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Long bone microstructure gives new insights into the life of pachypleurosaurids from the Middle Triassic of Monte San Giorgio, Switzerland/Italy

La microstructure des os longs donne un nouveau regard sur la vie des pachypleurosauridés du Trias moyen de Monte San Giorgio, Suisse/Italie

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ABSTRACT

The long bone microstructure of four pachypleurosaurid taxa from Monte San Giorgio (Switzerland/Italy) was studied. Pachypleurosaurids are secondarily aquatic reptiles that lived during the Middle Triassic in varying marine environments of the Tethys. All four pachypleurosaurids show high compactness values in their long bones based on a thick cortex and a calcified cartilaginous core, which remains in the medullary region throughout the ontogeny. Parts or even the entire embryonic bone layer composed of a mixture of woven-fibered bone tissue and parallel-fibered bone tissue is preserved in both pachypleurosaurid genera. The rest of the cortex consists of lamellar-zonal bone tissue type. Differences in the microstructure of the bones between the pachypleurosaurids are reflected in the occurrence of remodelling processes, which, if present, affect the innermost growth marks of the cortex or the calcified cartilaginous core. Further variation is present in the spacing pattern of the growth cycles, as well as in the degree of vascularisation of the lamellar-zonal bone tissue type. Our data on the microstructure of the long bones support previous studies on morphology and facies distribution, which indicated different habitats and adaptation to a secondary aquatic lifestyle for each pachypleurosaurid taxon. Life history data furthermore reflect different longevities and ages at sexual maturity. The bone histological data of the stratigraphically youngest and oldest pachypleurosaurid species might indicate possible climate-dependant reproductive seasons similar to Recent lacertilian squamates.

Mots clés :
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Squelettochronologie
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Histoire de vie
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RÉSUMÉ

La microstructure des os longs de quatre taxa de pachypleurosauridés de Monte San Giorgio (Suisse/Italie) a été étudiée. Les pachypleurosauridés sont des reptiles secondairement aquatiques qui ont vécu au Trias moyen, dans des environnements proches des rivages de la Téthys. Les quatre pachypleurosauridés présentent des valeurs de compacité élevées dans les os longs, basées sur un cortex épais et un cœur cartilagineux calcifié, qui persiste dans la région médullaire tout au long de l'ontogénie. Des parties ou même la totalité du feuillet

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osseux embryonnaire composé d'un mélange de tissu osseux à fibres entrelacées ou à fibres parallèles sont préservées dans les deux genres de pachypleurosauridés. Le reste du cortex consiste en un tissu osseux de type lamellaire-zonaire. Des différences dans la microstructure des os, entre pachypleurosauridés, sont perceptibles grâce à la présence de processus de remaniement qui, s'ils sont présents, affectent les marques de croissance les plus internes du cortex ou du cœur cartilagineux calcifié. Une variation ultérieure est observable dans le diagramme spatial des cycles de croissance, ainsi que dans le degré de vascularisation du tissu osseux de type lamellaire-zonaire. Nos données sur la microstructure des os longs corroborent des études antérieures sur la morphologie et la distribution des faciès, qui indiquent différents habitats et l'adaptation à un style de vie aquatique pour chaque taxon au sein des pachypleurosauridés. Les données histologiques sur les os reflètent, en outre, différents âges à maturité et différentes longévités. Les données histologiques sur les os des espèces de pachypleurosauridés stratigraphiquement les plus jeunes et les plus âgées indiquent, pour la reproduction, des saisons dépendant du climat, peut-être similaires à celles observées chez les squamates lacertiliens récents.

1. Introduction

Pachypleurosaurids are small secondarily aquatic reptiles which belong to the Triassic eusauropterygians (Klein, 2010; Rieppel, 2000). The UNESCO World Heritage site of Monte San Giorgio (Switzerland/Italy) has yielded pachypleurosaurids in great quantity and quality since excavations started in 1924. Two genera and four species of pachypleurosaurids, *Serpianosaurus mirigiolensis*, *Neusticosaurus pusillus*, *N. peyeri* and *N. edwardsii*, are recognised from four distinct horizons of Middle Triassic Ladinian carbonates (Peyer, 1932; Fig. 1). *S. mirigiolensis* is the stratigraphically oldest taxon and is known only from the Besano Formation (Furrer, 1995; Röhl et al., 2001; Sander, 1989; Fig. 1). The stratigraphically younger genus *Neusticosaurus* is found in the Lower Meride Limestone (Carroll and Gaskill, 1985; Sander, 1989; Fig. 1). *N. pusillus* is found in the beds of the Cava Inferiore horizon, whereas the beds of the Cava Superiore horizon exclusively yielded specimens of *N. peyeri* (Fig. 1). The stratigraphically youngest taxon, *N. edwardsii*, is preserved only in the Alla Cascina horizon (Sander, 1989; Fig. 1).

The Monte San Giorgio pachypleurosaurids lived in more or less near-shore environments in the Tethys (Sander, 1989) during a subtropical monsoonal climate (Röhl et al., 2001). Pachypleurosaurids clearly exhibit aquatic adaptations such as the loss of ossified carpal and tarsal elements, the ventral expansion of the girdles, the flattening of the forelimbs and hind limbs, and an isodont dentition that is typical for carnivorous animals (Carroll and Gaskill, 1985; Sander, 1989). However, the four species from Monte San Giorgio also show certain morphological variation which might be linked to different habitat preferences. *N. pusillus*, *N. peyeri* and *N. edwardsii* lived in a lagoonal environment with increasing restriction based on the evidence of facies and fossil distribution (Furrer, 1995). In contrast, *S. mirigiolensis* lived in a basin with more open marine influences (Furrer, 1995; Carroll and Gaskill, 1985; Rieppel, 1989). *N. pusillus* and *N. peyeri* differ morphologically from *S. mirigiolensis* and *N. edwardsii* by exhibiting anatomically pachyostotic vertebral centra and ribs (sensu Francillon-Vieillot et al., 1990), as well as elongated transverse processes of the caudal vertebrae (Sander, 1989). In contrast, *S. mirigiolensis* and *N. edwardsii* show less anatomically pachyostotic bones, but prominent insertion areas for strong muscles along the vertebral column, as well as higher dorsal spines of their caudal vertebrae (Carroll and Gaskill, 1985; Rieppel, 1989). Pachypleurosaurids also differ between each other by an advanced reduction of phalangeal formulae and body lengthening by increasing the vertebral count of the dorsals in the stratigraphically younger taxa compared to the stratigraphically older ones (Carroll and Gaskill, 1985; Nopcsa and Heidsieck, 1934; Rieppel, 1989; Sander, 1989). Histological studies of the limb bones and ribs of the pachypleurosaurids (Buffrénil and Mazin, 1989; Nopcsa and Heidsieck, 1934; de Ricqlès, 1976; de Ricqlès and Buffrénil, 2001; Sander, 1990; Zangerl, 1935) revealed that all four species exhibit pachyosteosclerotic bones in thin-section (sensu Francillon-Vieillot et al., 1990). Such dense and heavy bones are typically found in secondarily aquatic amniotes and are generally thought to

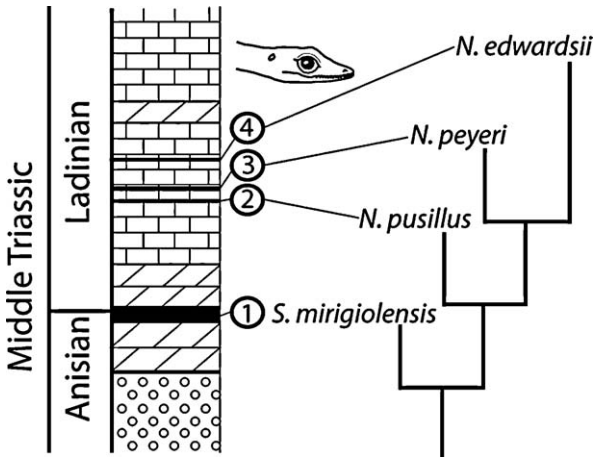

Fig. 1. Stratocladogram of the four pachypleurosaurids from Monte San Giorgio (after Furrer, 1995; O'Keefe et al., 1999; modified). 1: Besano Formation; 2: Cava Inferiore horizon; 3: Cava Superiore horizon; 4: Alla Cascina horizon.

Fig. 1. Stratocladogramme de quatre pachypleurosauridés de Monte San Giorgio (d'après Furrer, 1995 ; O'Keefe et al., 1999 ; modifié). 1 : Formation Bessano ; 2 : Horizon Cava inferiore ; 3 : Horizon Cava Superior ; 4 : Horizon Alla Cascina.

increase ballast, allowing a hydrostatic control of body density and body trim in water (Canoville and Laurin, 2010; Germain and Laurin, 2005; de Ricqlès and Buffrénil, 2001). Rothschild and Storrs (2003) suggested that *Neusticosaurus* spp. rarely dived into great depths or changed diving depths rapidly based on a few cases of avascular necrosis in the epiphyseal regions of the long bones, which was hypothesised to be caused by decompression syndrome. Avascular necrosis is also rarely indicated at the proximal epiphyseal regions of the humerus of *S. mirigiolensis* (Hugi and Scheyer, unpublished). New results obtained from the ossification sequences in *S. mirigiolensis* show that it shares slightly more similiarities with data from terrestrial reptiles, whereas the *Neusticosaurus* spp. resemble slightly more closely extant semi-aquatic and aquatic reptiles (Hugi and Scheyer, unpublished; Rieppel, 1993; Werneburg et al., 2009).

The aim of this study is to reveal further "terrestrial"- and "aquatic-like" features among the pachypleurosaurids from Monte San Giorgio based on new histological data. Questions include whether these new data show a gradual variation from stratigraphically older to younger specimens as seen in the reduction of the phalangeal formula and the elongation of the vertebral count, or whether new results reflect possible adaptations to different marine environments. Zangerl (1935) described the bone microstructure of *Neusticosaurus* spp. and de Ricqlès (1976) summarised his results in a comparative study of the histology of fossil and living reptiles. Sander (1990) then studied the skeletochronology of several specimens of *N. pusillus* and *N. peyeri* by counting the minimum number of lines of arrested growth (LAGs) (Castanet et al., 1993) in polished sections, as well as describing the spacing pattern of the growth cycles (Buffrénil and Castanet, 2000). This study is focused on the histological changes during the ontogeny of all four pachypleurosaurid taxa. It further expands the database, especially for *S. mirigiolensis* and *N. edwardsii*, and describes new histological and skeletochronological data for all pachypleurosaurids from Monte San Giorgio. The results are discussed in comparison to published accounts of fossil eosauropterygians and Recent lacertilian squamates.

2. Material and methods

We examined 11 specimens of *S. mirigiolensis*, nine of *N. edwardsii* (Table 1), 18 specimens of *N. pusillus* and nine of *N. peyeri*, all housed in the Palaeontological Institute of the University of Zurich (PIMUZ). These new data complement previous results of the bone histological studies of Sander (1990), where polished sections of *N. pusillus* and *N. peyeri* were used. Size classes of *S. mirigiolensis* and *N. edwardsii* were adopted from Sander (1989) (Rieppel, 1989). Diaphyseal thin-sections of the growth series of the bones of forelimb and hind limb (humerus, radius, ulna, femur, tibia, fibula, metacarpals, metatarsals; note that not all bones were available for each specimen) were processed and documented at the PIMUZ, following standard petrographic preparation techniques (Scheyer and Sánchez-Villagra, 2007). The diaphyseal region of the long bones is known to yield the strongest ecological signal

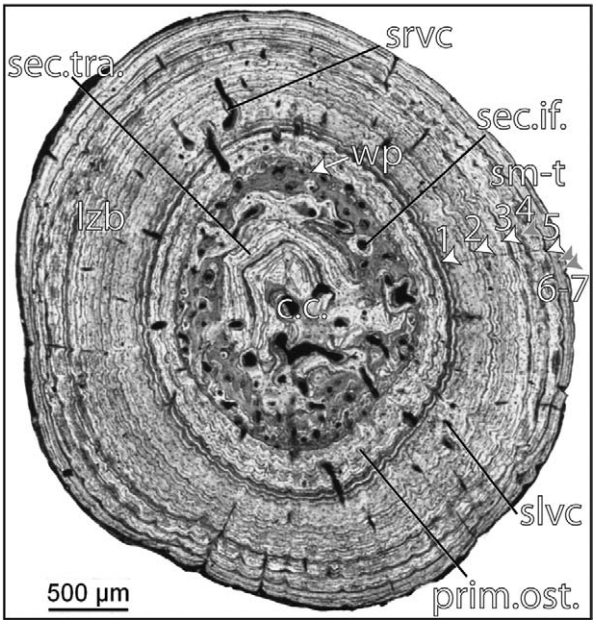

Fig. 2. Metaphyseal transverse section of the humerus of an adult *Neusticosaurus pusillus* (T4178) in normal transmitted light. The specimen shows seven LAGs (arrow heads). The sexual maturity (sm-t) is estimated at age three to four years due to the abrupt decline in thickness of growth cycle four (grey arrow head; Sander, 1990). The inner periosteal bone layers of the cortex are only partly affected by resorption processes and therefore, still reveal large parts of the first growth cycle which is composed of woven-fibered grading into parallel-fibered bone tissue type (wp). An external fundamental system (EFS) is developed in the outer cortex. The cortex shows lamellar-zonal bone tissue type (lzb) which is vascularised by simple longitudinal and radial vascular canals (slvc, srvc) and primary osteons (prim.ost.). The medullary region is filled with a calcified cartilaginous core (c.c.) and shows secondary trabeculae (sec.trab.), as well as widened and secondarily filled simple vascular canals (sec.if.).

Fig. 2. Section transversale de métaphyse de l'humérus d'un adulte de *Neusticosaurus pusillus* (T4178) en lumière transmise normale. L'échantillon montre sept LAGs (têtes de flèches). La maturité sexuelle (sm-t) est estimée à trois à quatre ans, en raison de la diminution abrupte de l'épaisseur du cycle de croissance quatre (tête de flèche grise ; Sander, 1990). Les feuillets internes d'os périostique du cortex ne sont qu'en partie affectés par les processus de résorption et, de ce fait, révèlent encore de grandes parties du premier cycle de croissance qui est composé d'un tissu osseux de type entrelacé fibreux passant à un type de fibres parallèles (wp). Un système fondamental externe (EFS) se développe dans la partie la plus externe du cortex. Le cortex montre un tissu osseux de type lamellaire-zonaire (lzb) qui est vascularisé par des canaux vasculaires simples, longitudinaux et radiaux (slvc, srvc) et des ostéones primaires (prim.ost.). La région médullaire est occupée par un cœur cartilagineux calcifié (c.c.) et montre des trabécules secondaires (sec.trab.), ainsi que des canaux vasculaires simples, élargis et remplis secondairement (sec.if).

(Canoville and Laurin, 2010) and generally preserves the most complete growth record of the bones among vertebrates (Francillon-Vieillot et al., 1990). The amount of vascularisation of the long bones, as well as the age of sexual maturity and the ontogenetic age of each individual (Table 1) was assessed qualitatively through the analysis of bone types and tissues, spacing patterns, as well as through the counting of LAGs. The bone compactness of one adult *S. mirigiolensis* and one adult *N. edwardsii* was also quantitatively analysed (Table 2) using the PC version of the program Bone Profiler (Girondot and Laurin, 2003). Paired *t*-tests were performed to report the probability (p) of the

Table 1
Skeletal elements of *Serpianosaurus mirigiolensis* and *Neusticosaurus edwardsii*.
Tableau 1
Éléments de squelette de *Serpianosaurus mirigiolensis* et *Neusticosaurus edwardsii.*

	Museum #	sc (hr)	Element	Length	Proximal width	Distal width	# LAGs (sm-t)
S. mirigiolensis	T131	A (0.89)	Humerus	12.7	2.8	4	2 (/)
			Radius	8.7	2.1	2.3	
			Ulna	7.1	1.8	2.5	
			Femur	11.3	3.3	2.3	
			Tibia	7.6	2.6	2.4	
			Fibula	8	1.7	2.9	
S. mirigiolensis	T0985a	A	Ulna	7.8	2.4	2.7	2 (/)
			Metacarpal-1	2.2	0.3	0.3	
			Metacarpal-2	3.4	0.4	0.4	
			Metacarpal-3	3.9	0.4	0.4	
			Metacarpal-4	4.1	0.4	0.4	
			Metacarpal-5	2.7	0.3	0.3	
S. mirigiolensis	T0985b	A	Radius	8.8	0.9	0.9	3 (/)
S. mirigiolensis	T1568	B	Humerus (right)	19.7	4.5	6.3	4 (2.-3.)
			Humerus (left)	19.3	4.3	6.3	
S. mirigiolensis	T114	B	Ulna	12.4	4.22	3.8	4 (2.-3.)
S. mirigiolensis	T1565	B (2.33)	Humerus	18.6	3.6	6.3	4 (2.-3.)
			Femur	17.1	4.8	2.6	
			Tibia	11.3	3.2	2.9	
			Fibula	11.2	3.5	2.7	
S. mirigiolensis	T119	C (1.54)	Humerus	21.3	4.6	5.4	5 (2.-3.)
S. mirigiolensis	T1564	C	Femur	18.8	4.2	2.6	6 (3.-4.)
			Tibia	10	2.6	2.2	
			Fibula	10.5	2	2.3	
S. mirigiolensis	T115	C	Humerus	ca. 23.5	5.3	?	7 (2.-3.)
S. mirigiolensis	T105	D	Femur	24.1	5.3	2.8	8 (2.-3.)
S. mirigiolensis	T4510	D (1.68)	Humerus	30	5.2	8.9	13 (2.-3.)
			Radius	17	3.3	2.1	
			Ulna	15.5	5.2	2.1	
N. edwardsii	T4748	B (2.0)	Humerus	29.8	6.5	8.4	4 (/)
			Radius	15.8	2.9	2.7	
			Ulna	ca. 13.5			
N. edwardsii	1u/342	B	Femur	19.9	4.9	2	6 (4.-5.)
N. edwardsii	1u/341	B	Humerus	35.6	5.7	?	6 (5.-6.)
N. edwardsii	T4758	B (1.98)	Humerus	28.7	8.3	9.9	5 (4.-5.)
			Radius	17.5	3.4	3.1	
			Ulna	13.5	5.2	4.1	
			Femur	ca. 18.1	6.9	3.3	
			Tibia	10.1	3.6	2.5	
			Fibula	10.3	2.6	3.6	
N. edwardsii	T4757	C (2.56)	Humerus	36.2	6	10	6 (4.-5.)
			Femur	20.5	7.2	3.9	
			Tibia	12.6	4.2	2.9	
			Fibula	11.3	2.8	4.8	
			Metatarsal-5	7.6	1.3	1.3	
N. edwardsii	Schicht L	C (2.61)	Humerus	39.7	9.3	15.4	8 (5.-6.)
N. edwardsii	T4752	D	Femur	47.6	19.1	10.5	10 (5.-6.)
			Tibia	20.4	7.3	6.1	
			Fibula	23.9	5.3	2.8	
			Rib (trunk)	34.3	2.08		
N. edwardsii	T3437	F (3.21)	Humerus	82.6	15.4	27.9	22 (6.-7)
			Radius	39.9	11.2	8	
			Ulna	35.7	12.1	10.9	
			Femur	45.5	18.9	10.1	
			Tibia	27.3	10	6.9	
			Fibula	29.1	7.2	10.1	

Question marks indicate non-preserved regions of bone. In those cases where the humerus was completely preserved, the ratio between maximum distal width/minimum width of humerus (sensu Sander, 1989) is given (hr). sc: size class (after Sander, 1989); LAGs: lines of arrested growth (after Castanet et al., 1993); sm-t: growth cycle at which sexual maturity is indicated; #: specimen number.
Les points d'interrogation (?) indiquent les régions non préservées de l'os. Dans les cas où l'humérus a été entièrement conservé, le rapport entre la largeur distale maximale et la largeur minimale de l'humérus (sensu Sander, 1989) est fourni (hr). sc : classe de taille (d'après Sander, 1989) ; LAGs : lignes d'arrêt de croissance (selon Castanet et al., 1993) ; sm-t : cycle de croissance auquel la maturité sexuelle est indiquée ; # : numéro de l'échantillon.

Table 2
Observed individual variation of compactness profile parameter values in several limb elements for two adult specimens of *Neusticosaurus edwardsii* and *Serpianosaurus mirigiolensis*, as retrieved from Bone Profiler (Girondot and Laurin, 2003). O.c.: observed compactness; n.a.: not available.
Tableau 2
Variation individuelle observée dans les valeurs paramétrées du profil de compacité, pour différents éléments de membre de deux spécimens adultes de *Neusticosaurus edwardsii* et de *Serpianosaurus mirigiolensis*, estimés à l'aide du logiciel *Bone profiler* (Girondot et Laurin, 2003). O.c. : compacité observée ; n.a. : non disponible.

Specimen	Elements	O.c.	S (SE)	P (SE)	Min (SE)	Max (SE)	R^2
N. edwardsii							
T3437	Humerus	0.925	0.4795578 (0.00623)	−0.2285325 (0.06981)	0.6532622 (0.03100)	0.9713988 (0.00117)	0.2106421
T3437	Radius	0.906	0.0017901 (0.00014)	0.0757331 (0.00013)	0.999999 (0)	0.9058535 (0.00023)	0.3786207
T3437	Ulna	0.899	0.1744264 (0)	−0.0985369 (0)	0.2962655 (0)	0.9175581 (0)	0.0904741
T3437	Femur	0.958	0.247114 (n.a.)	−36.06885 (n.a.)	0.0258474 (n.a.)	0.9577149 (n.a.)	4.101100e−11
T3437	Tibia	0.926	0.0492076 (n.a.)	−1.537819 (n.a.)	0.999999 (n.a.)	0.9261052 (n.a.)	2.929139e−12
T3437	Fibula	0.918	0.1111562 (n.a.)	−195.1377 (n.a.)	0.999999 (n.a.)	0.9181818 (n.a.)	2.277175e−12(n.a.)
S. mirigiolensis							
T1045	Humerus	0.959	0.0535338 (0.00061)	0.844583 (0.00099)	0.9426604 (0.00022)	0.999999 (0)	0.2782581
T1045	Radius	0.987	0.0179708 (0.00061)	0.3183357 (0.00093)	0.8924896 (0.00097)	0.9974831 (0.00005)	0.6657777
T1045	Ulna	0.985	0.1127315 (0.00061)	−0.0460759 (0.00182)	0.0000268 (0.00004)	0.999999 (0.00001)	0.4352688
T105	Femur	0.984	0.0067788 (0.00068)	0.1709579 (0.00143)	0.9154595 (0.00160)	0.986658 (0.00009)	0.3842736

two null hypotheses, which state that: (1) the limb bones of the sampled pachypleurosaurids all show an equal mean compactness value; and (2) *N. edwardsii* shows no statistically significant difference in the mean compactness values between the preaxial and postaxial elements of the zeugopodial region of both the forelimbs and hind limbs. The black and white sketches were prepared using Adobe Photoshop CS3.

3. Results

3.1. Bone histology, bone compactness and skeletochronology

The medullary region is entirely filled with a calcified cartilaginous core throughout the ontogeny of all pachypleurosaurids. In ontogenetically young pachypleurosaurids, primary endosteal bony infillings of the vascular canals and a few secondary trabeculae are preserved in the calcified cartilaginous core. Throughout ontogeny, secondary endosteal bony deposits progressively increase in all pachypleurosaurids (i.e. increased remodelling). Accordingly, the vascular canals are secondarily widened by resorption of the primary infillings, as well as of parts of the surrounding calcified cartilage. Subsequently, secondary deposition takes place (Fig. 2). These secondary endosteal infillings of vascular canals are similar to secondary osteons, which are usually found in bone tissue (Francillon-Vieillot et al., 1990), but are here surrounded by the calcified cartilage matrix. These remodelling processes (i.e. secondary endosteal infillings of the vascular canals and secondary trabeculae) are found only in the diaphyseal regions of the long bones in younger individuals, but progressively increase in abundance towards the epiphyseal regions in older specimens.

In both pachypleurosaurid genera, the LAGs are distinct in contrast to the growth zones and annuli, which in some cases change in thickness throughout the ontogeny of an individual (Fig. 3E–H, Fig. 4E–F). All the pachypleurosaurids show minor or no remodelling of the innermost wall of the cortex. The highest grades of remodelling occur within *N. pusillus* and *N. peyeri*, but even in these species only parts of the innermost periosteal bone layer are affected and therefore, the growth record can be entirely reconstructed.

The studied pachypleurosaurids show two bone tissue types in the cortex of their long bones; each is differently vascularised, contains a different quantity and shape of osteocyte lacunae, as well as shows a different birefringence based on the alignment of the crystallites (Glimcher and Muir, 1984). The innermost bone layers are composed of a mixture of woven-fibered bone tissue and parallel-fibered bone tissue (wp; Figs. 2, 3C–D, 4G–H), whereas a lamellar-zonal bone tissue type is found in the outer, more peripheral part of the cortex. The innermost bone layer (wp) is visible as an opaque zone in polarised light showing a high quantity of round osteocyte lacunae (Fig. 3D).

The stylopodial elements of both the forelimbs and hind limbs of pachypleurosaurids show constant and high compactness, as shown by compactness profiles (Table 2). The zeugopodial elements of all pachypleurosaurids, in contrast, show more and also larger erosion cavities in the medullary region. The amount of vascularisation appears

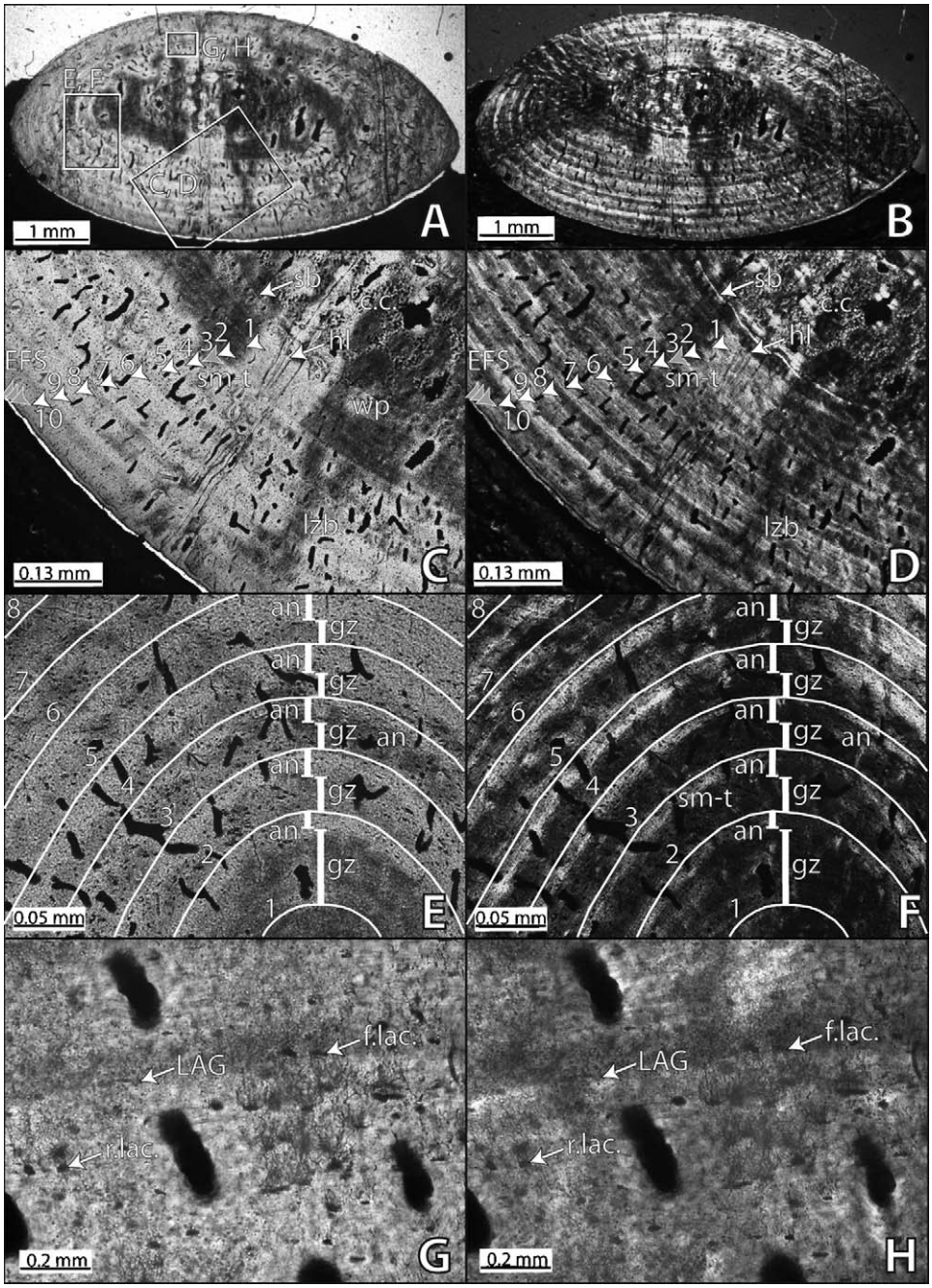

Fig. 3. Diaphyseal transverse section of the humerus of a large *Serpianosaurus mirigiolensis* (T4510). A, C, E, G is normal transmitted light; B, D, F is polarised light; H is polarised light and Lambda compensator. Images C and D show the sharp border (sb) between the cortex and the medullary region, the hatching line (hl) which borders the embryonic bone layer (wp), the growth cycle at sexual maturation (No. 3, grey arrow head) and 13 LAGs (lines of arrested growth; arrow heads). The EFS is composed of three LAGs (grey arrow heads in outermost cortex). Images E and F give a detailed look at the light and extinction pattern based on the different characteristics of the growth zones (gz) and annuli (an) within each growth cycle (redrawn by white lines). Images G and H show the different characteristics of the growth zones and annuli (birefringence, quantity and shapes of the osteocyte lacunae: flattened osteocyte lacunae: f.lac., round osteocyte lacunae: r.lac.). c.c.: calcified cartilage; wp: woven-fibered grading into parallel-fibered bone.

Fig. 3. Section transversale de diaphyse de l'humérus d'un grand *Serpianosaurus mirigiolensis* (TA4150). A, C, E, G sont en lumière transmise normale, B, D, F, en lumière polarisée, H en lumière polarisée et compensateur Lambda. Les images C et D montrent une limite nette (sb) entre le cortex et la région médullaire, la ligne hachurée (hl) qui borde le feuillet osseux embryonnaire (wp), le cycle de croissance dénotant l'arrivée à maturité sexuelle (No. 3, extrémité de flèche grise) et 13 LAGs (lignes de croissance arrêtée, têtes de flèches). L'EFS est composé de trois LAGs (têtes de flèches grises dans la partie la plus extérieure du cortex). Les images E et F donnent le détail du schéma d'extinction concentrique reflétant les caractéristiques des zones (gz) et anneaux (an) de croissance, au sein de chaque cycle de croissance, redessiné à l'aide de lignes blanches. Les images G et H montrent les différentes caractéristiques des zones et des anneaux de croissance (biréfringence, quantité et formes des lacunes d'ostéocyte : lacunes d'ostéocyte aplaties : f.lac, lacunes d'ostéocyte rondes, r.lac.). c.c. : cartilage calcifié ; wp : os à entrelacs fibreux passant au type à fibres parallèles.

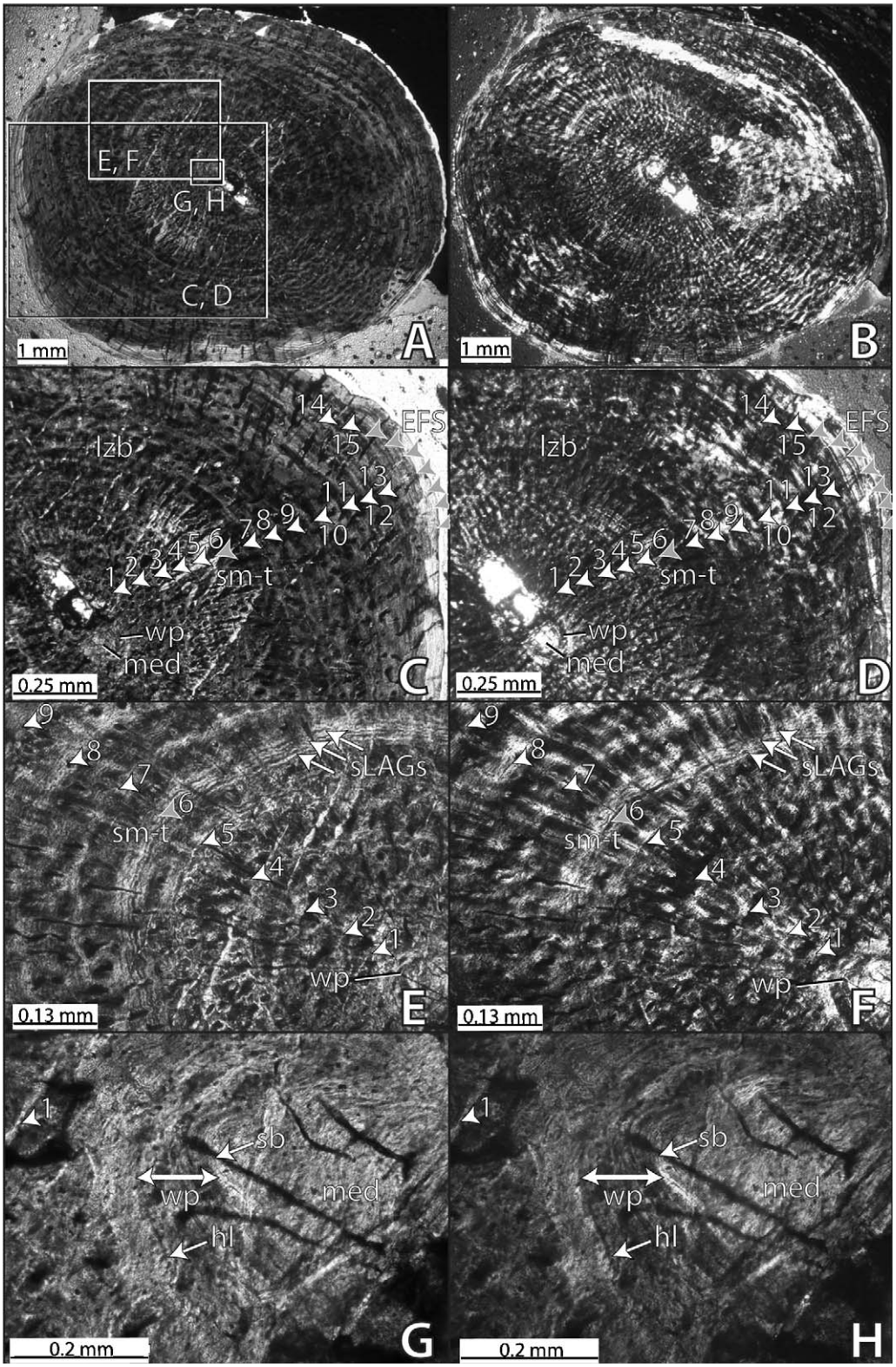

Fig. 4. Diaphyseal transverse section of the humerus of a large *Neusticosaurus edwardsii* (T3437). A, C, E, G is normal transmitted light; B, D, F, H is polarised light; H is polarised light and Lambda compensator. The calcified cartilaginous core of the medullary region (med), which is entirely replaced by secondary trabeculae, is visible in all the figure parts, whereas the sharp border (sb) between the medullary region is shown in more detail in images G and H. Images C and D show the number of LAGs (numbered, arrow heads). Images E and F give a detailed look at the growth cycle at sexual maturity (No. 6, grey arrow head) which shows supernumerary LAGs (sLAGs) and no decrease in the growth cycle thickness. Images G and H show the innermost periosteal region

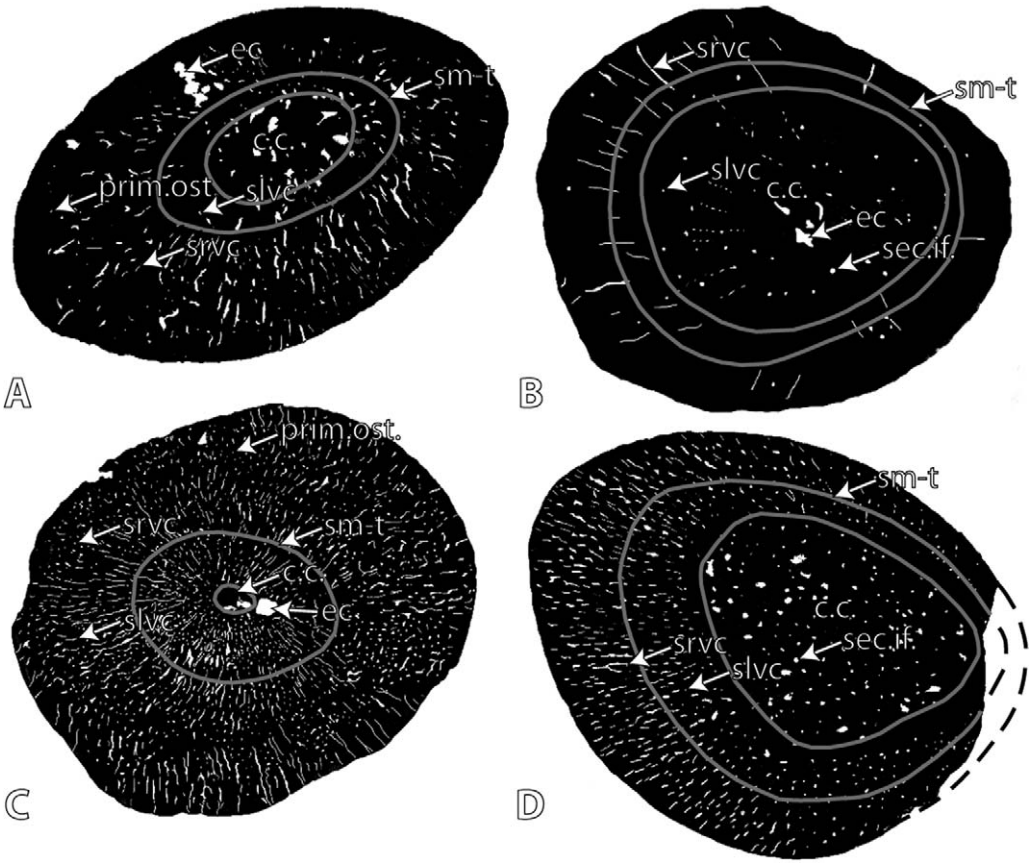

Fig. 5. Black and white sketches of the diaphyseal transverse sections through the humerus and femur of a large *Serpianosaurus mirigiolensis* (A: humerus, B: femur) and of a large *Neusticosaurus edwardsii* (C: humerus, D: femur) for which compactness values were established with the program Bone Profiler (Girondot and Laurin, 2003; Table 2). Although *N. edwardsii* generally shows higher vascularisation and more erosion cavities (ec), as well as slightly lower observed compactness values, compactness curves are still close to 1.0 and remain constant at least throughout the cortex. c.c.: calcified cartilage; wp: woven-fibered grading into parallel-fibered bone.

Fig. 5. Schémas en noir et blanc de sections transversales de diaphyse de l'humérus et du fémur d'un grand *Serpianosaurus mirgiolensis* (A : humérus, B : fémur) et d'un grand *Neusticosaurus edwardsii* (C : humérus, D : fémur), pour lesquelles les valeurs de compacité ont été établies à l'aide du programme « Bone profiler » (Girondot et Laurin, 2003, Tableau 2). Bien que *N. edwardsii* présente une vascularisation plus élevée et plus de cavités d'érosion (ec), ainsi que des valeurs de compacité observées légèrement plus faibles, les courbes de compacité sont encore proches de 1,0 et restent constantes au moins au travers du cortex. c.c. : cartilage calcifié ; wp : os à entrelacs fibreux passant au type à fibres parallèles.

to increase with stratigraphic age with *N. edwardsii* showing long bones with the highest degree of vascularisation (Fig. 5C–D), as is also indicated by the compactness indices (Table 2). The humerus, radius, ulna and femur show a statistically significant difference in the mean compactness between *N. edwardsii* and *S. mirigiolensis* (paired *t*-test, $t = 2.78$; $P < 0.05$) with the latter exhibiting slightly higher mean compactness values.

The four pachypleurosaurids differ between each other by the grade of the remodelling of the innermost periosteal bone layers of the cortex and the calcified cartilaginous core in the medullary region. They also show variation in the grade of the vascularisation of the cortex, the spacing pattern of the growth cycles, the age when sexual maturity is reached, as well as in their longevities.

Fig. 4. of the cortex which is composed of wp. This inner area is visible as opaque zone in polarised light with round osteocyte lacunae. c.c.: calcified cartilage; wp: woven-fibered grading into parallel-fibered bone.

Fig. 4. Section transversale de diaphyse de l'humérus d'un grand *Neusticosaurus edwardsii* (T3437). A, C, E, G sont en lumière transmise normale; B, D, F, H en lumière polarisée; H en lumière polarisée et compensateur Lambda. Le cœur cartilagineux calcifié de la zone médullaire (med), entièrement remplacé par des trabécules secondaires, est visible dans toutes les parties de la figure, tandis que la limite nette (sb) avec la région médullaire est montrée avec plus de détails sur les images G et H. Les images C et D montrent le nombre de LAGs (matérialisés par les têtes de flèches). Les images E et F donnent le détail du cycle de croissance à maturité sexuelle (No. 6, tête de flèche grise), qui présente des LAGs surnuméraires (sLAGs), mais pas de diminution d'épaisseur du cycle de croissance. Les images G et H montrent la région la plus interne du périoste du cortex, qui est composée de wp. La partie interne est visible en tant que zone noire en lumière polarisée, avec des lacunes d'ostéocyte rondes. c.c. : cartilage calcifié ; wp : os à entrelacs fibreux passant au type à fibres parallèles.

3.2. Serpianosaurus mirigiolensis

S. mirigiolensis specimens show highly compact limb bones except for the ulna (Table 2), which is the only limb bone exhibiting a relatively thin cortex and a larger medullary region.

The innermost region of the cortex is composed of a bone layer of wp (Fig. 3C–D), which is visible as an opaque zone in polarised light and characterised by a high quantity of round osteocyte lacunae (Fig. 3D).

Throughout ontogeny, the calcified cartilage often remains the main component in the medullary region of the long bones. Large specimens show more osseous deposits in the diaphyseal cartilaginous matrix and less towards the epiphyseal area of the medullary region. In a few large specimens, the calcified cartilaginous core is entirely replaced by secondary bone trabeculae in the diaphysis. The calcified cartilage of the medullary region generally shows a few simple vascular canals which are in some cases secondarily widened and filled with lamellar bone. On average, vascular canals of both the medullary region and cortex comprise 7% of the section area (Table 2).

The long bones reveal large parts of, or even a complete, distinct layer of endosteal lamellar bone (sb) that marks the separation between the medullary and cortical region (Fig. 3C, D) and is preserved along its entire border also at the meta- and epiphyseal regions (Fig. 6).

In *S. mirigiolensis*, the onset of sexual maturity is indicated by several microstructural changes within the corresponding growth cycles: an abrupt decrease in the growth cycle thickness (Fig. 3E–F), a change in the vascularisation pattern (Fig. 5A–B), a change in thickness of the growth zones and annuli (Fig. 3E–H; see light and extinction pattern). Based on those changes, the attainment of sexual maturity is indicated between the second to fourth year in *S. mirigiolensis* specimens (Table 1; Fig. 3C–F). The largest specimen (T4510) is also the ontogenetically oldest one with 13 LAGs, therefore being in its 14th year at death. It reached sexual maturity during its second to third year (Fig. 3). T4510 developed an external fundamental system (EFS after Horner et al., 2001; or outer circumferential layer, [OCL] after Chinsamy-Turan, 2005) from the age of 10 years onward. The EFS is marked by closely spaced LAGs and is composed of highly organised lamellar bone.

Generally, no or only a few primary osteons and no secondary osteons are found in the cortex of adult specimens. Before sexual maturity is reached, the long bones show several simple radial, and several simple longitudinal vascular canals (Fig. 3A–D; Fig. 5A–B). After sexual maturity, scattered primary osteons are displayed as well. On average of all the limb bones, the cortex is 5% less vascularised after the attainment of sexual maturity than before this event (average observed compactness before sexual maturity: 0.92).

In polarised light, the limb bones sometimes (e.g., T4510) reveal a distinct light and extinction pattern based on the different characteristics of the growth zones and the annuli within each growth cycle after the wp layers and before EFS. Although both growth zones and annuli are comprised of lamellar bone tissue, they can be differentiated by their thickness, by the alignment of the organisation of the crystallites which is reflected by the birefringence (Chinsamy-Turan, 2005; Glimcher and Muir, 1984), as well as by the quantity and shape of osteocyte lacunae (Francillon-Vieillot et al., 1990; Fig. 3E–H). Before the attainment of sexual maturity, the growth zones are thicker, exhibit more and rounder osteocyte lacunae and are less birefringent in polarised light, whereas the annuli are narrower and reveal a decreased number of flattened osteocyte lacunae, as well as higher birefringence (Fig. 3E–F). After sexual maturity, the growth zones become narrower, contain a lower number of flattened osteocyte lacunae and more equally directed crystallites, whereas the annuli are thicker with more rounder osteocyte lacunae and less birefringent. This distinct pattern continues until the EFS is reached. However, the resulting light and extinction pattern in polarised light is often poorly visible due to the strong coloration of the bone due to diagenesis.

3.3. Neusticosaurus pusillus and Neusticosaurus peyeri

Throughout ontogeny, the calcified cartilaginous core in the medullary region of the long bones of *N. pusillus* and *N. peyeri* is only partly replaced by secondary endosteal osseous infillings (Fig. 2). Only the medullary region in the diaphysis of the humerus in large specimens becomes entirely filled with secondary trabeculae (Sander, 1990).

Sander (1990) stated that *N. pusillus* and *N. peyeri* reached sexual maturity at the age of three to four. Upon reaching sexual maturity, the growth cycles abruptly decrease in thickness. The subsequent LAGs of the third and subsequent growth cycles gradually converge before the EFS, showing an asymptotic growth curve after sexual maturity (Fig. 2; Sander, 1990). The largest specimens of *N. pusillus* studied by Sander (1990) show seven LAGs (Fig. 2) and the age of *N. peyeri* is nine to ten years (Sander, 1990).

3.4. Neusticosaurus edwardsii

Some long bones (humerus, tibia and fibula) of the stratigraphically youngest pachypleurosaurid, *N. edwardsii*, entirely replace the calcified cartilaginous core of the diaphyseal, metaphyseal and even parts of the epiphyseal regions with secondary trabeculae and few secondary infillings of the vascular canals. In contrast, the radius, ulna and femur always show remnants of the calcified cartilage core of various sizes in the diaphyseal, metaphyseal and epiphyseal regions (Fig. 6A–H), closely resembling the composition of the long bones of the other pachypleurosaurids. Longitudinally oriented simple vascular canals (slvc) and secondary infillings of the vascular canals dominate the medullary region (when not entirely replaced by secondary trabeculae in large specimens; Fig. 5C–D). The long bones of *N. edwardsii* reveal large parts of, or even a complete distinct layer of endosteal lamellar bone (Figs. 4A–H, 6A–H) between the cortex and the medullary region. All the long bones show one innermost bone layer of wp (Fig. 4E–H).

The preaxial elements of both the forelimbs and hind limbs are slightly more compact than the corresponding postaxial elements, but show no statistically significant dif-

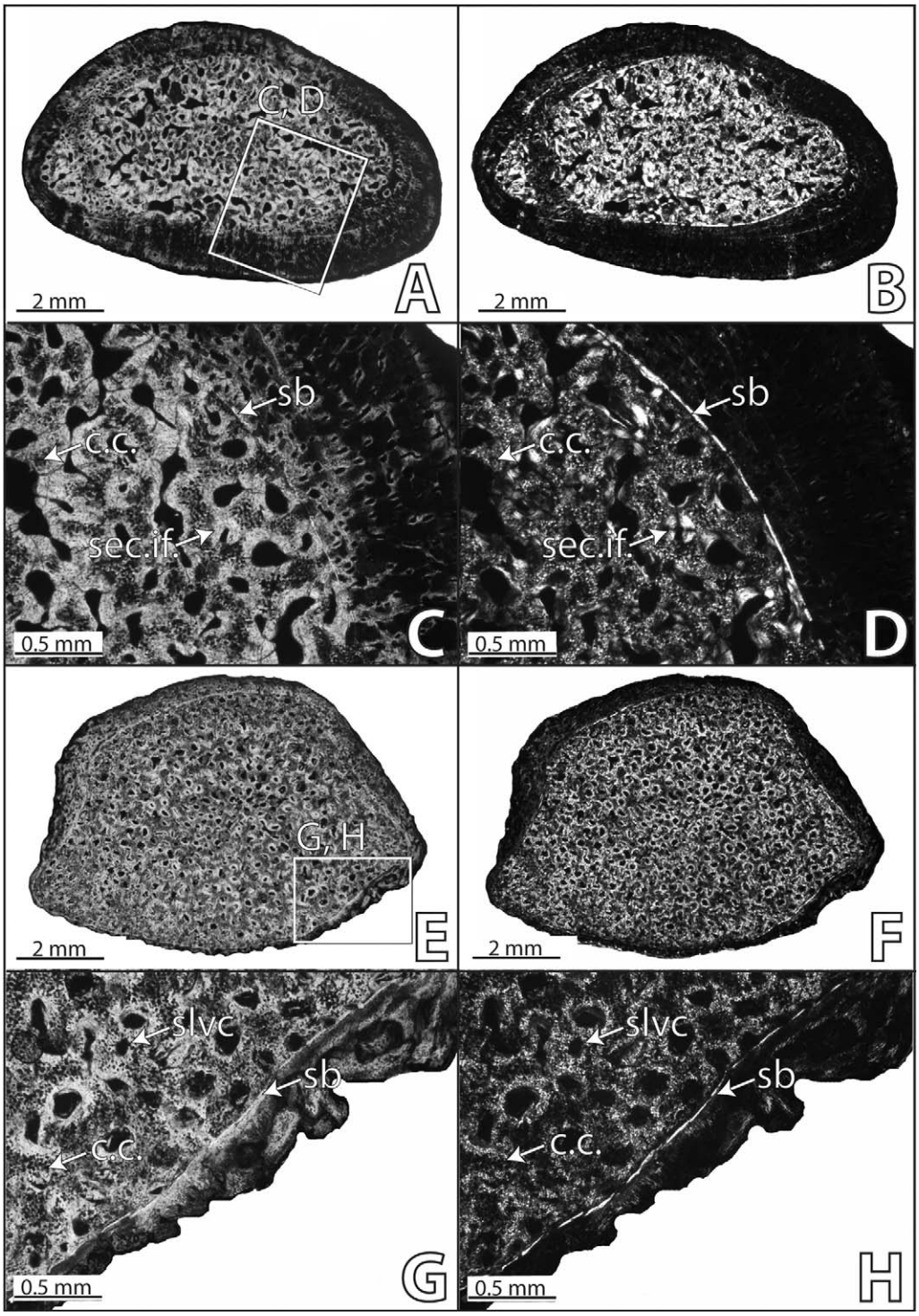

Fig. 6. Transverse sections of the femur of a large *Neusticosaurus edwardsii* (T4752). A, C, E, G in normal transmitted light; B, D, F, H in polarised light. Images A to D display the section through the metaphyseal region, whereas images E to H show the section through the epiphyseal region of the same individual. There is a sharp line (sb) bordering the medullary region and the cortex, which is composed of highly organised endosteal lamellar bone tissue showing high birefringence in polarised light. This sharp border is only preserved in *Serpianosaurus mirigiolensis* and *N. edwardsii*. The calcified cartilaginous core (c.c.) differs in the presence of secondary endosteal bony deposits (sec.if.). All the vascular canals of the metaphyseal region of the c.c. are widened and secondarily filled with bone (sec.if.), whereas the c.c. of the epiphyseal region still shows simple vascular canals (slvc).

ference in the mean compactness values and therefore, the null hypothesis cannot be rejected (paired *t*-test, $t = 4.3$; $P > 0.05$). In the limb bones of large *N. edwardsii*, erosion cavities are more numerous in the medullary region than in the equivalent region of the other pachypleurosaurids studied.

The attainment of sexual maturity is marked by the increased presence of double lines (sensu Castanet et al., 1993) or even more LAGs in the corresponding growth cycle (Fig. 4E–F). Accordingly, a change in the vascularisation (Fig. 4C–F; Fig. 5C–D), such as a higher number of srvc (simple reticular to radial vascular canals), a change in the light and extinction pattern (Fig. 4E–F), but no decrease in growth cycle thickness accompanies the onset of sexual maturity (Fig. 4C–F), contrary to what is observed in the vast majority of extant taxa. Before sexual maturity, the cortex houses a few primary osteons, several simple radial and longitudinal vascular canals (except for the humerus, Fig. 5C). After this event, shorter srvc and a few primary osteons are found in the cortex. Overall, the vascular canals of the cortex are more regularly distributed in *N. edwardsii* than in the other *Neusticosaurus* taxa and *S. mirigiolensis* (Figs. 2–5).

N. edwardsii appears to have reached sexual maturity between its fourth and seventh year (Table 1; Fig. 4C–F). The largest studied specimen of *N. edwardsii* (T3437) shows 22 growth cycles, indicating an age of 23 years (Fig. 4C–D). Sexual maturity is attained during its sixth to seventh year (Table 1; Fig. 4). The thickness of all growth cycles remains constant until the fifteenth growth cycle, time at which an EFS developed. Therefore, the EFS is the only event during the life time of *N. edwardsii* causing closely spaced LAGs.

4. Discussion

The pachypleurosaurids from Monte San Giorgio all exhibit pachyosteosclerotic limb bones with growth marks throughout the entire cortex. Differences among the four species are shown in the vascularisation of the cortex, as well as in the grade of remodelling processes which affect the innermost periosteal layers of the cortex and the calcified cartilaginous core. The pachypleurosaurids also differ in their life history traits, which are reflected in the spacing and light and extinction patterns of the growth cycles, as well as in the longevities and onset of sexual maturity. They all exhibit similar skeletal features when compared to those of Recent secondarily aquatic vertebrates, which live in shallow water and feed either on plants or slow moving/swimming prey (Buffrénil et al., 2010; Canoville and Laurin, 2009; Hugi and Sánchez-Villagra, unpublished; Laurin et al., 2009; Taylor, 2000). An increase in bone compactness is, in the case of the pachypleurosaurids, a result of the retention of the calcified cartilaginous core or its replacement by bony structures, as well as a result of the relatively thickened cortex.

The forelimb and hindlimb elements show similar compactness values in pachypleurosaurids, however, they vary in two aspects regarding: (1) the degree of vascularisation; (2) the replacement of the calcified cartilaginous core by bony tissue in the medullary region.

The histological differences between all four pachypleurosaurid taxa might result from size differences or adaptation to different habitats or different swimming modes. However, morphological (indicator of webbing in the forelimb and hind limb morphology, neural spines and transverse processes in the tail, snout-vent lengths and pachyostosis) and new skeletochronological data, such as the spacing pattern of the growth cycles, both clearly separate *S. mirigiolensis* and *N. edwardsii* from *N. pusillus* and *N. peyeri*. Data from sedimentary analyses support a habitat with more open marine influences for *S. mirigiolensis*, but more restricted lagoonal habitats for *N. pusillus*, *N. peyeri* and *N. edwardsii*.

All pachypleurosaurids from Monte San Giorgio lived in a subtropical climate with rhythmic monsoons as revealed by sedimentary analyses (Röhl et al., 2001). This strong seasonal climate may have led to the formation of distinct LAGs. Lamellar-zonal bone tissue type is found in generally slow growing ectothermic reptiles as well as in vertebrates showing synchronized endogenous rhythms (Castanet et al., 1993); with the latter often being enhanced when the animals live under strong environmental cycles (i.e. seasonality: effects on food availability and reproduction cycles).

The pachypleurosaurids from Monte San Giorgio give the rare opportunity to study the complete ontogenesis from embryos to adult specimens. All preserved embryos already show an advanced degree of ossification in their entire skeleton (e.g., Hugi and Scheyer, unpublished; Sander, 1988). *S. mirigiolensis* and *N. edwardsii*, which show no or only very little resorption of the innermost periosteal region of the cortex, always show the complete growth record and therefore, the presence of wp (caused by increased bone deposition rates) represents the ossification in this early stage of ontogenesis. Additionally, *S. mirigiolensis* and *N. edwardsii* always show a sharp border of lamellar bone between the cortex and the medullary region (Fig. 3C, D). This line is not preserved in *N. pusillus* and *N. peyeri*, because remodelling processes affect more parts of the innermost periosteal region of the cortex in these taxa. The sharp border is not referred to as a hatchling or neonatal line, since this line is continuously preserved within the entire bone and not only in the diaphyseal region (Fig. 6). With this first bone layer of wp being identified as embryonic bone, the first LAG bordering this layer

Fig. 6. Sections transversales dans le fémur d'un grand *Neusticosaurus edwardsii* (T4752). A, C, E, G sont en lumière transmise normale ; B, D, F, H en lumière polarisée. Les images A à D présentent la section à travers la région métaphysaire, tandis que les images E à H montrent la section à travers la région épiphysaire du même individu. Il y a une ligne nette (sb) à la limite de la région médullaire et du cortex, qui est composée d'un tissu osseux lamellaire de l'endoste, très organisé, montrant une forte biréfringence en lumière polarisée. Cette limite nette n'est conservée que chez *Serpianosaurus mirigiolensis* et *N. edwardsii*. Le cœur cartilagineux calcifié (c.c.) diffère par la présence de dépôts osseux secondaires d'endoste (sec.if.). Tous les canaux vasculaires de la région métaphysaire du c.c. sont élargis et secondairement remplis d'os (sec.if.), tandis que le c.c. de la région épihysaire présente encore des canaux vasculaires simples (slvc). c.c. : cartilage calcifié ; wp : os à entrelacs fibreux passant au type à fibres parallèles.

represents a possible hatching line. Parts of the wp are also very often preserved in *N. pusillus* and *N. peyeri* and, based on comparison with the histological characteristics in the other two pachypleurosaurids, are also identified as embryonic bone here.

4.1. Comparison with eusauropterygians

The eusauropterygian *Nothosaurus* from Winterswijk, for example, also shows the plesiomorphic lamellar-zonal bone tissue type, but with a varying spacing pattern of growth cycles (Klein, 2010). Basal pistosauroids often show constant growth cycle thicknesses up to a certain age, but, in contrast to the pachypleurosaurids sampled, also generally exhibit phases of less organised fibrolamellar layers between the parallel-fibered growth cycles (Klein, 2010). The lamellar-zonal bone tissue type generally results from a lower bone deposition rate, which indirectly reflects the metabolism of the animal (Castanet, 2006; Castanet et al., 1993). The frequent increase in the growth rate (i.e., presence of fibrolamellar bone tissue) might be linked to an increased metabolism, but could also be simply size-related (Cubo et al., 2005), which probably enabled at least the (basal) pistosauroids to spread further through the colder seas as they were the only sauropterygians not restricted to the Tethys (Klein, 2010; Rieppel, 2000).

4.2. Comparison with extant lepidosaurs

Lepidosaurs are the hypothesised living sister group of the Sauropterygia (Hill, 2005; Müller, 2003). The spacing pattern of the growth cycles of the pachypleurosaurids can be compared to that of Recent lacertilian squamates because they also show the lamellar-zonal bone tissue type (Castanet et al., 1993; Francillon-Vieillot et al., 1990). *N. pusillus*, *N. peyeri* and Recent terrestrial squamates show a similar spacing pattern of the growth cycles (Castanet and Baez, 1991; Castanet and Naulleau, 1985) and therefore, a similar life history (including the onset of sexual maturity) is inferred. The growth cycle thicknesses of *N. edwardsii* are the only ones that remain constant throughout the cortex until the EFS is developed; the condition closely resembling the one found in the marine iguanas, *Amblyrhynchus cristatus*, from the Galapagos islands (Hugi and Sánchez-Villagra, unpublished). These animals are the only living lacertilian squamates that forage exclusively in the sea (Trillmich and Trillmich, 1986). A comparison to the spacing pattern of terrestrial lizards shows that the distinct spacing pattern of constant growth cycle thicknesses of *A. cristatus* most possibly reflects secondary adaptation to a life in water, since it is not found in any of its terrestrial relatives (Hugi and Sánchez-Villagra, unpublished). In *A. cristatus,* sexual maturity is histologically indicated by the higher presence of supernumerary LAGs as well as a change in the light and extinction pattern, but neither by an abrupt decrease of growth cycle thickness nor by a change in the bone tissue type (i.e. lamellar-zonal bone tissue type is present throughout the cortex; Hugi and Sánchez-Villagra, unpublished).

Ecological data shows that this histological change occurs at the time when sexual maturity is reached (Trillmich and Trillmich, 1984). According to these authors, the onset of sexual maturity in marine iguanas is reflected in a change of the behaviour in both females and males. Males start to show strong territorial behaviour including male competition to monopolise the access to females. Females, on the other hand, aggregate once a year to lay their eggs on beach sites, which are geographically separated from the normal colony sites of all marine iguanas. In both sexes, these behavioural changes start with the onset of sexual maturity (Trillmich and Trillmich, 1984). In accordance with these observations, we conclude, that sexual maturity in *N. edwardsii* was reached well before the EFS developed. According to our interpretations the cortex of all the limb bones of *N. edwardsii* is about 7% less vascularised after the onset of sexual maturity, than before this event (average observed bone compactness before sexual maturity: 0.86).

In *A. cristatus* the histological changes, especially the light and extinction pattern, described above might reflect the annual reproduction cycle in this species. This hypothesis is supported by slight differences of this pattern between male and female specimens, as well as by the timing when the pattern changes (Hugi and Sánchez-Villagra, unpublished). The change of the growth zones and the annuli of *A. cristatus* may result from the changing availability of food, climate and the associated mating behaviour, which is sex-dependant (Trillmich and Trillmich, 1984). In pachypleurosaurids, this light and extinction pattern is indicated in *S. mirigiolensis* and in *N. edwardsii.* It is either not found in the other pachypleurosaurids or simply obscured due to the strong diagenetic coloration of the bones. *S. mirigiolensis* additionally shows a mixture of the spacing pattern between *N. pusillus* and *N. peyeri* on the one hand, and the stratigraphically youngest pachypleurosaurid *N. edwardsii* on the other. The prolonged phase of bone deposition after the onset of sexual maturity might be a result of the secondary aquatic adaptation in these rather small reptiles.

Shine and Charnov (1992) studied the growth rates in Recent squamates and showed that large forms tend to mature well before maximum body size is reached, which appears to be the plesiomorphic reptilian condition (Castanet et al., 1988; Sander, 2000). Phylogenetic analysis confirmed that the evolutionary increase in maximum body size is accompanied by a decrease in the relative size at sexual maturity (Andrews, 1982; Shine and Charnov, 1992). In summary, large squamates generally display a constant decrease in growth rate after sexual maturity is reached, whereas smaller ones terminate growth at sexual maturity or shortly after this event (Andrews, 1982; Shine and Charnov, 1992). Changes in growth rate are generally reflected in the bone deposition rate and therefore, in the histology of an individual (Castanet, 1985; Horner et al., 2000; Padian et al., 2001). Small lizard taxa often display an EFS, which is developed earlier during ontogenesis and which occurs soon after sexual maturity (Ortega-Rubio et al., 1993; Zug and Rand, 1987). The early development of the EFS exactly reflects the results of the growth analysis by Andrews (1982) and Shine and Charnov (1992). Large reptiles, in contrast, frequently increase their growth cycle thickness slightly again after this event for one or

more growth cycles. The subsequent LAGs then align slowly and continuously closer until an EFS is developed (Castanet et al., 1988; Chinsamy et al., 1995; Chinsamy-Turan, 2005; Erickson, 2005; Hugi and Sánchez-Villagra, unpublished; Saint Girons et al., 1989). However, in both cases, the first abrupt decrease in growth cycle thickness was verified as the time when sexual maturity is reached. This first abrupt decrease in growth cycle thickness occurs in size class B in *S. mirigiolensis* (Table 1) and in size class F in *N. pusillus* and *N. peyeri* (Sander, 1990). This histological change corresponds to the first appearance of sexual dimorphism in these taxa (Rieppel, 1989; Sander, 1989, 1990). The development of the EFS only occurs in larger size classes and therefore, does not coincide with the timing when sexual dimorphism is expressed for the first time (Fig. 2: T4178: size class I). The distinction between the two sexes in *S. mirigiolensis, N. pusillus* and *N. peyeri* are clearly expressed for example by the ratio of the maximum distal width of the humerus to the minimum width of the humerus (i.e., mid shaft width) (Sander, 1989). In Rieppel (1989) the same data were given, but the ratios were inverted, i.e., minimum width to maximum distal width of the humerus. This ratio, recorded for all four pachypleurosaurids, shows two morphotypes (summary given in Sander (1989): page 631, table 9), interpreted as sexual dimorphism between sex x and sex y. Measurements of *N. edwardsii* established from Carroll and Gaskill (1985) reveal a first occurrence of the sexual dimorphism at size class B. The histological data for the attainment of sexual maturity coincides with the timing of the first appearance of the sexual size dimorphism (Table 1). In contrast, the development of an EFS only occurs in larger specimens (Table 1) and therefore, does not indicate sexual maturity.

5. Conclusion

The four pachypleurosaurid taxa from Monte San Giorgio differ from one another in an increasing number of morphological and histological features. A comparison with the lacertilian squamates reveals a similar spacing pattern of the growth cycles in completely terrestrial members and suggests similar life history (age at sexual maturation, minimum age at death) for *N. pusillus* and *N. peyeri*. On the other hand, the spacing pattern of *N. edwardsii* resembles that of the marine iguana, *Amblyrhynchus cristatus*. *S. mirigiolensis* shows a mixture between the more "terrestrial-like" spacing pattern of *N. pusillus* and *N. peyeri* and the more "aquatic-like" one of *N. edwardsii*.

Skeletochronological data of *N. edwardsii* also support similarities to the marine iguana by revealing comparable elongated phases of low bone deposition rates, prolonged longevities and a delayed sexual maturity. Histology separates the pachypleurosaurids from *A. cristatus* by the vascularisation of the cortex and the retention of the calcified cartilaginous core in the medullary region. The stratigraphically youngest pachypleurosaurid, *N. edwardsii*, might be more adapted to a life in the pelagic zone compared to all the other pachypleurosaurids based on higher vascularisation of the bone tissue, the more extensive replacement of the calcified cartilaginous core by osseous deposits and based on the constant growth cycle thicknesses in the cortex. *S. mirigiolensis* was common in marine environments with higher open sea influences, although showing a mixture of the spacing pattern of *N. edwardsii* and the other *Neusticosaurus* spp. The data of *S. mirigiolensis* might reflect a less developed adaptation compared to *N. edwardsii* to a similar habitat as a result of its older stratigraphical age.

Therefore, the varying spacing pattern, the grade of the secondary replacement of the cartilaginous core and the vascularisation of the cortex are assumed to be linked to different habitat preferences among pachypleurosaurids.

Our interpretations of the sexual maturity and the EFS in *N. edwardsii* appear to be different from the usual reptilian pattern. Indeed, scepticism about the interpretation of the age of sexual maturity has been expressed in the case of *N. edwardsii*, which does not show an abrupt decline in the growth cycle thickness before the EFS. However, the first appearance of sexual dimorphism in *N. edwardsii* coincides with histological change in the characteristics of the growth zones and annuli, the change in vascularisation, as well as with the higher number of supernumerary LAGs.

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