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Structural, functional, and physiological signals in ichthyosaur vertebral centrum microanatomy and histology

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ABSTRACT

The first tuna-shaped amniotes evolved among ichthyosaurs, but this group exhibits in fact a wide diversity of morphologies and swimming modes. The histology and microanatomical features of vertebral centra of a diversity of ichthyosaur taxa from most basal to highly derived illustrating this variability were analyzed. The occurrence of unusual parallel fibered bone with platings of true parallel-fibered bone confirms high growth rate in all these taxa. Ichthyosaur vertebrae, which are deeply amphicoelous, show a limited endosteal territory associated with a limited growth in length. No bone mass increase nor decrease occurs. The vertebral centrum is spongious, and two microtypes are observed in the periosteal territory, with different degrees of organization of the trabecular network. The microtypes appear to be associated with the shape of the vertebral centrum, the organization of the spongiosa becoming homogeneous in the disk-shaped centra of cymbospondylids and Neoichthyosauria, rather than much more heterogeneous in spool-shaped centra of primitive Triassic forms. As opposed to what was previously suggested in other amniotes, the main switch in microanatomical organization appears thus to be correlated to the acquisition of deeply amphicoelous disk-like vertebral centra rather than to a shift in swimming mode from long and slender-bodied anguilliform swimmers to thunniform swimmers.

KEY WORDS
Ichthyosaur,
vertebral centrum,
histology,
microanatomy,
microtype,
amphicoelous.

RÉSUMÉ

Signaux struturel, fonctionnel et physiologique dans la microanatomie et l'histologie du centrum vertébral des ichtyosaures.

Les premiers amniotes thunniformes ont évolué au sein des ichtyosaures, mais ce groupe présente en fait une large diversité de morphologies et de modes de nage. Les caractéristiques histologiques et microanatomiques du centrum vertébral de divers taxons d'ichtyosaures, des plus basaux aux plus dérivés, et illustrant cette variabilité, ont été analysées. La présence d'os à fibres parallèles inhabituel avec des placages d'os à fibres parallèles vrai confirme un taux de croissance élevé chez ces taxons. Les vertèbres d'ichtyosaures, qui sont très amphicoèles, montrent un territoire endostéal réduit associé avec une croissance en longueur limitée. Il n'y a ni augmentation, ni réduction de la masse des os. Le centrum vertébral est spongieux et deux microtypes sont observés dans le territoire périostique, avec des degrés divers d'organisation du réseau trabéculaire. Les microtypes semblent associés à la forme du centrum vertébral, l'organisation de la spongiosa devenant homogène dans les centrums en forme de disques des cymbospondylidés et des Neoichthyosauria, plutôt que bien plus hétérogène dans les centrums en forme de bobines des formes primitives du Trias. Contrairement à ce qui était précédemment suggéré chez les autres amniotes, le principal changement dans l'organisation micro-anatomique apparait donc corrélé à l'acquisition de centrums vertébraux très amphicoèles et en forme de disques plutôt qu'à un changement dans le mode de nage entre les nageurs anguilliformes à corps long et mince et les nageurs thunniformes.

MOTS CLÉS
Ichtyosaure,
centrum vertébral,
histologie,
microanatomie,
microtype,
amphicoelie.

INTRODUCTION

Ichthyosaurs were one of the most successful groups of Mesozoic marine reptiles. They are the first amniote group that developed a thunniform mode of swimming, i.e., with propulsion only provided by a tail fluke (Sander 2000; McGowan & Motani 2003; Motani 2009). The strong morphological convergence between derived ichthyosaurs (Neoichthyosauria) and modern dolphins and lamnid sharks underlies their high degree of adaptation to a fully open-marine lifestyle. However, ichthyosaurs in fact display a wide range of morphologies illustrating diverse ecological grades, from early forms with a long and slender body and a long and straight tail (cf. *Utatsusaurus*) that were probably anguilliform swimmers (Motani *et al.* 1996), to thunniform tuna-shaped forms with an upright bilobate tail on a narrow peduncle (cf. *Ichthyosaurus*; McGowan & Motani 2003).

Ichthyosaur vertebrae display a neural arch and a centrum that are not fused. Vertebral centra are deeply amphicoelous (i.e., biconcave). Slightly elongated in primitive forms, they are particularly short (disk-like) in tuna-like forms (Sander 2000; Motani 2005).

Vertebral microanatomical features (i.e., the amount and distribution of the osseous tissue in the bone) have revealed themselves as encompassing an ecological signal and can be used to make paleoecological inferences about swimming abilities (Houssaye & Bardet 2012; Dumont *et al.* 2013; Houssaye 2013; Houssaye *et al.* 2014a). Previous microanatomical and histological studies on ichthyosaurs analyzed mainly limb bones and ribs (e.g. Buffrénil *et al.* 1987; Buffrénil & Mazin 1990; Kolb *et al.* 2011; Talevi *et al.* 2012; Talevi & Fernández 2012; Nakajima *et al.* 2014; Houssaye *et al.* 2014b). Only a few old studies described the internal structure of vertebrae (e.g. Kiprijanoff 1881; Fraas 1891; Seitz 1907). The aim of this study is to analyze in more detail the vertebral centrum microanatomical patterns encountered in ichthyosaurs and notably to see if there is any variation between the primitive and the most derived forms.

MATERIAL AND METHODS

MATERIAL

The material analyzed for this study consists of cervical and dorsal vertebral centra of various ichthyosaurs (see Table 1) showing distinct morphologies and representing a wide range of taxa, from basal to derived ichthyosaurs (Fig. 1). These morphologies thus illustrate various modes of adaptation to an aquatic life (especially swimming modes). Some of the centra derive from articulated material (NSM PV 23854, LACM DI 158109, Nevada C, PIMUZ T 1296, PIMUZ T 2004), while others were found in isolation (Nevada A, B, D, the Jurassic specimens).

Isolated ichthyosaur vertebral centra are relatively common in the fossil record, as compared to isolated long bones for example, and, as such, are of particular interest for comparative studies, especially destructive ones. Vertebral centra from different regions along the vertebral column vary in form and, especially, in the position of the facets for rib articulation (diapophyses and parapophyses), making it possible to approximately assign an isolated vertebral centum to a given region (McGowan & Motani 2003).

In the Triassic specimens in this study, assignment to taxon was based on diagnostic features in the skeletons and isolated vertebrae as well as on stratigraphic age. Centra that are sub-equal in length and height or longer than high are restricted to ichthyosaurs more basal than *Cymbospondylus* Leidy, 1868, i.e., Grippioidea. Grippioid centra are also small, not exceeding
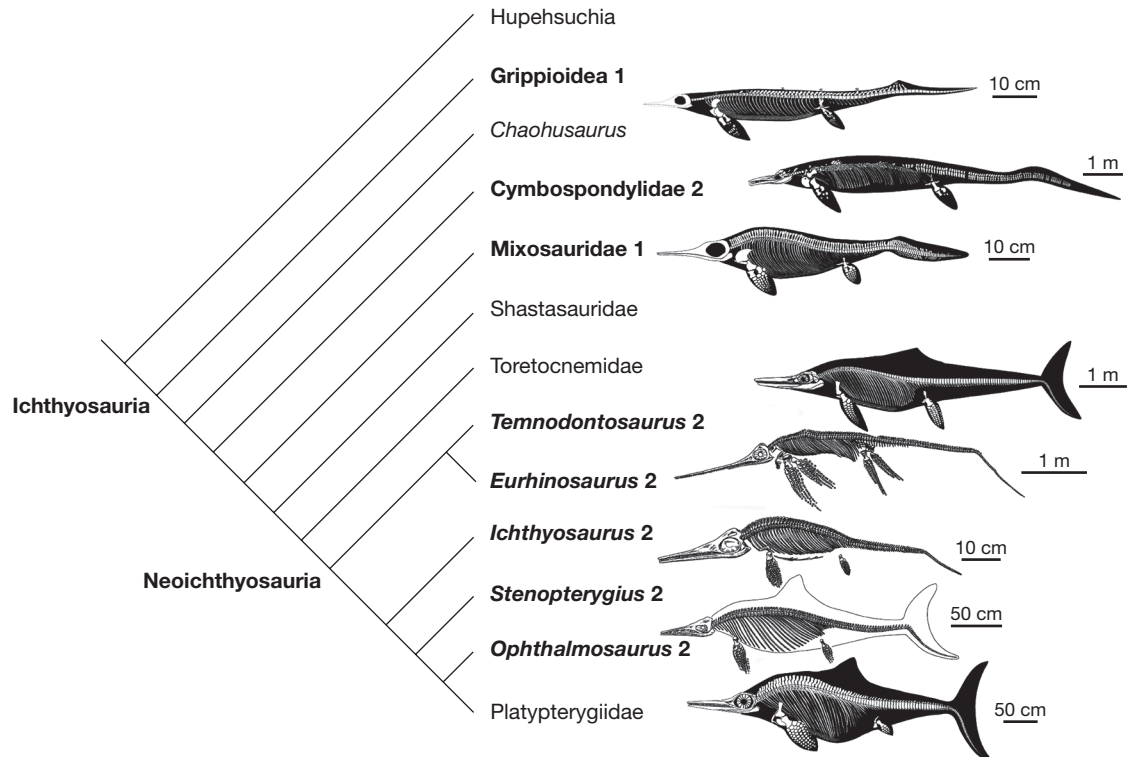
Fig. 1. — Consensus phylogenetic tree of Ichthyopterygia including (in bold) the taxa sampled for this study; modified from Ji *et al.* (2016); with associated silhouettes (from McGowan & Motani 2003) and label of the microanatomical type encountered.

4 cm in height. We assigned vertebrae meeting these criteria and being Early Triassic in age to this taxon (Table 1). There were also two isolated Middle Triassic vertebrae in our sample (Nevada B, D) that are elongate like the Early Triassic ones. Since they neither pertain to Cymbospondylidae nor to Mixosauridae, we also assigned them tentatively to Grippioidea. Nevada C can confidently be assigned to *Cymbospondylus* based on the anterior truncation of the single slanted rib articular facet in the dorsals (Sander 1992), large size, and Anisian age.

The Toarcian specimens from the SMNS collections we sectioned lack diagnostic features at the generic level but vary in size. We used the identifications provided to us in the early 1990s by the former curator of the marine reptile collection at the SMNS, Dr Rupert Wild. He presumably identified the vertebral material in comparison with complete skeletons, using the taxonomy available at the time. Thus, the material he identified as *Stenopterygius* Jaekel, 1904 could potentially also include the much rarer taxon *Hauffiopteryx* Maisch, 2008 which was described later (Maisch 2008).

METHODS

Most vertebral centra were physically sectioned using standard techniques along their mid-sagittal and neutral transverse planes (see Houssaye *et al.* 2008). The latter was easily identified as the one cutting the centrum core (point of minimal centrum length). In addition, conventional high-resolution computed tomography (GEphoenix|X-ray v|tome|xs 180 and 240; reconstructions performed using datox/res software) was used at the Steinmann Institute, University of Bonn (Germany) for the

Lower Triassic vertebral centra NMNS uncat. because of their rarity. Image segmentation and visualization were performed using VGStudioMax 2.2 (Volume Graphics Inc., Heidelberg, Germany). Scans of the physical thin sections were performed at high resolution (i.e., between 6400 and 12800 dpi) using an Epson V750-M Pro scanner. Centrum height and width were measured directly on the sagittal sections. The percentage of endochondral bone (%E), calculated as the ratio between the surface occupied by bone of endochondral origin and the total sectional area, was calculated based on the scans of the sagittal sections.

RESULTS

MICROANATOMICAL ORGANIZATION

Sagittal sections are hourglass-shaped (Fig. 2). The periosteal (resulting from periosteal [centrigugal] ossification, at the outer surface of the bone) and endochondral (resulting from [centripetal] endochondral ossification, i.e., the substitution of preformed cartilage [being destroyed] by bony tissue) territories can clearly be identified: like in all amniote vertebral centra, the two cones of periosteal bone extend from the center of growth dorsally and ventrally, whereas the two cones of endochondral bone extend anteriorly and posteriorly (see e.g. Buffrénil *et al.* 2008). Not surprisingly, both territories are spongious (Figs 2, 3), though with a distinct organization (see below). Thus, all of the studied ichthyosaur vertebral centra lack compact bone. The periosteal territory occupies most of

Table 1. — List of the material analysed for the current study, in phylogenetic order. Abbreviations: **Pos**, Position in vertebral colum; **C**, cervical vertebra; **D**, dorsal vertebra; **aD**, anterior dorsal vertebra; **pD**, posterior dorsal vertebra; **J**, juvenile; **SS**, sagittal section; **TS**, transverse section; **CH**, centrum height; **%E**, relative percentage of endochondral bone; **L/H**, length over height; **MT**, microanatomical type; **LACM**, Natural History Museum of Los Angeles County; **NSM**, National Museum of Nature and Science, Tokyo; **PIMUZ**, Paläontologisches Institut und Museum, Universität Zürich, Switzerland; **SMNS**, Staatliches Museum für Naturkunde Stuttgart, Germany. Symbol: *, intermediate between 1 and 2.

Taxon	Coll. nb.	Locality/Stratigraphy	Pos.	SS	TS	CH	%E	L/H	MT
Grippioidea	NSM PV 23854	Zhitkov Formation, Neocolumbites zone, middle Spathian, Lower Triassic, Zhitkov Cape, South Primorye, Russia	D	×	×	22.9	–	1.07	1
Grippioidea, same individual as above	NSM PV 23854	Zhitkov Formation, Neocolumbites zone, middle Spathian, Lower Triassic, Zhitkov Cape, South Primorye, Russia	D	×	×	24.2	–	0.82	1
Grippioidea – Nevada A	LACM uncat.	Tobin Formation, Subcolumbites zone, late Spathian, Lower Triassic, Augusta Mountains, Nevada, USA	?	×	×	28.0	49.0	0.74	1
?Grippioidea – Nevada B	LACM uncat.	Fossil Hill Member, Favret Formation, Anisian, Middle Triassic, Favret Canyon, Augusta Mountains, Nevada, USA	?	×	×	28.3	–	1.20	1
?Grippioidea – Nevada D	LACM uncat.	Fossil Hill Member, Favret Formation, Anisian, Middle Triassic, Favret Canyon, Augusta Mountains, Nevada, USA	?	×	×	33.5	–	0.73	1
?Cymbospondylidae new taxon A, mother	LACM DI 158109	upper part of Fossil Hill Member, Favret Formation, Anisian, Middle Triassic, Favret Canyon, Augusta Mountains, Nevada	D	×	×	42.0	–	0.56	2
?Cymbospondylidae new taxon A, fetus	LACM DI 158109	upper part of Fossil Hill Member, Favret Formation, Anisian, Middle Triassic, Favret Canyon, Augusta Mountains, Nevada	D	×	×	14.4	–	0.62	*
Cymbospondylus sp. – Nevada C	LACM uncat.	upper part of Fossil Hill Member, Favret Formation, Anisian, Middle Triassic, Favret Canyon, Augusta Mountains, Nevada	D	×	×	89.0	–	0.47	2
Mixosaurus sp.	PIMUZ T 2004	Besano Formation, latest Anisian, Middle Triassic, Monte San Giorgio, Ticino, Switzerland	?	×	×	9.0	34.4	0.66	1
Mixosaurus sp.	PIMUZ T 1296	Besano Formation, latest Anisian, Middle Triassic, Monte San Giorgio, Ticino, Switzerland	?	×	×	–	–	–	1
Temnodontosaurus sp.	SMNS uncat.	Posidonienschiefer Formation, Toarcian, Lower Jurassic, Holzmaden area, Germany	C?	×	×	69.0	19.0	0.67	2
Eurhinosaurus sp.	SMNS 50913	Posidonienschiefer Formation, Toarcian, Lower Jurassic, Zell unter Aichelberg, Germany	pD	×	×	74.0	21.0	0.50	2
Ichthyosaurus sp.	SMNS 13080	Lias, Hettangian or Sinemurian, Lower Jurassic, Lyme Regis, England	pD	×	×	32.0	38.9	0.60	2
small thunnosaurian ichthyosaur, probably *Stenopterygius*	SMNS uncat. SMNS uncat. SMNS uncat.	Posidonienschiefer Formation, Toarcian, Lower Jurassic, Holzmaden area, Germany	C, J ? ?	× – ×	× × –	19.0 – 52.0	45.2 – 36.1	0.51 – 0.50	2 2 2
Ophthalmosaurus sp.	SMNS uncat.	Oxford Clay, Callovian, Middle Jurassic, England	aD	×	×	79.0	24.6	0.48	2

the section. The relative proportion of the endochondral territory varies according to taxon (from 19 to 49%; see %E in Table 1). The transverse sections essentially correspond to the periosteal territory because they were cut through the center of the centrum. However, paired cone-shaped territories spread from the centrum core towards the facets for articulation with the neural arch and the ribs, similar to what is observed in shark vertebral centra (Romer 1961: 172, fig. 99).

Two main organizations can be observed: a) Microtype 1. In these vertebral centra (see Table 1; Fig. 2A-D), the spongiosa in the periosteal territory is not homogeneous and intertrabecular spaces are relatively large. Trabeculae are not well organized despite a roughly dominantly radial and sagittal orientation in transverse and sagittal sections, respectively; trabeculae are of various thicknesses so that intertrabecular spaces are randomly sized and shaped; and b) Microtype 2. In these vertebral centra the trabeculae are essentially oriented along the sagittal plane. The difference in main orientation in the two microtypes is obvious in transverse section where the radial orientation of the trabeculae in microtype 1 is replaced by a circumferential one in microtype 2. In this microtype, the spongiosa is relatively homogeneous with fairly regularly dorsoventrally spaced thin trabeculae and reduced intertrabecular

spaces. In longitudinal section, the endochondral territory is strictly limited to the anterior and posterior borders of the centrum, i.e., its intervertebral articular surfaces (Fig. 2G). In transverse section, the inner organization of the vertebral centra clearly evokes a honeycomb (Fig. 2H).

Although there seems to be a size effect in this microtype distinction (significant correlation when only adult specimens are taken into consideration – but small sample size), the largest vertebral centra showing the highest degree of organization, it is clearly not the unique factor. Indeed, the *Ichthyosaurus* sp. (as in Fig. 2F-G) and LACM uncat. Grippioidea indet. Nevada D (Fig. 2C, D), despite showing a similar centrum height, clearly show distinct microanatomical organizations, suggesting that there is also a taxon-specific effect.

Juvenile material was available for two taxa: *Stenopterygius* and a still undescribed Middle Triassic ichthyosaur, for which a fetus is preserved inside an adult skeleton. In the case of the *Stenopterygius* vertebral centrum, the material, although from the same locality, was not found associated and the isolated vertebral centrum can only be identified at the genus level. A clear change in microanatomical organization is observed between the fetal/juvenile and adult specimens, with a strong increase in the tightness of the spongiosa with size (Fig. 3). The surface
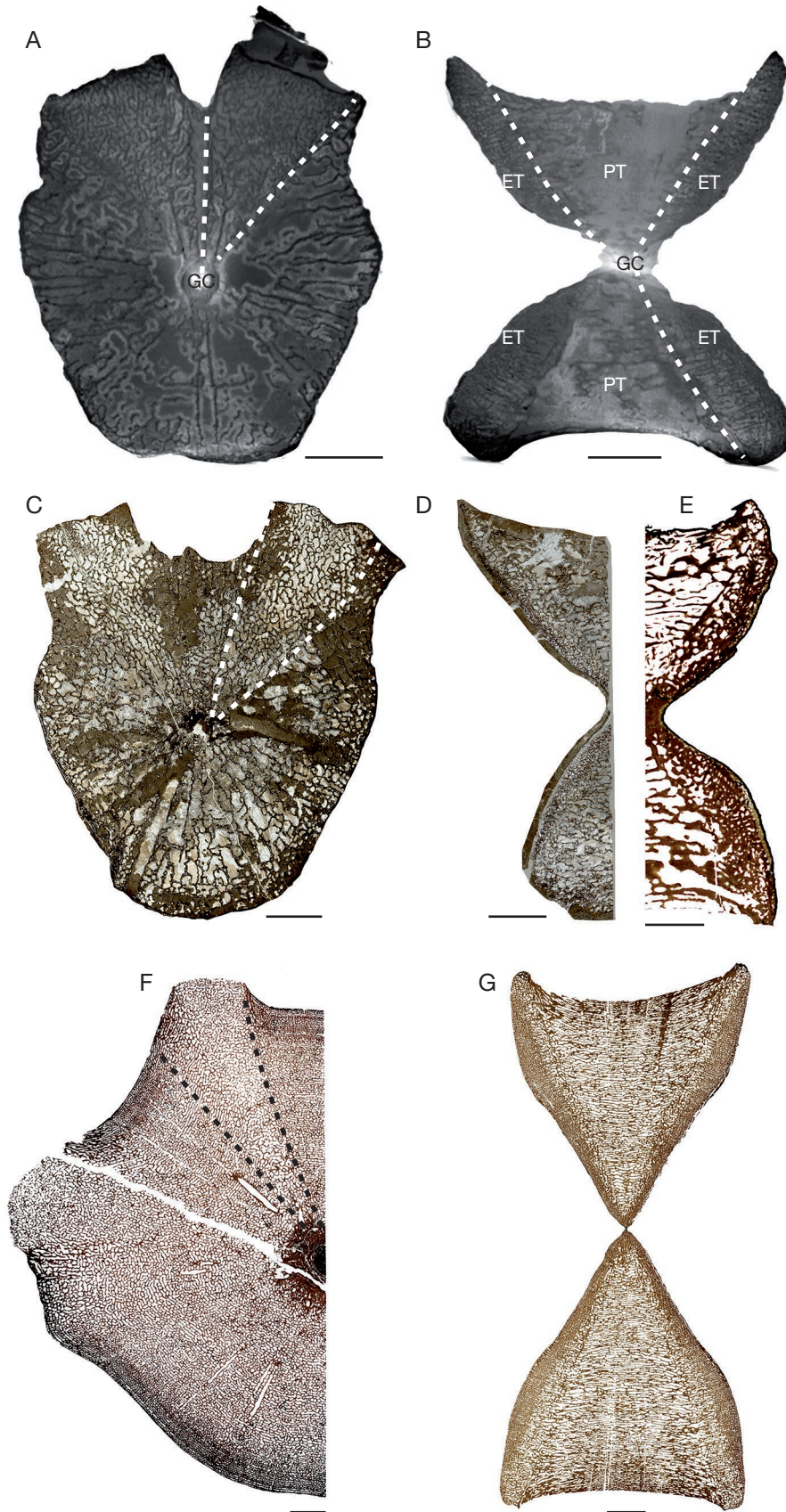
FIG. 2. — Virtual (**A**, **B**) and classical (**C-G**) sections of ichthyosaur vertebral centra illustrating the microanatomical types 1 (**A-E**) and 2 (**F**, **G**): **A**, **B**, Grippioidea indet., Lower Triassic, Russia, NSM PV 23854; **C**, **D**, Grippioidea indet., Middle Triassic, Nevada, LACM uncat. Nevada; **D**, **E**, *Mixosaurus* sp. PIMUZ T 2004; **F**, *Temnodontosaurus* sp. half transverse section; **G**, *Eurhinosaurus* sp. SMNS 50913 sagittal section; **A**, **C**, **F**, transverse sections; **B**, **D**, **E**, **G**, sagittal (and half mid-sagittal) sections. Abbreviations: **GC**, growth center; **ET**, endochondral territory; **PT**, periosteal territory. Scale bars: 5 mm.
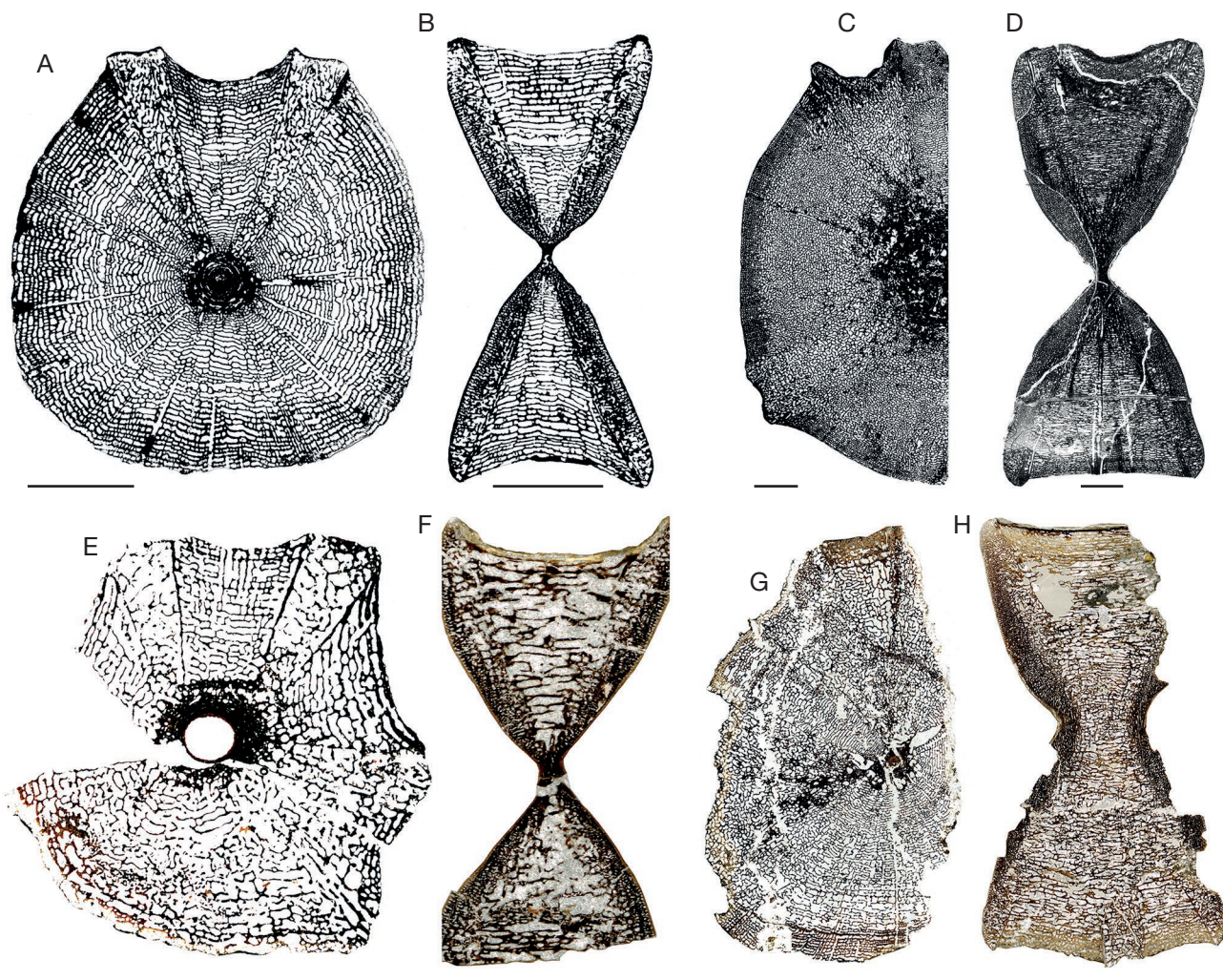
Fɪɢ. 3. — Classical sections of ichthyosaur vertebral centra illustrating the intraspecific size variation (interpolated as possible ontogenetic variation): **A**-**D**, *Stenopterygius* sp.; **A**, **B**, juvenile vertebra SMNS uncat.; **C**, **D**, adult vertebra SMNS uncat.; **E**-**H**, Ichthyosauria new taxon A, Middle Triassic, Nevada, LACM 8031; **E**, **F**, fetal vertebra LACM DI 158109; **G**, **H**, adult vertebra DI 158109; **A**, **C**, **E**, **G**, transverse (and half-transverse) sections; **B**, **D**, **F**, **H**, sagittal sections. Scale bars: 5 mm.

occupied by bone of endochondral origin is higher in the fetal/juvenile specimens than in adult specimens (45.2% vs 36.1% in *Stenopterygius*; 33.2% and 33.7% [two sections] vs 28.7% in the undescribed Middle Triassic specimen).

HISTOLOGICAL FEATURES

In the endochondral territory, the centrum anterior and posterior borders naturally exhibit calcified cartilage cells (Fig. 4A). From these surfaces, trabeculae are naturally oriented, in continuity with the cartilaginous cells, approximately perpendicular to the articular surface (Fig. 4A). However, this primary orientation is modified by bone deposits partly filling the intertrabecular spaces, so that the organization of the spongiosa becomes less homogeneous in the endochondral territory away from the bone anterior and posterior borders (Fig. 4B). The osseous trabeculae are essentially made of a core of fibrous bone with linings of parallel-fibered bone (Fig. 4C). Remodeling is limited, although a few secondary osteons can sometimes occur (Fig. 4C). In the periosteal territory, bone trabeculae essen-tially consist of unusual parallel-fibered bone sensu Houssaye *et al.* (2013) (i.e., with large and randomly shaped osteocyte lacunae [as in fibrous bone]; Fig. 4D-F). With the additional deposits of parallel-fibered bone in the intertrabecular spaces, this tissue evokes fibrolamellar bone (Fig. 4D-F) without truly corresponding to this tissue type, since there is no true fibrous bone. Numerous Sharpey's fibers insert between the two ter-ritories, following the direction of the limit between the ter-ritorial (periosteal and endochondral) cones (Fig. 5A). Around the center of the centrum, that is in its antero-posteriorly thin-nest portion, bone histological features are different from the above descriptions. In parasagittal sections, slightly away from the perfect mid-sagittal plane, periosteal bone consists of true parallel-fibered bone (Fig. 5B, C). The organization is much more compact than in the rest of the bone. Remodeling occurs in the center of the section (Fig. 5B, C). This is in accordance with the observation, in transverse sections, of a ring of more compact periosteal deposits of parallel-fibered bone surround-ing the centrum core (notochordal canal; Fig. 5D).
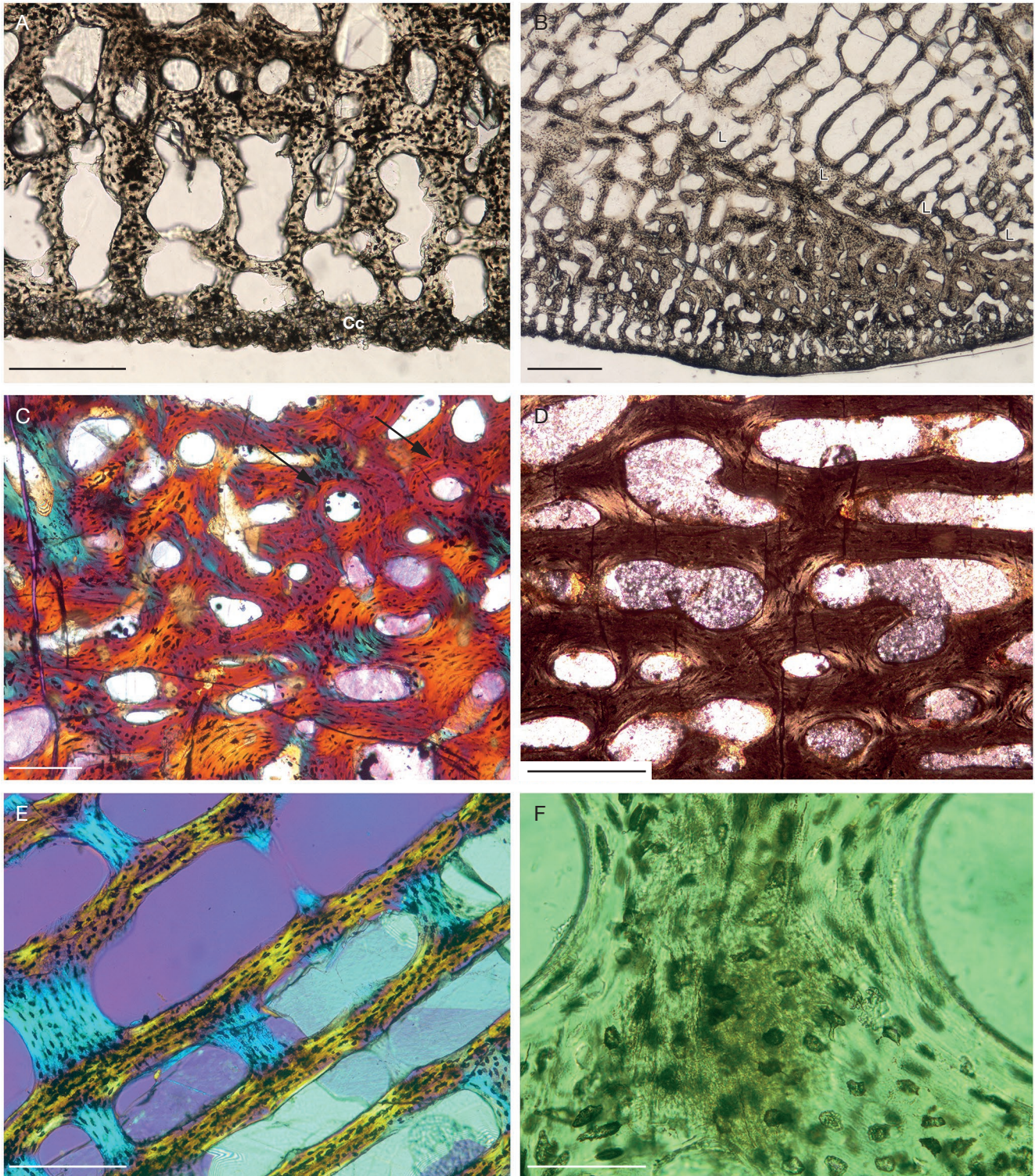
Fig. 4. — Histological features of ichthyosaur vertebral centra: **A**, **B**, **E**, **F**, *Stenopterygius* sp. SMNS uncat. longitudinal section; **C**, *Stenopterygius* sp. SMNS uncat. longitudinal section; **D**, *Temnodontosaurus* sp. SMNS uncat. longitudinal section; **A**, cartilaginous cells (**Cc**) and osseous trabeculae in the periphery of the endochondral territory in natural light (**NL**); articular surface at the bottom; **B**, illustration of the change of organization/orientation of the trabeculae from the periphery (**bottom**) to the core of the endochondral territory and limit (**L**) with the peripheral territory (from **bottom** to **top**) in NL; **C**, inner endochondral bone showing some degree of remodelling in polarized light (**PL**) with gypsum filter; arrows point to secondary osteons; **D**, **E**, unusual parallel fibered bone with platings of parallel-fibered bone in the periosteal territory in PL (**D**) and additional gypsum filter (**E**); **F**, core of a trabeculae of unusual parallel fibered bone showing the large randomly shaped osteocyte lacunae and the elongated cells in the platings of parallel-fibered bone in NL. Scale bars: A, D, E, 200 µm; B, C, 500 µm; F, 50 µm.
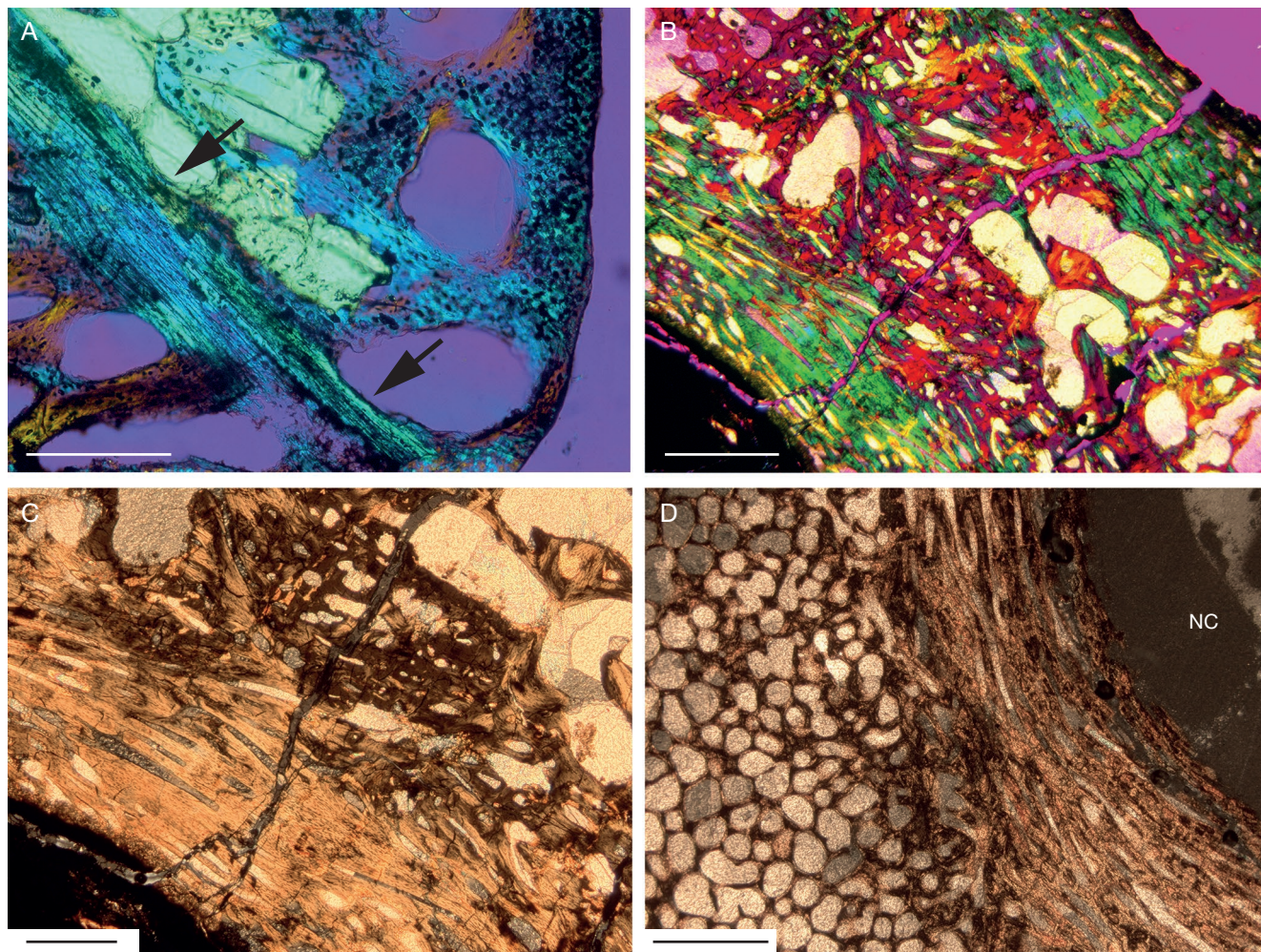
FIG. 5. — Histological features of ichthyosaur vertebrae: **A**, *Stenopterygius* sp. SMNS uncat. Longitudinal section. Numerous Sharpey's fibers at the limit between the periosteal (**left**) and endochondral (**right**) territories in PL with gypsum filter; pointed by **arrows**; **B**, **C**, *Temnodontosaurus* sp. SMNS uncat. parasagittal section showing the compact deposits of parallel-fibered bone (**blue**) in the outer core of the vertebra and secondary bone (**red**) in its inner core in PL with gypsum filter (**B**) and NL (**C**); **D**, *Temnodontosaurus* sp. SMNS uncat. Transverse section showing on the right the layer of rather compact parallel-fibered bone lining the centrum core (**NC**, notochordal canal), whereas the rest of the centrum is spongious (**left**) in NL. Scale bars: A, 200 μm; B, D, 1 mm; C, 500 μm.

DISCUSSION

GROWTH FEATURES

Ichthyosaur vertebral centra are characterized by an extremely limited endochondral territory, as compared to other known amniote vertebral centra. In Cymbospondylidae and ichthyosaurs more derived than mixosaurs, growth in length of the vertebral centrum, as compared to growth in diameter, was extremely limited, conferring upon them their typical "hockey puck" disk shape.

Primary bone deposits consist of unusual parallel fibered bone with platings of true parallel-fibered bone, mimicking a fibro-lamellar complex. This shows that growth was quick in these taxa, although slightly slower than in long bones, where true fibro-lamellar bone is observed (Kolb *et al.* 2011; Houssaye *et al.* 2014b).

The spongiosa is of primary origin, as in long bones, and not resulting from primary bone resorption (see Houssaye *et al.* 2014b contra Buffrénil & Mazin 1990).

STRUCTURAL REQUIREMENTS

All ichthyosaur vertebral centra are deeply amphicoelous. Our observations highlight the occurrence of a local increase in bone deposits surrounding the centrum growth center, circumferentially, probably reinforcing the bone and preventing any breakage in this thin region.

MECHANICAL ROLE

As opposed to what was described for some ribs (Talevi & Fernández 2012; Massare *et al.* 2014) and long bones (Houssaye *et al.* 2014b), no general or local increase in bone compactness is observed in ichthyosaur vertebral centra, which are exclusively spongious, as compared to what is observed in most amniotes (Houssaye *et al.* 2014a). As noted above, there is no peripheral layer of compact cortex as in most amniotes (Dumont *et al.* 2013; Hayashi *et al.* 2013; Houssaye *et al.* 2014b). This is not specific to ichthyosaurs and has been documented in several aquatic amniotes (Hayashi *et al.* 2013; Dumont *et al.* 2013; Hous-

saye *et al.* 2016). Compactness appears slightly lower than in mosasaurs (Houssaye & Bardet 2012) but not particularly low as compared to other amniotes (Houssaye *et al.* 2014b, 2016). Vertebral centra are lightly built but not extremely light and so do not show an osteoporotic-like pattern.

Trabeculae are essentially sagittaly oriented, i.e., in the direction of maximum stress (Fig. 2B, E, G). The limited remodelling suggests that the primary organization is optimized for functional requirements even later in life. There is a strong increase in the number of trabeculae during ontogeny (observed in microtype 2 vertebral centra) and evolution. The increase in the tightness (i.e., smaller intertrabecular spaces and thinner trabeculae) of the spongiosa through ontogeny and with taxon size is consistent with the trend already described in mosasaurs (Houssaye & Tafforeau 2012) and amniotes in general (Houssaye *et al.* 2010; Dumont *et al.* 2013). The two main microtypes here described for ichthyosaur vertebral centra seem to rather follow the evolutionary history of the group (except for *Cymbospondylus*; Fig. 1). The less organized and looser spongiosa is observed in the most primitive ichthyosaur forms: the Lower Triassic Russian vertebral centra and the Lower and Middle Triassic indet. Grippioidea specimens from Nevada. These specimens also correspond to proportionally longer vertebral centra (0.66 < L/H < 1.2 vs 0.47 < L/H < 0.67), and they show the highest calculated percentages of endochondral bone in adult specimens (Table 1).

As noted, more derived ichthyosaurs have disk-shaped vertebral centra that are distinctly shorter than tall (Sander 2000; McGowan & Motani 2003). This is the case for all the Jurassic Neoichthyosauria in our sample but also for *Cymbospondylus* with vertebral centra showing a length/height ratio up to 1:3. However, the Neoichthyosauria have fewer vertebrae (around 45 presacrals) than the Triassic cymbospondylid and shastasaurid ichthyosaurs (commonly > 60 presacrals; Sander 2000; McGowan & Motani 2003; Sander *et al.* 2011).

These various observations suggest a joint shift in vertebral centrum anatomical and microanatomical features between primitive ichthyosaurs with spool-shaped vertebral centra and more derived ones (and *Cymbospondylus*) with disk-shaped vertebral centra. However, microanatomy does not appear to correlate with body shape and swimming style because it is essentially the same in the long and slender-bodied cymbospondylids and tuna-shaped neoichthyosaurs.

An increase in the length (assumed total length of all trabeculae placed end to end) of the trabecular network in large pelagic oscillators is hypothesized to enable vertebral centra to better resist the extreme mechanical stresses they have to face (Dumont *et al.* 2013). However, our results seemingly falsify this hypothesis, suggesting a more simple relationship between vertebral centrum proportions and microanatomy instead, in ichthyosaurs, possibly resulting from morphogenesis of the disk-shaped vertebral centra that was achieved by faster osteogenesis in the periosteal domain compared to the endochondral domain.

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