Bone histology and microanatomy of *Edaphosaurus* and *Dimetrodon* (Amniota, Synapsida) vertebrae from the Lower Permian of Texas

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Abstract

Here we describe the histology and microanatomy of vertebral centra of the iconic pelycosaur-grade synapsids *Edaphosaurus boanerges* and *Dimetrodon* spp. Vertebrae from different axial positions and, in the case of *Dimetrodon*, from different ontogenetic stages were selected. For the histological description, we produced histological petrographic thin sections ground to a thickness of 50–80 μm of the vertebrae in the sagittal and transversal cutting planes. After the preparation process, the thin sections were examined under transmitted and cross-polarized light in a polarized microscope. The analyzed vertebrae reveal similar bone tissues, where both taxa have cortical parallel-fibered bone (PFB). PFB and lamellar bone (LB) forms in the cancellous part. However, in juvenile *Dimetrodon*, woven-fibered bone (WFB) is also deposited and shows a high degree of vascularity. This suggests that *Dimetrodon* had slightly faster bone growth than *Edaphosaurus*, which is mainly made of PFB and LB and shows poorly developed vascular canals. In addition, one specimen of *Dimetrodon* displays the preservation of an ossified notochord, which can be assumed to be indicative of how the intervertebral tissues were developed. Historically, evidence of how the joint between *Dimetrodon* vertebral centra was built was lacking until this specimen appeared. If the notochord ran persistently through the vertebrae, it would have possibly increased the stiffness of the vertebral column and would have affected the limbs and locomotion. Furthermore, the organization of trabeculae and relative thickness of the vertebral cortex gives insights into how the animals were adapted to their habitat.

Keywords

basal synapsids, bone histology, growth pattern, microanatomy, Texas Permian red beds, vertebra

Abbreviations: FLB, fibrolamellar bone; iFLB, incipient fibrolamellar bone; IGPB, Goldfuβ Museum, Institute of Geosciences, Bonn; LAG, line of arrested growth; LB, lamellar bone; LZB, lamellar-zonal bone; PFB, parallel-fibered bone; WFB, woven-fibered bone.
INTRODUCTION

The “sail-backed lizards” Dimetrodon and Edaphosaurus are iconic early amniote fossils, mainly known from the Lower Permian of Texas, but also from other localities in North America and from Europe (Berman, Reisz, Martens, & Henrici, 2001; Fröbisch, Schoch, Müller, Schindler, & Schweiss, 2011; Reisz, 1986). The dorsal vertebrae have extremely elongated neural spines that in life were connected by a dermal “sail” (Romer & Price, 1940) which served in display (Huttenlocker, Mazierski, & Reisz, 2011; Shelton, 2015) and possibly thermoregulation (Bennett, 1996). Dimetrodon and Edaphosaurus traditionally were known as Pelycosauria but now are recognized to represent a grade, not a clade. Thus, they are commonly subsumed under the informal name “pelycosaurs” and, more properly, non-therapsid synapsids. Pelycosaurs can be divided into two major clades: Caseasauria and Eupelycosauria, where Caseasauria includes Eothyrididae and Caseidae, and Eupelycosauria are represented by Varanopidae, Ophiacodontidae, Edaphosauridae, and Sphenacodontidae (Chinsamy-Turan, 2012). Although varanopids have traditionally been classified as the most basal synapsids, a recent study has suggested that they are in fact diapsids (Ford & Benson, 2020). The sister clade to Sphenacodontidae is Therapsida, the “mammal-like reptiles.” The earliest known synapsids are the ophiacodonts Protoelepsydrops haplous (Carroll, 1964) and Archaeothyris florensis (Reisz, 1972) from the Late Carboniferous.

The Edaphosauridae were an important group of herbivorous tetrapods in the Late Carboniferous and especially the Early Permian and together with Diadectomorpha and Caseidae represent the earliest evolution of cellulose herbivory (Reisz & Fröbisch, 2014). This early assemblage of herbivores was replaced by therapsids and pareiasaurs in the Middle Permian. Fossils of edaphosaurids are known from North America and Europe. The elongate edaphosaur neural spines differ from those of sphenacodonts (Romer & Price, 1940) in having lateral tubercles. Four genera of edaphosaurids are currently recognized: Ianthasaurus, Glaucosaurus, Edaphosaurus, and Lupeosaurus (Benson, 2012; Mazierski & Reisz, 2010).

Sphenacodontids were the apex predators in their ecosystems. Fossilized specimens of this group were found in the Upper Carboniferous and Lower Permian of the United States and in Europe. The most famous and iconic sphenacodontid is Dimetrodon, which also had a sail formed by elongated neural spines. However, not every member of the clade featured this trait. Eleven genera are currently recognized in the sphenacodontid clade (Berman et al., 2001; Fröbisch et al., 2011). As described in detail by Romer and Price (1940), basal synapsid (“pelycosaur”) vertebral centra are generally deeply amphicoelous and notochordal, meaning the articular surfaces form an inwardly directed funnel structure and that the two funnel tips are connected by a foramen. The main vertebral body is the pleurocentrum (hereafter referred to as centrum), with small intercentra seen in the cervical vertebrae. Pelycosaur taxa differ mainly in proportions and in the shape of the ventral surface of the centrum. Whereas Edaphosaurus has a rounded ventral keel flanked by depressions, Dimetrodon centra are characterized by a sharp and deep keel. Edaphosaurus vertebrae tend to be shorter than their sphenacodontid counterparts (Romer & Price, 1940). The margins of the articular surfaces are wider and more rounded in Edaphosaurus than in Dimetrodon in which the endochondral surface is more restricted around the margin. However, it is unknown whether basal synapsids had evolved an intervertebral disk or what other type of intervertebral joint would have been present. In adult pelycosaurs, the neurocentral suture is fused and obliterated (Romer & Price, 1940). Presacral vertebral centra show little morphological variation from anterior to posterior, and it even may be difficult to distinguish dorsal from cervical centra if the neural arch and spine are missing (Romer & Price, 1940).

Despite the detailed knowledge of pelycosaur vertebral centrum morphology, little is known about their internal structure, microanatomy, and histology, and how morphogenesis relates to histology, although the first histological descriptions of pelycosaur bone tissues were provided by Enlow and Brown (1957). This limited knowledge is in contrast to that about the histology of the neural spines which have recently been studied in both Dimetrodon and Edaphosaurus (Huttenlocker et al., 2011; Huttenlocker, Rega, & Sumida, 2010). However, the skeletal elements histologically studied most are the large long bones of the limbs (Ričqlés, 1974a, 1974b, 1976a, 1976b, 1978; Enlow & Brown, 1957; Shelton, 2015; Shelton & Sander, 2017; Shelton, Sander, Stein, & Winkelhorst, 2012). Dimetrodon long bone histology has been described by Shelton et al. (2012) and Shelton (2015). That of Edaphosaurus was only recorded by Shelton (2015). Ricqlés (1974a, 1974b, 1976a, 1976b, 1978) provided the first histological inferences on the metabolism in selected pelycosaurs. Dental histological analysis was done in several basal synapsids and derived therapsids by LeBlanc (2016). Here we document the microanatomy and histological traits of the vertebral centrum structure and relate it to the histology of the other skeletal elements, considering implications for growth, life history, and metabolism.
1.1 | Background on fossil bone histology

Bone is a hard tissue principally formed by hydroxyapatite, and the apatite crystallite arrangement is preserved in the bone structure during fossilization, making the comparison with living animals possible. Bone histology can be studied in biology and paleontology to draw conclusions about the bone tissue and its fossilized remnants. Histological microanatomy describes the distribution of tissues in a bone. Fossil bone histology is examined in petrographic thin sections of bone in a polarized light microscope in normal light, cross-polarization, and λ filter (Padian & Lamm, 2013).

Bone histology is paleobiologically informative because local bone growth rate is indirectly tied to metabolic rate and records life history and growth of the animal. For instance, it is possible to calculate the age of an animal by counting the lines of arrested growth (LAGs) in the long bones (Padian & Lamm, 2013; Zhao, Benton, Sullivan, Sander, & Xu, 2013).

A bone such as a vertebral centrum is divided into two domains, the endochondral and the periosteal domain. In endochondral bone, a cartilaginous precursor is formed by chondroblasts (cartilage-producing cells) and functions as a scaffold-like structure (Francillon-Vieillot et al., 1990). This cartilage forms a three-dimensional network of trabeculae which is then lined by bone tissue through osteoblast activity. Soon after the onset of bone deposition, the cartilage precursor is eroded and replaced by bone matrix, retaining the spongy architecture (Francillon-Vieillot et al., 1990; Padian & Lamm, 2013).

The second domain is the periosteal domain which forms the cortex (Francillon-Vieillot et al., 1990, Padian & Lamm, 2013). Cortical bone is generally deposited centrifugally by an epithelium of bone-forming cells, the periosteum, around the bone shaft. The function of cortical bone is to provide a protective mantle and stability which the cancellous endochondral bone cannot offer. In cross-section, the bone cortex reveals the cyclical deposition mentioned above (Padian & Lamm, 2013). During periosteal bone formation, blood vessels and osteoblasts are incorporated into the bone tissue, forming vascular canals and osteocyte lacunae as non-mineralized areas in the bone. Vascular canal density is higher in juveniles than in adults (Francillon-Vieillot et al., 1990), and organization of bone crystallites increases with decreasing bone apposition rate, grading from woven-fibered bone (WFB) via parallel-fibered bone (PFB) to lamellar bone (LB). Highly organized crystallites mark the insertion fibers of connective tissue as thin collagenous bundles, known as Sharpey's fibers. Sharpey's fibers are obliquely aligned to the cortex growth direction and are independent of the cortical layering. Sharpey's fibers mark the insertion of ligaments and tendons that run down the vertebral column and connect adjacent vertebrae, for example, intercentral ligaments (Francillon-Vieillot et al., 1990).

As a bone expands during growth, the bone inner structure may undergo rebuilding, a process known as remodeling. Strong cortical remodeling is often associated with endothermy and large body size (Francillon-Vieillot et al., 1990). Bone resorbing cells (osteoclasts) resorb the primary bone tissue, either of endochondral or periosteal origin, followed by redeposition of bone on the walls of the resorption cavities. Thus, a centrifugal front of bone remodeling follows the bone apposition front (Mitchell & Sander, 2014). The resulting bone tissue is termed secondary bone. When resorption exceeds redeposition, a net loss of bone tissue occurs, commonly along a third front, the resorption front (Mitchell & Sander, 2014). Mainly in long bones, the resorption front may create a cavity inside the bone, the medullary cavity. The medullary cavity is filled with the bone marrow in the living animal but becomes filled by diagenetic minerals during fossilization. In vertebrae, the resorption front commonly is not distinct from the remodeling front and both together produce cancellous bone.

1.2 | Geological background on the Texas Lower Permian red beds

The Texas Lower Permian red beds are the oldest fluvial deposits that preserve abundant fossil terrestrial tetrapod remains, including diverse pelycosaurs, most prominently Dimetrodon and Edaphosaurus, and a host of temnospondyl amphibians, including the iconic Eryops (e.g., Reisz, 1997; Romer & Price, 1940; Sander, 1987, 1989). These classical faunas were extensively studied by leading American vertebrate paleontologists for a century, from about 1870 to 1970, including such illustrious names as E.D. Cope, S.W. Williston, E.C. Case, A.S. Romer, and E.C. Olson. The North Texas red beds are subdivided into several stratigraphic formations. The pelycosaur material studied by us originates from the Artinskian-aged (286.5–290.1 my) Nocona Formation (Lucas, 2018; Reisz & Fröbisch, 2014; Shelton et al., 2012). Traditionally, this formation has been known as Admiral Formation (Romer & Price, 1940), although formation boundaries differ somewhat between the two.

2 | MATERIAL AND METHODS

2.1 | Material

This study is based on 13 pelycosaur presacral vertebral centra consisting of two adult Edaphosaurus centra and
eight juvenile and three adult Dimetrodon centra from two different classical localities in the Nocona Formation. These are the Rattlesnake Canyon Bonebed (Sander, 1989) in northwestern Archer County and the Briar Creek Bonebed (Case, 1915) in western Archer County (Shelton, 2015). The fossils were excavated by one of the authors (P.M.S.) during two field seasons (2010 and 2011) at the Briar Creek Bonebed (Shelton, 2015) and surface collected in 2013 from the Rattlesnake Canyon Bonebed. Although it is difficult to assign isolated vertebrae to species, the Edaphosaurus material from Briar Creek shows no differences from E. boanergeres, the typical Nocona Formation edaphosaur. The Dimetrodon vertebrae from Briar Creek either pertain to D. limbatus (Romer & Price, 1940) or to the smaller D. natalis (Romer & Price, 1940). The latter species was recently validated histologically (Shelton et al., 2012). The Rattlesnake Canyon vertebrae are assigned to D. limbatus based on large size and stratigraphic position (Shelton, 2015). All vertebral centra were found in isolation, but all regions of the column are represented in the collection except for atlas and axis, sacral vertebrae and posterior caudals. The centra were identified to taxon, position in the vertebral column, and ontogenetic stage based on the criteria reviewed above. The neural arches of the adult centra are generally damaged, and the neural spines are broken off. The neural arches of the juveniles were not fused to the centra and thus are lacking. However, we selected centra that preserve sufficient morphology to assign them to position in the column, based on the descriptions in Romer and Price (1940). The fossils are accessioned to the collections of the Goldfuss Museum, Institute of Geosciences, University of Bonn, Bonn, Germany (acronym IGPB).

2.2 | Methods

The entire vertebral centra were embedded in epoxy resin (Araldite 2020) and were cured for 24 hours at room temperature. Next, the centra were cut with a precision rock saw (Isomet 4000) along the sagittal plane to obtain a sagittal section and along the transverse plane intersecting the growth center (marked by the notochordal foramen) to obtain a transverse section. Because of the lower sample number ($n = 2$) for Edaphosaurus, sagittal and transverse sections were cut from the same vertebrae. This means that either of the two section planes was not in its optimal location because the given thickness of the finished thin section and the saw cuts means that only one of these sections can intersect the center of growth. In Dimetrodon, each vertebra ($n = 11$) was either cut sagittally or transversally. After sectioning, the samples were glued to a petrographic glass slide and then cut apart from the epoxy resin block. Next, the sections were ground by hand with a slush of water and silicon carbide powder of decreasing grain sizes to a thickness of 50–80 μm (Padian & Lamm, 2013). The sections were finished with a cover slip, using UV glue cured for 5 min under UV light. Thereafter, the thin sections were examined for histological study in normal transmitted and cross-polarized transmitted light using a Leica DMLP polarized microscope configured with a 360° rotating stage. Images were taken with a Leica DFC420 color camera attachment and ImageAccess easyLab (Version 7) software. The microanatomical documentation and overview image of the sections were generated with a high-resolution scanner (EPSON V750) and were edited with Photoshop (Version CSS). Histological terminology follows Francillon-Vieillot et al. (1990).

3 | RESULTS

3.1 | Edaphosaurus adult

The centra of the specimens are dorsoventrally and laterally composed of a moderately thick layer of periosteal bone, which forms the cortex. The remaining volume of the centra is generally highly spongious with secondary trabeculae. The trabeculae of the endochondral bone form a dense cancellous network and are filled anteroposteriorly with longitudinal broad beams in the medullary region (Figure 1a–c). The transverse section reveals a honeycomb structure of secondary trabeculae in the periosteal domain (Figure 1c), indicating longitudinal orientation of the trabeculae in this region. The trabeculae are mostly made of PFB; LB forms only at the marginal surface of the trabeculae in thin layers (Figure 1d). A delimiting line which separates secondary cancellous bone from cortical bone in the periosteal domain is difficult to observe and possibly was obliterated in the bone remodeling process. Similarly, the boundary between the periosteal domain and the endochondral domain is poorly defined. However, it is clear that the endochondral domain contributes substantially to the bone tissue of the centra.

The primary periosteal bone of the cortex consists of PFB. The vascularization of the primary periosteal bone of the cortex is ventrally and ventrolaterally poorly developed and shows longitudinal vascular canals, that is, paralleling the body axis, as well as radial ones, that is, arranged in the direction of growth. Osteocyte lacunae appear highly concentrated in parts of the bone, for instance in the endosteal bone, which also features a dense network of canaliculi. The dorsal cortex forming the floor of the neural canal is avascular but is also rich in osteocyte lacunae. In the inner area of the cortical
FIGURE 1  Histological petrographic thin sections of adult *Edaphosaurus* centrum IGPB R-654. (a) Scanned sagittal (IGPB R-654b) and transverse (IGPB R-654a) sections. (b) Close-up of the medullary region with secondary trabeculae. (c) Close-up of transverse section. Note the honeycomb organization with PFB and LB. (d) Close-up of panel (b) in cross-polarization. LB is found on the marginal surface of a trabecula and is present along with PFB in the trabeculae. (e) Close-up of panel (a) with $\lambda$ filter. The arrow indicates ligament insertion fibers, that are preserved through the cortex. Anterior is to the left. LB, lamellar bone; PFB, parallel-fibered bone.
bone, there are erosion cavities that represent the remodeling front, transitioning the primary compact bone of the cortex into the secondary trabecular bone. Sharpey’s fibers representing ligament insertions are distinctive in the cortical bone of the anterior and posterior ends of the periosteal domain. The fibers are densely packed and obliquely angled to the bone surface in both specimens, paralleling the boundary between the periosteal and endochondral domains (Figure 1e).

### 3.2 Dimetrodon juvenile

The inner architecture of the *Dimetrodon* juvenile vertebrae is spongy. The distribution of endochondral and periosteal bone is similar in all specimens, with the former contributing substantially to the bone tissue of the centrum. The endochondral bone shows a dense organization of primary trabeculae (Figures 2b and 3a). In some specimens, these trabeculae are made of WFB which is deposited along with PFB (Figure 2b–d). Some specimens reveal preservation of chondrocytes in the growth zone of the primary trabeculae (Figure 2d). In older juveniles (e.g., IGPB R-653c), secondary trabeculae already have formed in the endochondral bone by remodeling, with the trabeculae consisting of PFB and LB, and some even consisting entirely of LB (Figure 2c,e). The boundary between periosteal and endochondral bone is distinguished by a sharp delimiting line made of LB. The medullary cavity is large in some specimens (e.g., IGPB R-657) and smaller or lacking in others; in these cases, the inner part of the periosteal domain consists of secondary trabeculae instead. The secondary trabeculae are generally coarser than the primary ones of the endochondral bone. Where present, the medullary cavity is lined by secondary LB (Figure 2e).

The cortex of all specimens is of periosteal origin and consists of PFB. The centra display a major dorsoventral asymmetry in the deposition of cortical bone; the ventral cortex is generally much thicker than in the dorsal one (Figures 2a and 4a). The thin dorsal cortex is an expression of the deep, anteroposteriorly elongate pocket on the floor of the neural canal which is typical for pelycosaurs (Romer & Price, 1940). All juvenile specimens display a highly vascularized cortex, with a predominantly radial arrangement of the vascular canals. It appears that the dorsal cortex of juveniles is also vascularized, but without the high density of the ventral cortex (Figure 4b). The vascular canals of the dorsal cortex are mostly arranged radially which can be seen especially well in the transverse sections.

There are high osteocyte lacuna concentrations in the cortex of several specimens. The organization of the osteocyte lacunae in juveniles is either an ordered deposit or diffuse (Figure 4c). Mineralized ligamental insertion fibers (Sharpey’s fibers) are already strongly developed in the juveniles, especially in the caudal centra and in one dorsal vertebra (Figure 4c). These fibers show the same oblique orientation relative to the bone surface as in *Edaphosaurus* and traverse the entire cortical bone.

### 3.3 Dimetrodon adult

The pattern of distribution of compact and cancellous bone, that is, the microanatomy, in the adult *Dimetrodon* is the same as in the juveniles, consisting of dominantly spongy bone and a tendency to dorsoventral asymmetry of the cortical bone. The endochondral bone retains the dense trabecular network of the juveniles (Figure 5a). The trabeculae are entirely secondary and are either made of PFB or sometimes LB (Figure 5c). In some specimens, a high osteocyte lacuna concentration is observed in the cancellous bone, but this feature seems to be randomly distributed. In the periosteal domain, secondary trabeculae are strongly developed and fill in the medullary region. Trabecular architecture is diffusely organized trabeculae, especially in the ventral part of the centra. The trabeculae dorsal to the growth center are less dense, possibly because the dorsal part of the centra is more flattened than the ventral part. The sharp delimiting line between the endochondral and periosteal domains remains distinctive, even after bone remodeling, and is entirely made of LB.

The periosteal cortical bone is thin, especially in the dorsal region, and mainly composed of PFB in a layered deposit. In general, the cortical bone is thinner than in juveniles. The cortical bone is made up of PFB which shows one or two LAGs separating zones. Because of the thinness of the cortex, a longer growth record is not preserved. Vascularity is poorly developed, even in the ventral part of the cortex (Figure 5b), but the vascular canals retain the radial orientation of the juveniles. Growth marks, for instance LAGs, are absent or difficult to observe. Osteocyte lacunae are well preserved and tend to follow a layered organization in the cortex, with an abundance of complex canalicular networks. Insertion fibers of ligamental origin are preserved in the cortex. They show the same depositional orientation as in the juveniles.

A peculiar feature is present in dorsal centrum IGPB R-652a from Rattlesnake Canyon. This feature is a bony tube of the diameter of the notochordal foramen. The tube is oriented longitudinally and extends through the growth center into the intervertebral space both anteriorly and posteriorly for a couple of millimeters. The tube is about 200 μm in diameter, with a wall thickness of about 80 μm and a thin bore of 40 μm. The wall is composed of PFB with osteocyte lacunae, several of which are elongated (Figure 5d,e). The wall of the tube appears fibrous, with the preservation of
FIGURE 2  Histological petrographic thin section of juvenile *Dimetrodon* (IGPB R-657). (a) Scanned sagittal section. (b) Close-up of the endochondral bone with $\lambda$ filter. (c) Close-up of panel (b), also with $\lambda$ filter. The image shows deposition of PFB and LB and implies an advanced resorption of primary bone. (d) Close-up of panel (b). Arrows indicate preserved remnants of chondrocytes. The whole bone area around the chondrocytes is the growth zone of WFB. (e) Close-up of panel (a) in cross-polarization. The medullary cavity is surrounded by LB at the endosteal margin of endochondral bone. Anterior is to the left. LB, lamellar bone; PFB, parallel-fibered bone; WFB, woven-fibered bone.
fibers along the anteroposterior direction. Possibly, the tube represents an ossified notochord.

4 | DISCUSSION

4.1 | Histological comparison of ontogenetic stages in Dimetrodon

The developmental histology of juvenile and adult Dimetrodon vertebrae is well documented by our samples, and the way the bone tissue has changed during ontogeny is clearly shown. First, the deposition of WFB and the occurrence of chondrocytes in juveniles indicates that the vertebral centra were still growing at the time of death of the animal. Thus, as the animal grew, the primary endochondral bone would have been resorbed and subsequently replaced by secondary trabeculae, which are either made of LB or PFB. It seems that at an early ontogenetic stage, the animal grew relatively fast, as evidenced by fast-growing tissue, both in the periosteal domain (evidenced by relatively high vascularity and the
radial arrangement of vascular canals) and in the endochondral domain (woven bone in the primary trabeculae). Shelton et al. (2012) identified incipient FLB (iFLB) in the long bones of *Dimetrodon* and suggested a slightly faster metabolism than in modern reptiles, which is consistent with the vertebral growth record in the juveniles studied by us. Furthermore, it appears that the growth rate of endochondral bone tended to be faster than that of the cortical bone, leading to a faster increase of the bone in length rather than in diameter until growth stopped when the animal reached adulthood.

The parallel-fibered periosteal cortex was deposited both in juvenile and adult specimens, but a decrease of vascularity is observable when the bone, and therefore the animal, reached maturity. These observations agree with those in the vertebrae of *Edaphosaurus* because of a similar low vascularity in adult *Edaphosaurus*. Sharpey’s fibers in the cortical bone occur more frequently in juveniles than in adult specimens.

*Figure 4*  
Histological petrographic thin section of juvenile *Dimetrodon* (IGPB R-652b). Panel (a) shows the scanned centrum in the sagittal plane. This centrum is highly spongious. (b) Close-up of the dorsal cortex. The dorsal cortex is thicker than in other specimens and shows vascular canals in a radial pattern. (c) Close-up of panel (a). The image shows the ventral cortex. The bone features an abundance of osteocyte lacunae and fibers. Anterior is to the left.
FIGURE 5  Histological petrographic thin sections of adult *Dimetrodon* centrum (IGPB R-652a). (a) Scanned sagittally (left) and transversely (right) sections of the same specimen. (b) Close-up of the cortex which is poorly vascularized, as is typical for adults. (c) Close-up of (a) with λ filter. The image provides a closer view of the development of secondary trabeculae made of PFB and fine bone lamellae. The entire vertebra is formed by this kind of secondary bone. (d) Close-up of panel (a) in cross-polarization. The possible ossified notochord is indicated by the red dotted line. The structure is made of PFB and distinctive fibers. (e) Close-up of (d) in normal light. Both arrows indicate elongated osteocyte lacunae. Anterior is to the left. LB, lamellar bone; PFB, parallel-fibered bone
4.2 | Bone growth

In both taxa, there clearly is a dominance of certain bone tissue types in the primary bone: PFB and LB in the endochondral and cortical bone. In early ontogenetic stages, WFB is primarily formed to sustain fast growth and provide a scaffold-like structure (Padian & Lamm, 2013). Because of the sparse and random distribution of fibrils, the mechanical strength of WFB is likely weak. Consequently, this bone tissue is often replaced by PFB and LB while the individual is still a juvenile. No FLB was found in any of the vertebral centra we studied, although Shelton et al. (2012) reported incipient FLB with incipient primary osteons for D. natalis limb bones from Briar Creek. Shelton and Sander (2017) found FLB in the limb bones of Ophiacodon (Marsh, 1878), a more basal relative of Edaphosaurus and Dimetrodon and described the histology as “mammal-like” or more derived than in any other pelycosaur taxon.

The incipient FLB reported by Shelton et al. (2012) is intermediate between FLB and lamellar-zonal bone (LZB). LZB is mostly found in modern reptiles and small mammal species (<10 kg), but seemingly true FLB evolved independently in modern mammals (>10 kg), extinct therapsids, archosaurs (Padian & Lamm, 2013), and plesiosaurs (Wintrich, Hayashi, Houssaye, Nakajima, & Sander, 2017). Crocodylians were also reported to produce FLB in their histology (Turmarkin-Deratzian, 2007), even as ectothermic animals, but these observations were not reproducible (Woodward, Horner, & Farlow, 2014).

Descriptions of Edaphosaurus long bone histology were given by Shelton (2015). He found PFB and LB and observed lower vascularity with longitudinal canals compared to other pelycosaurs. These observations agree with the findings in the centra. Shelton (2015) suggested low growth rates for Edaphosaurus based on the histology of its extremities and correlated this feature with its herbivorous diet, arguing that a high metabolic rate is not required by a plant-eating animal. Furthermore, Shelton (2015) compared Edaphosaurus long bone histology with that of Caseidae, earlier-branching herbivorous synapsids, which also have LB and low vascularity, but show even lower growth rates than Edaphosaurus (Shelton, 2015).

On the other hand, Dimetrodon may have evolved higher metabolic rates as a predatory animal. A faster metabolism may have conferred a selective advantage by allowing higher activity levels. It is possible that this correlation appeared early in synapsid evolution, already in the Late Carboniferous, when edaphosaurid species size increase stagnated at some point but Dimetrodon evolved larger species over time with higher metabolic rates (Brockehurst & Brink, 2017). Perhaps this situation reflects the predator–prey dynamics in the Late Carboniferous and Early Permian terrestrial ecosystem. In any case, our study of vertebral centra confirms the disparity in growth rates between herbivorous and carnivorous clades (Shelton, 2015).

Local bone apposition rates appear to have been lower in the vertebral centra of Dimetrodon compared to its long bones, which is suggested by the higher vascularity and incipient primary osteons of the latter (Shelton et al., 2012). However, these observations need proper quantification of vascularity to be sure that bone tissue was indeed deposited faster in the long bones than in the vertebral centra.

4.3 | Comparison with other amphicoelous vertebrae

Our study revealed that the endochondral domain contributed substantially, if not primarily, to the bone tissue of the amphicoelous centra of pelycosaurs. This is also seen in captorhinid reptiles, as nicely illustrated by LeBlanc, McDougall, Haridy, Scott, and Reisz (2018). The important contribution of endochondral bone to the vertebral centrum might seem counterintuitive in an amphicoelous centrum because an amphicoelous, funnel-shaped condition could simply be produced by a lack of endochondral bone formation. This is seen, for example, in the poorly ossified, funnel-shaped ends of amphibian limb bones (Francillon-Vieillot et al., 1990). Little endochondral contribution is also seen in the disk-shaped vertebral centra of all but the most basal ichthyosaurs (Houssaye, Nakajima, & Sander, 2018). However, basal ichthyosaur vertebral histology shows a greater contribution of the endochondral domain (Houssaye et al., 2018) and thus resembles the situation in pelycosaurs and early reptiles.

4.4 | Possible ossified notochord

The notochord is an elementary structure in the evolution of the axial skeleton and defines the phylum Chordata. In morphology, the notochord is a rod-like structure with high flexibility provide by a material similar to cartilage (Romer, 1956; Stemple, 2005). The notochord is located dorsally in the body, but ventrally to the neural canal. The notochord serves three functions in the embryonic stage of higher vertebrates: First, the notochord provides signals for the neighboring tissues as a midline tissue. Second, it is responsible for muscle
attachments during development and third, it plays the role as vertebral column precursor. Thus, the embryonic formation of the vertebral column begins with a cartilaginous structure extending down the notochord which subsequently ossifies. During further development, the vertebral centra form around the notochord as hollow bony shells with a small axial opening, the notochord foramen (Romer, 1956), as seen in the pelycosaurs studied here. In vertebrae that lack a notochord foramen, the cavity left by the notochord closes up in the next stage of development while the notochord is constricted between the vertebral segments. In the last stage, however, the notochord persists as a part of the intervertebral disk, known as the nucleus pulposus (Stemple, 2005). Intervertebral joints of the synovial ball-and-socket type completely lack notochordal tissue.

The possible preservation of an ossified notochord in specimen IGPB R-652a may provide evidence of how the intervertebral tissues in a derived pelycosaur developed. Due to its position, the bony tube is best interpreted as the remnant of the notochord, which ossified during adulthood in this Dimetrodon individual. Although the amphicoelous vertebrae were already described in detail by Romer and Price (1940), these authors did not mention any feature resembling what we observed in IGPB R-652a. This suggests that the thin bony tube is either a very rare feature or that it normally was destroyed by weathering and preparation of the fossil.

Examining the vertebrae of Dimetrodon, the whole morphology suggests a stiff vertebral column (Gadow, 1933) caused by the vertebral arrangement and the development of elongated neural spines which carry the sail. An ossified notochord extending through the vertebrae could have supported the stiffness of the column which would not have been involved much in locomotion by lateral undulation (different from the plesiomorphic tetrapod condition). This would be in agreement with an increasing importance of the limbs in locomotion, consistent with the distinctly longer limbs of sphenacodontids compared to edaphosaurs (Romer & Price, 1940).

Judging from its histology, the vertebra showing the bony tube belonged to an adult animal. It is odd that this vertebra is the only specimen revealing a preserved possible notochord, and no tube was discovered in any other specimens. A genetic mutation or pathology causing the ossification are possible explanations as well because in coelacanth fishes, the notochord persists through adulthood and ossifies (Griffith et al., 1975; Symmons, 1979). This fact might suggest that vertebra IGPB R-652a was from a senescent individual, but ontogenetic stage estimation of isolated vertebrae is currently not possible.

If the bony tube indeed represents an ossified notochord, it suggests that the notochord persisted in pelycosaurs in general, as also suggested by the notochord foramen. Some gekkonid lizards and Sphenacodontidae have amphicoelous vertebrae, similar to the vertebral anatomy of Dimetrodon, with a complete notochord persisting through adulthood (Winchester & Bellairs, 1977). However, in these taxa, the notochord is constricted by the centra and expanded in the intervertebral space, different from what is suggested by the bony tube showing a uniform diameter. Clearly, a search of collections for more material showing a bony tube should confirm the existence of a persisting notochord in sphenacodonts and in any other pelycosaur taxa.

5 | CONCLUSIONS

The histological analysis of the vertebrae of Edaphosaurus and Dimetrodon provides baseline data on the growth and morphogenesis of the axial skeleton of these iconic early non-therapsid synapsids. Whereas both taxa show a similar centrum morphology, and their histology has a similar developmental origin, the centra are different in histology and in microanatomy, especially in trabecular organization and vascularity. Adult periosteal and endochondral bone is composed of PFB and LB in both taxa, but juvenile Dimetrodon also show WFB. Periosteal bone in all centro is concentrically deposited on the dorsoventral and lateral surfaces and consists of PFB. Microanatomy differs between both taxa in the structural development of secondary trabeculae and in vascularity. Additional differences are the sharp delimiting line which separates the endochondral domain from the periosteal domain in Dimetrodon, whereas the determination of the delimiting line is difficult in Edaphosaurus.

The vertebral histology provides clues about the growth rate, suggesting that Edaphosaurus vertebrae grew distinctly more slowly than those of Dimetrodon. This is consistent with previous observations that Edaphosaurus had slow growth in limb bones and in total in body size, whereas Dimetrodon had a higher growth rate than in any other pelycosaur, except Ophiacodon (Shelton, 2015; Shelton & Sander, 2017). These differences may have been related to the mode of life of the herbivorous Edaphosaurus versus the predatory Dimetrodon.

It thus is remarkable that already early relatives of mammal-like reptiles such as Dimetrodon evolved an elevated metabolism, despite the primitive amphicoelous notochordal condition observed in the vertebrae. If the unique bony tube observed in one Dimetrodon centrum were to represent an ossified notochord, this would suggest a stiff vertebral column, possibly indicating a stronger contribution of the limbs to locomotion.
Comparing histology and microanatomy, such as trabecular architecture of vertebral material, of recent taxa with those of Edaphosaurus and Dimetrodon would further improve our understanding of how the pelycosaurs were adapted to their habitat and possibly our inferences about their behavior as well as help in understanding interactions between different pelycosaurs.

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REFERENCES
Bennett, S. C. (1996). Aerodynamics and thermoregulatory function of the dorsal sail of Edaphosaurus. Paleobiology, 22, 496–506.
Benson, R. B. (2012). Interrelationships of basal synapsids: Cranial and postcranial morphological partitions suggest different topologies. Journal of Systematic Palaeontology, 10, 601–624.
Berman, D. S., Reisz, R. R., Martens, T., & Henrici, A. C. (2001). A new species of Dimetrodon (Synapsida: Sphenacodontidae) from the lower Permian of Germany records first occurrence of genus outside of North America. Canadian Journal of Earth Sciences, 38, 803–812.
 Brocklehurst, N., & Brink, K. S. (2017). Selection towards larger body size in both herbivorous and carnivorous synapsids during the Carboniferous. Facets, 2, 68–84.
Carroll, R. L. (1964). The earliest reptiles. Zoological Journal of the Linnean Society, 45, 61–83.
Case, E. C. (1915). The Permocarboniferous red beds of North America and their vertebrate fauna. Publication 207 (pp. 1–176). Washington, DC: The Carnegie Institution of Washington.
Chinsamy-Turan, A., (Ed.) (2012). Forerunners of mammals: Radiation, histology, biology. Bloomington: Indiana University Press.
Enlow, D. H., & Brown, S. O. (1957). A comparative histological study of fossil and recent bone tissues. Part II. Reptilian and bird bone tissues. The Texas Journal of Science, 9, 186–204.
Ford, D. P., & Benson, R. B. J. (2020). The phylogeny of early amniotes and the affinities of Parareptilia and Varanopidae. Nature Ecology & Evolution, 4, 57–65.
Francillon-Vieillot, H., de Buffrénil, V., Castanet, J. D., Géraudie, J., Meunier, F. J., Sire, J. Y., ..., de Ricqlès, A. J. (1990). Microstructure and mineralization of vertebrate skeletal tissues. Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends. (Vol. I, pp. 175–234) New York: Van Nostrand Reinhold.
Fröbisch, J., Schoch, R. R., Müller, J., Schindler, T., & Schweiss, D. (2011). A new basal sphenacodontid synapsid from the latest carboniferous of the Saar-Nahe Basin, Germany. Acta Palaeontologica Polonica, 56, 113–120.
Gadow, H. F. (1933). The evolution of the vertebral column. A contribution to the study of vertebrate phylogeny (pp. 1–356). Cambridge: Cambridge University Press.
Griffith, R. W., Mathews, M. B., Umminger, B. L., Grant, B. F., Pang, P. K., Thomson, K. S., & Pickford, G. E. (1975). Composition of fluid from the notochordal canal of the coelacanth, Latimeria chalumnae. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 192, 165–171.
Houssaye, A., Nakajima, Y., & Sander, P. M. (2018). Structural, functional, and physiological signals in ichthyosaur vertebral centrum microanatomy and histology. Geodiversitas, 40, 161–171.
Huttenlocker, A. K., Mazierski, D., & Reisz, R. R. (2011). Comparative osteohistology of hyperelongate neural spines in the Edaphosauridae (Amniota: Synapsida). Palaeontology, 54, 573–590.
Huttenlocker, A. K., Rega, E., & Sumida, S. S. (2010). Comparative anatomy and osteohistology of hyperelongate neural spines in the sphenacodontids Sphenacodon and Dimetrodon (Amniota: Synapsida). Journal of Morphology, 271, 1407–1421.
LeBlanc, A. R. H. (2016). Amniote dental histology, development, and variation: Perspectives from the fossil record. (PhD Dissertation). University of Alberta, Edmonton.
LeBlanc, A. R. H., McDougall, M. J., Haridy, Y., Scott, D., & Reisz, R. R. (2018). Caudal autotomy as anti-predatory behaviour in Palaeozoic reptiles. Scientific Reports, 8, 3328.
Lucas, S. G. (2018). Permian tetrapod biochronology, correlation and evolutionary events. Geological Society, London, Special Publications, 450, 405–444.
Marsh, O. C. (1878). Notice of new fossil reptiles. American Journal of Science, 89, 409–411.
Mazierski, D. M., & Reisz, R. R. (2010). Description of a new specimen of Ianthasaurus hardei Storion (Eupelycosauria: Edaphosauridae) and re-evaluation of edaphosaurid phylogeny. Canadian Journal of Earth Sciences, 47, 901–912.
Mitchell, J., & Sander, P. M. (2014). The three-front model: A developmental explanation of long bone diaphyseal histology of Sauropoda. Biological Journal of Linnean Society, 112, 765–781.
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Padian, K., & Lamm, E. T. (2013). Bone histology of fossil tetrapods: Advancing methods, analysis, and interpretation (pp. 1–285). Berkeley: University of California Press.

Reisz, R. R. (1972). Pelycosaurian reptiles from the Middle Pennsylvanian of North America. Bulletin of the Museum of Comparative Zoology, 144, 27–62.

Reisz, R. R. (1986). Encyclopedia of paleoherpetology. Part 17A: Pelycosaurs. Stuttgart: Gustav Fischer Verlag.

Reisz, R. R. (1997). The origin and early evolutionary history of amniotes. Trends in Ecology & Evolution, 12, 218–222.

Reisz, R. R., & Fröbisch, J. (2014). The oldest caseid synapsid from the Late Pennsylvanian of Kansas, and the evolution of herbivory in terrestrial vertebrates. PLoS One, 9, e94518.

Ricqlès, A. de. (1974a). Paleohistological research on the long bones of tetrapods IV: Eotheriodonts and pelycosaurs. Annales de Paléontologie, 60, 3–39.

Ricqlès, A. de. (1974b). Evolution of endothermy: Histological evidence. Evolutionary Theory, 1, 51–80.

Ricqlès, A. de. (1976a). Recherches paléohistologiques sur les os longs des Tétrapodes: VII. Sur la classification, la signification fonctionnelle et l’histoire des tissus osseux des Tétrapodes. Deuxième partie. Annales de Paléontologie, 62, 71–126.

Ricqlès, A. de. (1976b). On bone histology of fossil and living reptiles, with comments on its functional and evolutionary significance. Linnean Society Symposium Series, 3, 123–149.

Ricqlès, A. de. (1978). Recherches paléohistologiques sur les os longs des tétrapodes: VII. Sur la classification, la signification fonctionnelle et l’histoire des tissus osseux des tétrapodes. Troisième partie. Annales de Paléontologie, 64, 153–176.

Romer, A. S. (1956). Osteology of the reptiles (pp. 1–772). Chicago: University of Chicago Press.

Romer, A. S., & Price, L. I. (1940). Review of the Pelycosaurs. Special Papers, Geological Society of America, 28, 1–538.

Sander, P. M. (1987). Taphonomy of the Lower Permian Geraudine bonebed in Archer County, Texas. Palaeogeography, Palaeoclimatology, Palaeoecology, 61, 221–236.

Sander, P. M. (1989). Early Permian depositional environments and pond bonebeds in central Archer County, Texas. Palaeogeography, Palaeoclimatology, Palaeoecology, 69, 1–21.

Shelton, C. D. (2015). Origins of endothermy in the mammalian lineage: the evolutionary beginning of fibrolamellar bone in the “mammal-like” reptiles. (Ph.D. Dissertation). University of Bonn, Bonn. http://hss.ulb.uni-bonn.de/2015/4054/4054.htm

Shelton, C. D., & Sander, P. M. (2017). Long bone histology of Ophiacodon reveals the geologically earliest occurrence of fibrolamellar bone in the mammalian stem lineage. Comptes Rendus Palevol, 16, 397–424.

Shelton, C. D., Sander, P. M., Stein, K., & Winklerhorst, H. (2012). Long bone histology indicates sympatric species of Dimetrodon (Lower Permian, Sphenacodontidae). Earth and Environmental Science Transactions of the Royal Society of Edinburgh, 103, 217–236.

Stemple, D. L. (2005). Structure and function of the notochord: An essential organ for chordate development. Development, 132, 2503–2512.

Symmons, S. (1979). Notochordal and elastic components of the axial skeleton of fishes and their functions in locomotion. Journal of Zoology, 189, 157–206.

Tumarkin-Deratzian, A. R. (2007). Fibrolamellar bone in wild adult Alligator mississippiensis. Journal of Herpetology, 41, 341–345.

Winchster, L., & Bellairs, A. A. (1977). Aspects of vertebral development in lizards and snakes. Journal of Zoology, 181, 495–525.

Winrich, T., Hayashi, S., Houssaye, A., Nakajima, Y., & Sander, P. M. (2017). A Triassic plesiosaurian skeleton and bone histology inform on evolution of a unique body plan. Sciences Advances, 3, e1701144.

Woodward, H. N., Horner, J. R., & Farlow, J. O. (2014). Quantification of intraskeletal histovariability in Alligator mississippiensis and implications for vertebrate osteohistology. PeerJ, 2, e422.

Zhao, Q., Benton, M. J., Sullivan, C., Sander, P. M., & Xu, X. (2013). Histology and postural change during the growth of the ceratopsian dinosaur Psittacosaurus lufijiensis. Nature Communications, 4, 2079.

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