investigations including both intercentrum and pleurocentrum would be of great interest.

VERTEBRAL MICROANATOMICAL ORGANIZATION

Based on the present study, two trends can be distinguished. The first one is present in *Eryops* and *Trimerorhachis*, the two taxa with a typical rhachitomous centrum. Here, the external part of the cortex is very thin and almost avascular (Fig. 4). It then abruptly progresses into a strong remodelled area with large erosion cavities. Between the cavities, primary tissue is still preserved. The endochondral bone consists of a network of irregularly arranged, but rather thick, trabeculae with large cavities in between (Fig. 4). A distinct limit between the two areas is not visible. The second type is represented by the Stereospondyli in which a thick, well-organized cortex is visible (Figs 5–7). The two types of organization of trabeculae can be observed. The first one, visible in middle-sized plagiosaurids, except *Plagiosuchus*, is characterized by thick trabeculae (Fig. 5). In the second type, a tendency to produce more numerous, but thinner trabeculae (Figs 6, 7) is visible. This system is present in the large-sized

Stereospondyli such as *Cyclotosaurus* and *Mastodonsaurus*. The Middle Triassic *Mastodonsaurus giganteus* is one of the largest known temnospondyls, reaching a snout-to-tail length well beyond 5 m (Schoch & Milner, 2000). Similar trend to increase the number and decrease the size of cavities together with general body size increase has also been observed in Amniota vertebrae (Houssaye *et al.*, 2010; Houssaye & Bardet, 2012; Houssaye *et al.*, 2014).

AGE DETERMINATION

Clear skeletochronological marks, visible as zones, annuli or LAGs, are present in the intercentra of *Plagiosuchus* and *Plagiosternum* (Fig. 5B, L). On the basis of these characters the individual age can be calculated as follows: 5 years for *Plagiosuchus* and 3 years for *Plagiosternum*. In other specimens, like *Gerrothorax* or *Mastodonsaurus*, a specific periodicity is visible by the succession of layers with different orientations and sizes of vascular canals (Figs 5E, G and 7B, C, D). However, due to the lack of comparative studies, it is difficult to conclude if the described periodicity represents an important skeletochronological information. The individual age of the *Metoposaurus* intercentrum has been discussed in Konietzko-Meier *et al.* (2013). Based on characteristics typical for different ontogenetic stages (HOS – histological ontogenetic stages), the age of the investigated specimen could be determined as a late juvenile.

THE PRESENCE OF CALCIFIED CARTILAGE

Another difference between the microstructure of the investigated taxa is the presence and distribution of calcified cartilage. Here again, there is a discrepancy between *Eryops* and *Trimerorhachis* on the one hand and the Stereospondyli on the other hand. In the former, calcified cartilage is only present on the dorsal side of the intercentrum (Fig. 8E). In all other taxa, however, remains of calcified cartilage can be found between the trabeculae in the endochondral bone, even deep in the centre (Fig. 8F). This distinction may be interpreted as a manifestation of different ontogenetic stages or of a pedomorphic condition (de Ricqlès, 1979). We are not able to determine an exact individual age of the intercentra, except of *Plagiosternum* and *Plagiosuchus*, but the amount of calcified cartilage, even in the large *Mastodonsaurus* intercentrum, is very high. After Konietzko-Meier *et al.* (2013), calcified cartilage is still preserved in the centra of *Metoposaurus* that are determined as sub-adults. By contrast, in long bones of a similar ontogenetic stage, calcified cartilage is not preserved (Konietzko-Meier & Klein, 2013). In the femur of a

5-year-old *Plagiosuchus*, the cartilage is preserved only as a Kastschenko line (Konietzko-Meier & Schmitt, 2013); in the intercentrum sectioned here numerous remains of calcified cartilage are still visible. It may suggest that calcified cartilage in vertebrae preserves long into ontogeny as a result of a slow ossification process of the endochondral domain. This seems to be an important character typical for Stereospondyli. It also agrees with the postulated paedomorphic postcranial skeleton for Stereospondyli (de Ricqlès, 1979; Pawley & Warren, 2004, 2006). However, it is not possible to state in this study for how long the calcified cartilage is preserved.

MODE OF LIFE

The mode of life of fossil vertebrates is to some extent indicated by skeletal morphology and by bone histology. Several studies have shown that a correlation exists between bone microanatomy and lifestyle of an animal (de Ricqlès & de Buffrénil, 2001; Laurin, Girondet & Loth, 2004). In Temnospondyli, the microstructure of the cortex of long bones resembles that of the cortex of intercentra in those taxa in which both bones are known (Sanchez *et al.*, 2010; Konietzko-Meier & Sander, 2013; Konietzko-Meier & Schmitt, 2013; Quémeneur, de Buffrénil & Laurin, 2013; Sanchez & Schoch, 2013). This indicates the potential usefulness of axial bone histology in further analyses as it may provide a new perspective in histological studies for temnospondyls and other basal tetrapods, as long bones are rare and vertebrae are highly abundant in the fossil record.

In mostly all investigated taxa, the mode of life, previously based on the morphology or long bones microstructure, matches the microanatomy of the vertebrae. The nine investigated temnospondyl taxa represent an amphibious to aquatic mode of life, indicated by both morphological and histological parameters. The mode of life of *Eryops megalcephalus* has been under much controversy. The fairly well-ossified limb bones suggest an amphibious mode of life (Pawley & Warren, 2006). Despite the well ossified skeleton, the spongy structure observed in the long bones of *Eryops* resembles essentially aquatic animals (Sanchez *et al.*, 2010; Quémeneur *et al.*, 2013). However, the high torsional resistance of the long bones suggested that *Eryops* might have used its limbs to crawl on the bottom of a shallow sea or lake floor or to frequently venture on land (Sanchez *et al.*, 2010). The spongy structure of the *Eryops* intercentrum (Fig. 4A) is clearly different to that observed in predominantly aquatic Stereospondyli (Schoch, 2009). This may support the idea of a more amphibious mode of life for *Eryops* in comparison to aquatic Stereospondyli.

Trimerorhachis after Case (1935) and Pawley (2007) represents a primarily aquatic temnospondyl, an interpretation that is in accordance with the presence of fish-like internal gills (Schoch & Witzmann, 2011). Sander (1989) considered *Trimerorhachis* to have had a semi-aquatic lifestyle. Sanchez *et al.* (2010), based on long bone histology, interpreted it as a swimmer of the pelagic realm with a relatively active use of its limbs during locomotion. In the study of Quémeneur *et al.* (2013), *Trimerorhachis* was situated on the most aquatic end of the spectrum of the amphibious category. The similarities of *Trimerorhachis* intercentrum to the *Eryops* structure may confirm the amphibious biology for both taxa (Fig. 4).

Stereospondyli appear to have been predominantly aquatic (Schoch, 2009), but their behaviour, as active swimmers or bottom dwellers, cannot be reconstructed for every taxon. The microstructure of the vertebral centra may add new information to the lifestyle interpretation of these taxa.

The plagiosaurids are characterized by a broad skull, weak limbs and a flattened body shape with dermal armour (Schoch & Witzmann, 2011) and are interpreted as being bottom dwellers. The histology of the femur is only known for two taxa: *Gerrothorax* (Sanchez *et al.*, 2010; Sanchez & Schoch, 2013) and *Plagiosuchus* (Konietzko-Meier & Schmitt, 2013). An increase of bone mass can be observed in the appendicular skeleton of *Gerrothorax* (Sanchez *et al.*, 2010), supporting a bottom-dwelling mode of life. A similar tendency is visible in both vertebrae of *Gerrothorax* studied here (Fig. 5C, F). The more osteoporotic condition of long bones of *Plagiosuchus* represents an adaptation to the pelagic habitat (Konietzko-Meier & Schmitt, 2013). The intercentrum of *Plagiosuchus* compared to *Gerrothorax* represents also a more osteoporotic state (Fig. 5A). The long bone histology is not known for *Plagiosternum* and *Plagiosaurus*. Based on the microanatomical organization of the intercentra (Fig. 5H, K), a similar ecological position as *Gerrothorax* is assumed. Furthermore, it is important to consider the osteodermal cover of the belly, the gill region and neck of all plagiosaurids as well as the large dermal pectoral girdle (Witzmann & Soler-Gijón, 2010; Witzmann, 2011). This may have adjusted the body density as well.

Mastodonsaurus giganteus has a heavily ossified and elongated trunk, a flat skull, dorsally oriented orbits, a propulsive tail and very small limbs (Schoch, 1999). These morphological adaptations for an aquatic lifestyle coincide with the bone histology (Sanchez *et al.*, 2010). Due to an increase in compactness, the bones get heavier, which may facilitates staying at the bottom (de Buffrénil *et al.*, 1990). In the

intercentrum, an extremely large amount of calcified cartilage preserved in the endochondral part (Fig. 8F) may also play a role as ballast in buoyancy (de Buffrénil *et al.*, 1990).

Cyclotosaurus intermedius is interpreted as a fully aquatic animal on the basis of its well known postcranial skeleton (Sulej & Majer, 2005). The long bone histology is not known for *Cyclotosaurus*. However, the remodelling process of its vertebral centra is very intense. In *Cyclotosaurus intermedius*, the primary trabeculae are thin; thicker, secondary trabeculae are observed in the deep cortex and in the endochondral domain. In *Cyclotosaurus* sp., osteosclerosis produced very compact bone without any cavities (Fig. 6C, E). It is not possible to conclude if the process is more dependent on individual variation or ontogenetic stage. The increase in bone mass seems to be an adaptation to a benthic mode of life similar to *Masodontosaurus*, and *Cyclotosaurus* may constitute its ecological equivalent in the Late Triassic.

PHYLOGENETIC SIGNAL

Based on the microstructure, the intercentra of the stereospondyl taxa can be clearly distinguished from the more basal taxa *Eryops* and *Trimerorhachis*. Among the Stereospondyli, the cortex is always well developed and highly vascularized, despite some variation of its thickness. The cortex with regularly arranged trabeculae can be distinguished from the endochondral region which is characterized by a chaotic network of trabeculae. As the study of Konietzko-Meier *et al.* (2013) has shown, the thickness of the cortex is variable in the same taxon depending directly on the ontogenetic stage. However, in the specimens analysed for this study, clear and stable taxon dependent growth pattern can be observed. Both specimens of *Gerrothorax*, which differ in geological age and locality (Table 1), have the same organization (Fig. 5C, F). The plasticity of the histological framework as a result of adaptations to a local environment, as it is observed in long bones of *Gerrothorax* (Sanchez & Schoch, 2013) or metoposaurids (Konietzko-Meier & Klein, 2013), does not play an important role in the organization of the intercentra. Intercentra seem to be more conservative compared to long bones and in their microanatomy and may possess an important taxonomical signal. An important characteristic among all stereospondyls is the slow ossification of the endochondral area. A large amount of calcified cartilage is preserved between the trabeculae in the endochondral region (Fig. 8F).

Contrary to this, in the intercentra of *Eryops* and *Trimerorhachis* calcified cartilage is only visible on

the dorsal side (Fig. 8E), it probably also covers the anterior and posterior articular surface, but never is preserved between the trabeculae.

Interestingly, the described histological framework seems to match established phylogenetic relationships (Schoch, 2013), except in the position of *Eryops*. As the study of Cubo *et al.* (2005) has shown, there are a lot of signals in the inner structure of bones, including a phylogenetic one. In order to clarify if the visible architecture is related to environment, mode of life or if it represents indeed a phylogenetic signal, it would be of interest to analyse a larger number of specimens.

ORIGIN OF PLAGIOSAURID VERTEBRAL CENTRA

Vertebrae of four plagiosaurid taxa have been studied and an uncommon feature has been found within *Gerrothorax* and *Plagiosaurus*. As already mentioned, the assignment of plagiosaurids vertebrae either as intercentrum, pleurocentrum or fusion of both remain yet unresolved (Hellrung, 2003). Based on the results derived from this study, two types of vertebral architecture can be discussed. In *Plagiosternum* and *Plagiosuchus*, the centrum consists of a ventro-lateral region of periosteal bone and a dorsal area of endochondral bone (Fig. 5A, K). As this histological structure has also been found in the other investigated temnospondyls, we suggest that the centrum consists only of the intercentrum. In contrast, in *Gerrothorax* and *Plagiosaurus*, periosteal bone is additionally present on the dorsal side of the intercentrum (Fig. 5C, F, H). A similar structure has already been described in squamates (de Buffrénil *et al.*, 2008; Houssaye *et al.*, 2010); however, the origin of Amniota vertebrae is pleurocentral. This specific structure has not been found in the intercentrum of temnospondyls. Independently of the sectioning plane, the bottom of the neural canal is always solely covered by endochondral bone (Konietzko-Meier *et al.*, 2013). This may suggest that the vertebral bodies of *Gerrothorax* and *Plagiosaurus* have a pleurocentral origin, as proposed by Panchen (1959). The presence of parapophyses on the vertebral body, however, confirms the intercentral origin of the whole body. The microstructure present here could be an indication of a fusion of large intercentrum and strongly reduced pleurocentra. The cortex which is present on the ventral and lateral side belongs to the intercentrum, whereas the dorsal part corresponds to the pleurocentrum. This would support Shishkin's suggestion of a fusion (1986, 1989). This hypothesis, however, should be tested with further specimens of different plagiosaurid taxa including ontogenetic series.

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