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Bone shell microstructure of *Condorchelys antiqua* Sterli, 2008, a stem turtle from the Jurassic of Patagonia

Microstructure de la carapace de Condorchelys antiqua *Sterli, 2008, une tortue souche du Jurassique de Patagonie*

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ABSTRACT

The histology of the turtle shell has proven to be a valuable source of characters for the study of development and shell origin, as well as early turtles' lifestyles and systematics. Here we describe and discuss the shell bone microanatomy and histology of *Condorchelys antiqua*, a stem turtle from the Lower Jurassic of Patagonia. Examination of several elements (including costals, neurals, peripherals and plastral plates) reveals that the shell bones consist of a diploe structure in which the interior area of cancellous bone is framed by external and internal cortices. Whereas the external cortex is mostly composed of structural fiber bundles, histological composition of the internal cortex is more variable, exhibiting structural fiber bundles and/or parallel fibered bone. This histological variation in the internal cortex allows discerning between different types of plates. The cancellous bone is mostly well developed and consists of fine trabeculae composed of secondary lamellar bone tissue. Predominance of structural fiber bundles in the shell bones suggests that metaplasia plays an important role during the development of the shell in this taxon. Comparison with other stem Testudines (including *Proganochelys quenstedti*, *Proterochersis robusta*, *Kayentachelys* sp., *Eileanchelys waldmani*, and *Heckerochelys romani*) reveals that the shell histology of *C. antiqua* resembles more *H. romani* and *E. waldmani* than the other stem taxa. The obtained values of compactness of *C. antiqua* shell bones suggest an aquatic lifestyle for this taxon.

Mots clés :
Groupe souche Testudinés
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RÉSUMÉ

Il n'est plus à prouver que l'histologie de la carapace de tortue dans les taxons fossiles est un outil précieux pour résoudre de nombreuses questions concernant l'origine et le développement de la carapace, le mode de vie et la systématique. Nous décrivons et discutons ci-dessous la micro-anatomie et l'histologie de la carapace de *Condorchelys antiqua*, une souche tortue du Jurassique inférieur de Patagonie. L'observation de plusieurs éléments (notamment les plaques osseuses costales, neurales, périphériques et du plastron)

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révèle que la carapace consiste en une structure diploë dans laquelle la partie interne de l'os spongieux est entourée par des cortex interne et externe. Bien que le cortex externe soit principalement composé des fibres structurales, la composition histologique du cortex interne est plus variable, montrant des fibres structurelles et/ou pseudo-lamellaires. Cette variation histologique du cortex interne permet de discerner différents types de plaque osseuse. L'os spongieux est principalement bien développé et consiste en fines trabécules composées de tissu osseux lamellaire secondaire. La prédominance des fibres structurelles dans la carapace suggère que la métaplasie de l'os joue un rôle important pendant le développement de la carapace pour ce taxon. Les comparaisons avec d'autres testudinés du groupe souche (incluant *Proganochelys quenstedti*, *Proterochersis robusta*, *Kayentachelys* sp., *Eileanchelys waldmani*, et *Heckerochelys romani*) révèlent que l'histologie de la carapace de *C. antiqua* ressemble plus à celles de *H. romani* et *E. waldmani* qu'à celle d'autres taxons souches. Les valeurs de densité de la carapace de *C. antiqua* suggèrent un mode de vie aquatique.

1. Introduction

The turtle shell is the most striking anatomical feature of the turtles and one of the most specialized skeletal structures in vertebrates. This unique structure is composed of dorsal and ventral portions, termed the carapace and plastron, respectively (e.g., Zangerl, 1969). Generally, the carapace consists of eight neurals, eight costals, twenty-two peripherals (eleven on each side), a nuchal, one or two suprapygals and a pygal plate. From anterior to posterior, the plastron is organized into the paired epiplastra, a single entoplastron, two hyo-, two hypo- and two xiphiplastra. In some extinct taxa, the plastron also had one pair (e.g., *Kayentachelys aprix*) or two pairs (e.g., *Proterochersis robusta*) of mesoplastra between the hyo- and hypoplastra. In general, the morphology and anatomical variation of the turtle shell in extinct taxa has been historically well studied. However, its microstructure has just recently attracted the interest of palaeontologists, alongside a renewed interest in paleohistological studies in vertebrates in general.

The histology of turtle shell bones in fossil taxa has proven to be a very important tool for addressing several issues, including: shell origin and development (Delfino et al., 2013; Scheyer and Sánchez-Villagra, 2007; Scheyer et al., 2007), paleoecology (Scheyer and Sander, 2007; Scheyer et al., 2014a), and systematics (Lyson et al., 2013; Scheyer and Anquetin, 2008; Scheyer, 2009). Given the relevance of the microstructure of the shell bones, histological descriptions of these elements have also been included in recent anatomical and systematic studies of extinct turtles (e.g., Cadena et al., 2013; Gaffney et al., 2008; Marmi et al., 2009; Scheyer et al., 2014b; Slater et al., 2011; Sterli et al., 2013a, 2013b). Whereas the shell microstructure of several taxa of the crown Testudines has been studied (e.g., Scheyer, 2009; Scheyer and Sánchez-Villagra, 2007; Scheyer and Sander, 2007; Scheyer et al., 2007, 2014a), detailed histological studies on stem taxa are rather scarce, particularly in those from the Triassic and Jurassic periods. In this regard, histological features of *Proganochelys quenstedti* and *Pr. robusta* provided evidence for terrestrial palaeoecology in these Triassic taxa (Scheyer and Sander, 2007). On the contrary, recent detailed histological descriptions of *Eileanchelys waldmani* and *Heckerochelys romani* corroborated the aquatic life style of these Middle Jurassic stem Testudines (Scheyer et al., 2014a).

In this contribution we perform a detailed study of the bone shell histology of *Condorchelys antiqua*, a stem testudine from the Lower Jurassic of Patagonia, Argentina (Cúneo et al., 2013; Sterli, 2008). This taxon was named and described on the basis of several cranial and postcranial elements, all of them collected from Queso Rallado locality (Chubut Province, Argentina) (Sterli, 2008; Sterli and de la Fuente, 2010). *Condorchelys antiqua* is a small turtle (shell length circa 20 cm) showing a mixture of plesiomorphic (e.g., interpterygoid vacuity, absence of processus trochlearis) and derived characters (e.g., absence of palatal teeth). Its characteristics and geographic and stratigraphic location make *C. antiqua* the most complete, continental turtle from the Jurassic of Gondwana yielding valuable information to understand the early evolution of the clade in the southern continents. Furthermore, the relative abundance of material assigned to *C. antiqua* offers an excellent opportunity to document the bone shell histology of a Jurassic stem Testudines, allowing to discern some important paleobiological and systematic issues.

The main goals of the current work include:

- characterizing the bone shell microstructure of *C. antiqua*;
- determining the degree of histological variation among different elements (e.g., costal and neural plates) and the same element in different individuals;
- recognizing the presence of histological characters with systematic value;
- comparing the shell histology with other Jurassic stem Testudines;
- inferring the lifestyle of *C. antiqua* from its shell bone microstructure.

Institutional abbreviations: MPEF-PV: Museo Paleontológico Egidio Feruglio, Paleontología de Vertebrados, Trelew, Chubut, Argentina.

2. Materials and methods

All specimens were recovered from the Queso Rallado locality (Rauhut et al., 2002; Sterli, 2008), 5.5 km west of Cerro Cóndor Village, Chubut Province, Argentina. Queso Rallado consists of outcrops of the Cañadón Asfalto Formation (Stipanicic et al., 1968), which is considered to be Early to Middle Jurassic in age (Cúneo et al., 2013; Nullo and Proserpio, 1975; Tasch and Volkheimer, 1970; Volkheimer et al., 2009). The Cañadón Asfalto Formation is represented by calcareous, silicoclastic, and volcanic deposits related to the evolution of the Cañadón Asfalto Basin (Figari et al., 1996, and references cited therein). Rougier et al. (2007) interpreted the depositional environment of this formation as a perilittoral flood plain and meandering fluvial deposits in association with small, shallow lacustrine bodies. Specifically, the bone-bearing bed of Queso Rallado locality is a restricted horizon of 50 cm of thickness composed of thin interlaminated tuffaceous and calcareous deposits (Rougier et al., 2007). In general, the vertebrate remains found in this bone-bed are usually isolated, showing evidence for a certain amount of transportation (Rougier et al., 2007). The turtles, in particular, are sometimes an exception, because many specimens have been found articulated (e.g., MPEF-PV 1783A, 3132, 3147; see Sterli and de la Fuente, 2010). All the known shells or skulls attributed to *C. antiqua* show similar characteristics, not showing morphological or size variation. This homogeneity found in the most common elements (e.g., carapaces, skulls) allowed Sterli (2008) and Sterli and de la Fuente (2010) to assign all the turtle remains found up to now in Queso Rallado to *C. antiqua*.

For the microstructural analysis of *C. antiqua* 41 thin sections from 23 shell bones have been sampled, including three neurals (MPEF-PV 10883, 10852, 10853), 13 costals (MPEF-PV 1783A, 10845-10851A, 10856A-10858A, 10859), two peripherals (MPEF-PV 10854, 10855) and five hyo-hypoplastra (MPEF-PV 10841-10844, 10851B) (Table 1). To date, this is the largest histological sample for a single fossil turtle taxon. Planes of sectioning varied among the different shell bone elements: neurals were cut perpendicular to their anteroposterior axis (perpendicular to the vertebrae); costals were cut parallel to the anteroposterior axis (perpendicular to the incorporated ribs); peripherals were cut perpendicular to the anteroposterior axis of the turtle shell; plastron elements were mainly cut parallel or slightly oblique to the anteroposterior axis of the turtle shell (Fig. 1). Specimens were prepared for thin sections based on the methodology outlined in Chimsamy and Raath (1992). The preparation of the histological sections was carried out in the MPEF (Trelew, Argentina). The slices were studied using petrographic polarizing microscopes (LabKlass and Nikon E400). Nomenclature and definitions of structures used in this study are derived from Francillon-Vieillot et al. (1990). The terms “external” and “internal” are used throughout the text instead of “dorsal” and “ventral” to prevent confusion between dorsal carapacial and ventral plastral bones of the turtle shell (e.g., the “dorsal” surface of a neural plate corresponds to the external surface of the bone, whereas the “dorsal” surface of a plastral plate corresponds to the visceral surface of the bone). The terms “inner/outer” refer to the relative position within the cortical bone, toward the core or toward the surface of the plate, respectively.

To infer the lifestyle of *C. antiqua* based on its shell bone histology, a compactness analysis was carried out. For this, compactness parameters were calculated using the Windows version 4.5.5 of Bone Profiler (Girondot and Laurin, 2003). To be used with the program, images of the sectioned bones were transformed into black and white (black for bone and white for vascular spaces). Matrix was digitally erased from the intertrabecular spaces using Photoshop CS2. Because several of the sampled plates are incomplete or crushed, we performed the compactness analysis for four complete elements, including three costals (MPEF-PV 10847, 10849, 10851) and one peripheral (MPEF-PV 10854). The obtained results were compared with those published previously for other crown-group taxa (Pérez-García et al., 2013; Scheyer et al., 2014a).

Histological Abbreviations: CB: cancellous bone; ECO: external cortex; ICO: internal cortex; ITS: inter-trabecular spaces; LB: lamellar bone; LSFB: longitudinally sectioned structural fiber bundles; ShF: Sharpey’s fibers; SFB: structural fiber bundles; TSFB: transversally sectioned structural fiber bundles.

Table 1
Shell bones of *Condorchelys antiqua* sectioned for this study.
Tableau 1
Os de la carapace de *Condorchelys antiqua* sectionnée pour cette étude.

Element	Collection number	Number of sections
Costal plate	MPEF-PV 10857	1
Costal plate	MPEF-PV 10850	1
Carapace fragment (costal plates)	MPEF-PV 1783A	2
Small costal plate	MPEF-PV 10845	1
Distal portion of a costal plate	MPEF-PV 10846	1
Proximal portion of a costal plate	MPEF-PV 10847	1
Distal portion of a costal plate	MPEF-PV 10848	2
Medial portion of a costal plate	MPEF-PV 10849	4
Costal plate	MPEF-PV 10851A	2
Costal plate	MPEF-PV 10858A	2
Costal plate	MPEF-PV 10856A	1
Costal plate	MPEF-PV 10859	3
Neural plate	MPEF-PV 10883	2
Neural plate	MPEF-PV 10852	2
Neural plate	MPEF-PV 10853	2
Peripheral plate	MPEF-PV 10854	4
Peripheral plate	MPEF-PV 10855	2
Hyo/hypoplastron	MPEF-PV 10841	4
Hyo/hypoplastron	MPEF-PV 10842	1
Hyo/hypoplastron	MPEF-PV 10843	1
Hyo/hypoplastron	MPEF-PV 10844	2
Plastron?	MPEF-PV 10851B	2

3. Results

Costal, neural and plastral plates of *C. antiqua* are thin elements. The sectioned bridge peripheral plates exhibit a sub-triangular or “L” shape in cross section, with a concave internal surface and a convex external surface. A dorsal projection is observed in both sampled peripheral plates. All shell bones of *C. antiqua* show a diploe structure with external cortex and internal cortex framing an interior area of cancellous bone (Fig. 2). In peripheral plates,

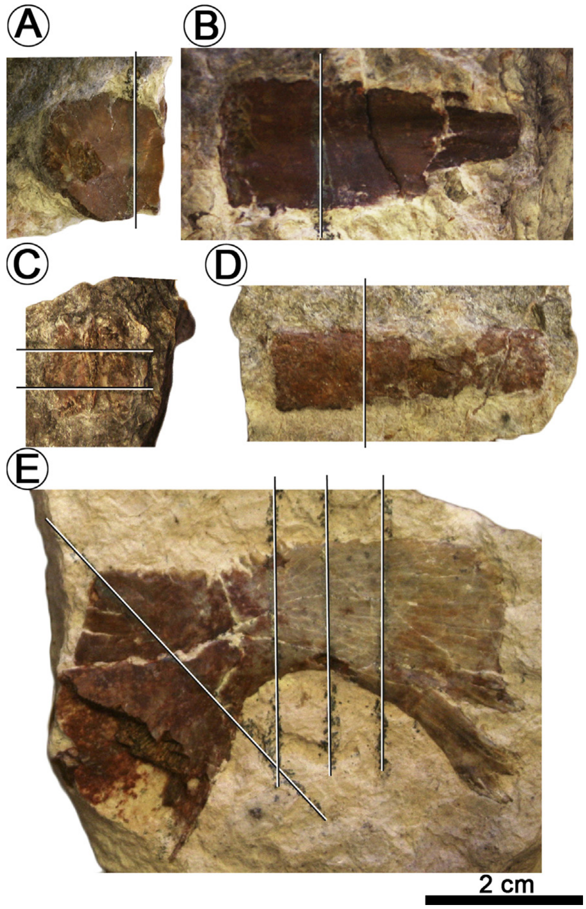

Fig. 1. (Color online.) Some of the selected bony plates before sectioning. A. MPEF-PV 10847, proximal portion of costal plate. B. MPEF-PV 10846, medial and distal portions of costal plate. C. MPEF-PV 10853, neural plate. D. MPEF-PV 10854, peripheral plates. E. MPEF-PV 10841, hyo-hypoplastron. Straight lines indicate the plane of sections.

Fig. 1. (Couleur en ligne.) Quelques plaques osseuses sélectionnées avant section. A. MPEF-PV 10847, partie proximale de l'écaille costale. B. MPEF-PV 10846, parties médiane et distale de l'écaille costale. C. MPEF-PV 10853, écaille neurale. D. MPEF-PV 10854, plaques osseuses périphériques. E. MPEF-PV 10841, hyo-hypoplastron. Les lignes droites indiquent le plan des sections.

the external cortex can be divided in dorsal and ventral portions, which merge in the lateral side of the plate. The cancellous bone is well developed in most of the samples and occupies the majority of the cross-sectional area. Because the sampled shell elements share several histological features, we describe them together. Histological variation among samples is mentioned if applicable.

3.1. External cortex

The external cortex is thinner than the internal cortex in plastral plates, whereas cortices of the costal, neurals and peripherals are of similar thickness. In most of the costal, neural, peripheral and plastral plates the external cortex consists entirely of a compact bone tissue of structural fibers, which commonly exhibits two main levels of organization (Fig. 3A–C). The first level corresponds to small bundles with diameters of 8–10 μm. These fiber bundles can be distinguish from each other when they are transversely sectioned (Fig. 3C). The second level of organization corresponds to larger bundles of structural fibers, in which the smaller bundles are arranged parallel to each other. These larger bundles of structural fibers exhibit a variable pattern of arrangement. To describe the spatial patterns in more detail, we refrain from uniformly classifying them as 'interwoven structural collagenous fiber bundles' (sensu Scheyer and Sánchez-Villagra, 2007) herein. Whereas in some areas the structural fibers are loosely packed, oriented parallel or sub-parallel to the external surface, in others, these fibers are tightly packed and oriented in different directions, forming an interwoven meshwork. Despite the observed variation in the arrangement of structural fibers, they are predominantly arranged sub-parallel to the external surface. In neural plates, the structural fibers are usually more loosely packed toward the lateral margins where they resemble typical parallel fibered bone. In the lateral margin of the peripheral plates, the intrinsic fibers of the external cortex become oriented parallel to the antero-posterior carapace axis (perpendicular to the plane of section) (Fig. 3D). Bone cell lacunae possess an elongated shape, which appears to correspond to a scalene ellipsoid (D'Emic and Benson, 2013). These lacunae are commonly arranged following the orientation of the structural fibers in which they are embedded. Branching canaliculi are mostly well preserved. Although the distribution of the bone cell lacunae is rather homogenous, they cluster in some regions, principally toward the inner portion of the cortex. In this sense, clusters of bone cell lacunae are also present in the lateral border of the peripheral plates.

The degree of vascularization of the external cortex is variable, even within a single type of plate. Whereas costal and peripheral plates commonly exhibit moderate to low vascularization, neural and plastral plates are mostly well vascularized. In general terms, vascularization consists of small simple canals and primary osteons. Larger vascular spaces are present in some specimens (e.g., costal MPEF-PV 10851A and neural MPEF-PV 10853). Although the vascular spaces are mainly longitudinally oriented, oblique and vertical canals are also present. Vascular spaces are commonly more abundant in the inner portion of the cortex. Vertical canals open to the outer surface are abundant in few specimens (e.g., MPEF-PV 10859).

Sharpey's fibers are present only in some samples (e.g., costal MPEF-PV 10859, neural MPEF-PV 10883). When present, these extrinsic fibers are short and they are oriented mostly perpendicularly or oblique to the outer surface (Fig. 3E). Growth marks are rather uncommon in the external cortex. When present (e.g., costal MPEF-PV 10859, neural MPEF-PV 10883) they resemble typical lines of arrested growth (i.e. kind of cementing lines that represent temporary arrests of local osteogenesis; Castanet et al., 1993).

Variations to the general histological pattern of the external cortex described above were recorded in some samples. In some cases (e.g., costal MPEF-PV 10847), the intrinsic fibers exhibit diffuse variation in their arrangement and they are roughly organized as typical parallel fibered bone (Fig. 3F). The external cortex in the costal plate

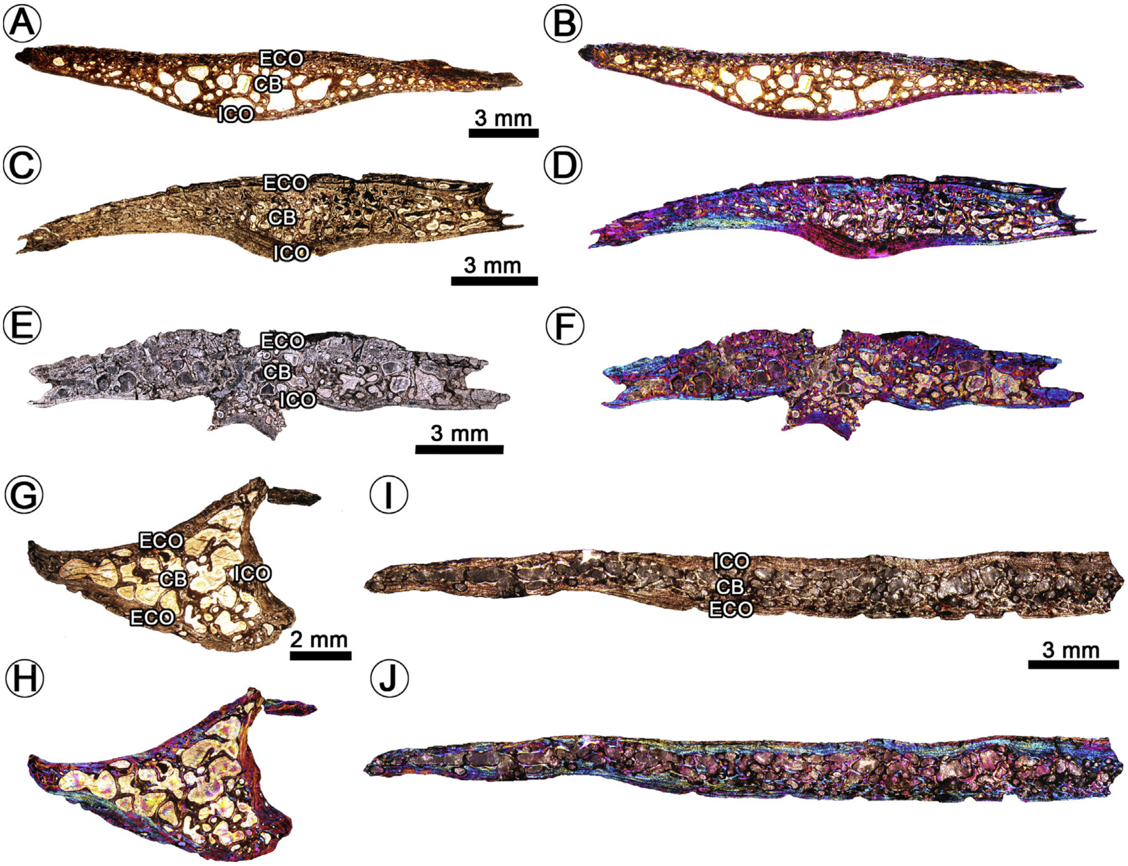

Fig. 2. (Color online.) General view of selected shell bones of *Condorchelys antiqua* in cross section. A, B. MPEF-PV 10849, costal plate. C, D. MPEF-PV 10851A, costal plate. E, F. MPEF-PV 10883, neural plate. G, H. MPEF-PV 10854, peripheral plate. I, J. MPEF-PV 10844, hyo/hypoplastron. A, C, E, G, I. Normal transmitted light. B, D, F, H, J. Cross-polarized light with lambda compensator.

Fig. 2. (Couleur en ligne.) Vue générale de certaines plaques osseuses de la carapace de *Condorchelys antiqua* en coupe transversale. A, B. MPEF-PV 10849, écaille costale. C, D. MPEF-PV 10851A, écaille costale. E, F. MPEF-PV 10883, écaille neurale. G, H. MPEF-PV 10854, écaille périphérique. I, J. MPEF-PV 10844, hyo/hypoplastron. A, C, E, G, I. Lumière transmise naturelle. B, D, F, H, J. Lumière polarisée avec compensateur lambda.

MPEF-PV 10859 (Fig. 3G) can be differentiated in two distinct zones, outer and inner. The outer zone is composed of lamellar bone and parallel fibered bone (Fig. 3H). This bone appears to be formed during successive episodes of cortical resorption and redeposition, which is evidenced by the presence of resorption lines. The bone tissue located in the inner zone is composed of structural fibers. A similar pattern is observed in the plastral plate MPEF-PV 10844, in which the external cortex constitutes parallel fibered bone formed during different stages of bone erosion and formation. The cortical bone in this plate is almost avascular, with the sole exception of large scattered resorption cavities, some secondary osteons, and few simple vascular spaces.

3.2. Cancellous bone

The cancellous bone is well developed in most of the samples and commonly occupies the majority of the cross sectional area. It comprises slender trabeculae (Fig. 4A) of different sizes. Inter-trabecular spaces are circular or subcircular in section. Some of these spaces coalesce to form larger cavities. The trabeculae themselves are composed of centripetally deposited secondary lamellar bone formed over different growth generations (Fig. 4B, C). Flattened bone cell lacunae follow the centripetally deposited lamellar bone linings. Independent of the plane of section, the bone cell lacunae are strongly flattened. Canaliculi of the lamellar bone tissue are radially oriented from the inter-trabecular cavity. There is no apparent variation in the fiber orientation between successive lamellae deposited in a single generation. Not all spaces are entirely lined by lamellar bone. Interstitial areas within the trabeculae are both composed of secondary and primary bone. The primary bone resembles that described for the external cortex. Histological variation among the sampled specimens is mostly attributed to the relative length and width of the bony trabeculae and the size of the inter-trabecular spaces (Fig. 2). In this sense, larger inter-trabecular spaces were observed in costal MPEF-PV 10856, in which some cavities extend to both the internal and external cortices. On the other hand, in the costals MPEF-PV 10851 and MPEF-PV 10848, MPEF-PV 10849 and in the neural MPEF-PV 10852, the bony trabeculae are short and thick and the inter-trabecular spaces are relatively smaller in comparison to the other plates. A particular feature was observed in the cancellous

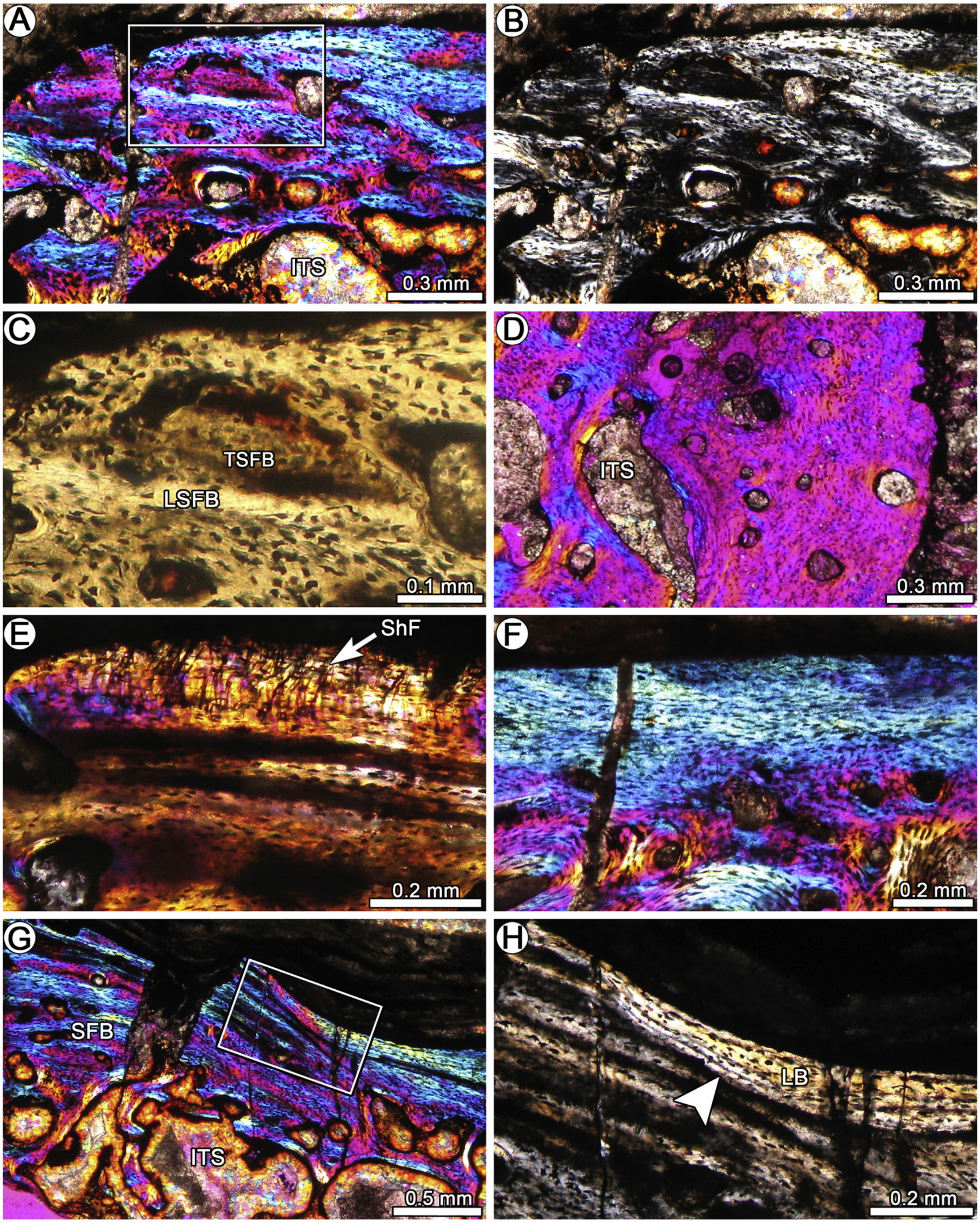

Fig. 3. (Color online.) Histology of external cortex of *Condorchelys antiqua* shell bones. A, B. Cortical bone of costal MPEF-PV 10859 composed of structural fiber bundles. C. Detailed view of the structural fibers (box inset in A). D. Compact bone of lateral region of peripheral MPEF-PV 10855. E. Sharpey's fibers in outer cortex of costal MPEF-PV 10859. F. Cortical bone of costal MPEF-PV 10847 composed of parallel fibered bone. G. General view of the partially remodeled cortex in costal MPEF-PV 10859. H. Enlarged view of the same picture (box inset in G). The arrowhead indicates the presence of a resorption line. A, D–G. Cross-polarized light with lambda compensator. B, H. Cross polarized light. C. Normal transmitted light.

Fig. 3. (Couleur en ligne.) Histologie du cortex externe des plaques osseuses de la carapace de *Condorchelys antiqua*. A, B. Cortex de l'écaille costale MPEF-PV 10859 composé de faisceaux de fibres structurales. C. Vue détaillée des fibres structurales (provenant de A). D. Os compact de la région latérale de l'écaille périphérique MPEF-PV 10855. E. Fibres de Sharpey dans le cortex externe de l'écaille costale MPEF-PV 10859. F. Cortex de la plaque costale MPEF-PV 10847 composé de tissue osseux à fibres parallèles. G. Vue générale du cortex partiellement remodelé de l'écaille costale MPEF-PV 10859. H. Vue rapprochée de la même photographie (provenant de G). La flèche indique la présence d'une ligne de résorption. A, D–G. Lumière polarisée avec compensateur lambda. B, H. Lumière polarisée. C. Lumière transmise naturelle.

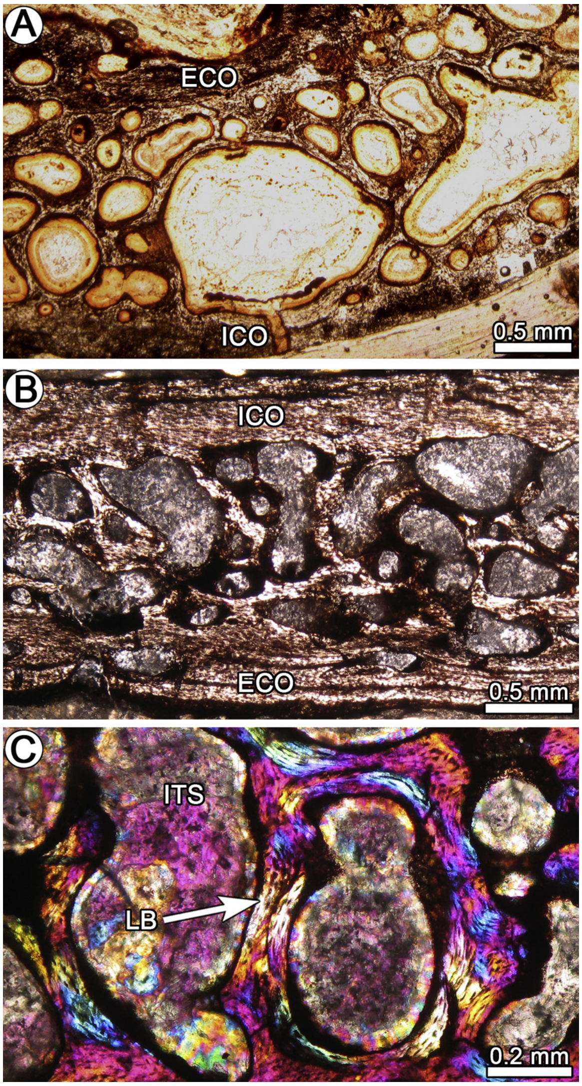

Fig. 4. (Color online.) Cancellous bone in *Condorchelys antiqua* shell plates. A. General view of a costal plate (MPEF-PV 10849) showing a large subcircular space in the inner core of the bone. B. General view of a plastral bone (MPEF-PV 10844). C. Detailed view of the cancellous bone. Bony trabeculae are mostly composed of secondarily deposited lamellar bone. A, B. Normal transmitted light. C. Cross-polarized light with lambda compensator.

Fig. 4. (Couleur en ligne.) Os spongieux des plaques osseuses de la carapace de *Condorchelys antiqua*. A. Vue générale d'une écaille costale (MPEF-PV 10849) montrant un large espace subcirculaire au centre de l'os. B. Vue générale d'un os du plastron (MPEF-PV 10844). C. Vue détaillée de l'os spongieux. Les travées osseuses sont principalement composées d'os lamellaire secondaire. A, B. Lumière transmise naturelle. C. Lumière polarisée avec compensateur lambda.

bone of the costals MPEF-PV 10848 and MPEF-PV 10849, which exhibits a relatively large, roughly circular shaped inter-trabecular space in the central area (Fig. 4A).

3.3. *Internal cortex*

Except for neural plates, in which the internal cortex is reduced in comparison to the external cortex, in all the other plates the internal cortex is commonly well developed, occupying an important portion of the total plate thickness in some cases. The maximum thickness of the internal cortex is observed in the plastral elements. In costal plates, the internal cortex is usually thicker in the mid-part of the section (where the rib is incorporated) and thinner towards the sutural margins of the bones (Fig. 2A–D). The internal cortex in all plates is typically poorly vascularized (avascular in several instances). When present, vascular spaces are mostly scattered in the central area and they are mainly longitudinally oriented.

A distinct degree of histological variation was recorded among different plate types. In the costal plates, the cortical bone is composed of parallel fibered bone. Although the intrinsic fibers of the parallel fibered bone are commonly oriented parallel to the outer surface, their orientation is variable with regard to the element main axis. In this sense, intrinsic fibers of costal bones are parallel to the lateromedial axis in the central area (which corresponds to the rib), but gradually change their arrangement toward the suture margins, where they become parallel to the anteroposterior axis (Fig. 5A, B). This change is not only evidenced by a variation in the optic properties of the bone (monorefringent in the center, birefringent toward the margins), but also by a change in the shapes of the bone cell lacunae, which are circular/sub-circular in section in the center and strongly elongated and flattened toward the suture margins. The inner cortex of several costal plates (e.g., MPEF-PV 10847, MPEF-PV 1783A) also shows a degree of variation with regard to their intrinsic fiber arrangement, which changes in the inner portion of the compacta. In the plastral elements, the internal cortex is composed of two distinct zones (an inner and an outer) (Fig. 5C–E). The inner zone occupies most of the total thickness of the internal cortex and consists of parallel fibered bone (Fig. 5D). As in the costal plates, the intrinsic fibers, which are oriented parallel to the element main axis in the central area, gradually change their arrangement and they become perpendicular to the main axis in the edges of the plates. The outer zone of the cortex is composed of a thin layer of lamellar bone that maintains its arrangement in all the compacta (Fig. 5E). Compared with the bone tissue of the inner area, this tissue exhibits a more pronounced birefringence and its bone cell lacunae are less abundant and more flattened. In the plastral plate MPEF-PV 10844, a third bone tissue is exhibited in a reduced portion of the internal cortex, between the cancellous bone and the inner zone previously described. Contrary to the intrinsic fibers observed in the inner and outer zone, the intrinsic fibers of this tissue possess an irregular arrangement, similar to the structural fibers developed in the external cortex. In neural plates, the internal cortex is composed of structural fibers (Fig. 5F). Similar to what is observed in the external cortex, structural fibers in these plates are organized in bundles oriented mostly parallel or sub-parallel to the surface. Finally, the intrinsic fibers of the internal cortex in peripheral plates exhibit a heterogeneous arrangement. In this sense, local variation from typical parallel fibered bone (homogeneous mass birefringence) to intercrossed structural fibers is clearly observed in the cortex (Fig. 5G, H).

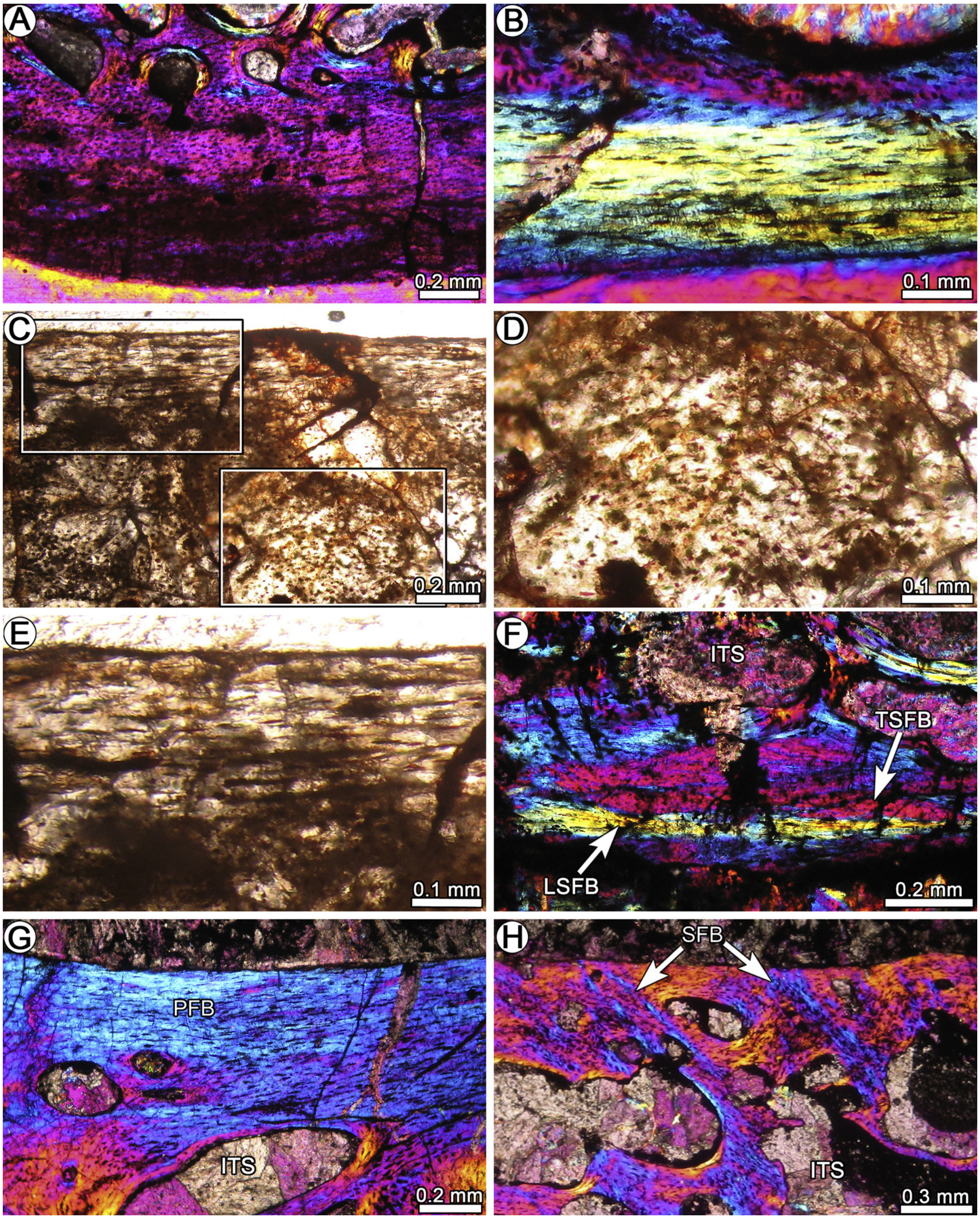

Fig. 5. (Color online.) Histology of internal cortex of *Condorchelys antiqua* shell bones. A, B. Cortical bone in MPEF-PV 10847 in the region that corresponds with the rib (A) and near the suture (B). Note the variation in the optical properties and in the bone cell lacunae shape between both regions. C. General view of the cortex in the plastral bone MPEF-PV 10841. D. Detail of the inner region of the same specimen (box inset in the inferior area of C). Note that the bone cell lacunae exhibit a circular shape in section. E. Detail of the outer portion of the cortex (box inset in the superior portion of C). In this area the bone cell lacunae are strongly flattened. F. Structural fiber bundles in the cortex of the neural plate MPEF-PV 10883. G, H. External cortex of peripheral plate MPEF-PV 10854, which is composed of parallel fibered bone (G) and structural fiber bundles (H). A, B, F–H. Cross-polarized light with lambda compensator. C–E. Normal transmitted light.

Fig. 5. (Couleur en ligne.) Histologie du cortex interne des plaques osseuses de la carapace de *Condorchelys antiqua*. A, B. Cortex de MPEF-PV 10847 dans la région correspondant à la côte (A) et près de la suture (B). Les propriétés optiques du tissu osseux, ainsi que la forme des logettes ostéocytaires varient entre les deux régions. C. Vue générale du cortex de l'os du plastron MPEF-PV 10841. D. Détail de la région interne du même spécimen (provenant de la partie inférieure de C). Les logettes ostéocytaires ont une forme circulaire en section. E. Détail de la partie externe du cortex (provenant de la partie supérieure de C) ; les logettes ostéocytaires sont très aplaties dans cette zone. F. Faisceaux de fibres structurales dans le cortex de la plaque neurale MPEF-PV 10883. G, H. Cortex externe de la plaque périphérique MPEF-PV 10854, composé de tissu osseux pseudo-lamellaire (G) et de fibres structurales (H). A, B, F–H. Lumière polarisée avec compensateur lambda. C–E. Lumière transmise naturelle.

Despite the observed variation in the intrinsic fiber organization in primary bone matrix, important variation regarding the overall shapes of bone cell lacunae is not observed in the sample. These lacunae exhibit abundant branching canaliculi and they are arranged according to the intrinsic fiber orientation. Clustered lacunae are more commonly observed in the inner portions of the compacta. Growth marks were only recorded in a few costals (e.g., MPEF-PV 10859) and a plastral plate (MPEF-PV 10843). The growth marks observed in the costals correspond with lines of arrested growth (Fig. 3H). On the other hand, the internal cortex of the plastral plate exhibits a tenuous, stratified pattern.

3.4. Sutures

The internal structure of sutural areas could only be studied in several costal and neural plates. The margins show sutural areas with short protrusions and sockets (Fig. 6A, B). Both protrusions and sockets are commonly lined by a thin layer of birefringent tissue (Fig. 6C). The bone tissue adjacent to the sutures is similar to the tissue described for the external cortex, but shows more signs of remodeling. In some instances, growth marks, which are deflected from the cortical bone, can be followed in the bone tissue of the sutures, thus showing an increasing sutural relief during ontogeny. Sharpey's fibers are observed in several samples, but not in all (possibly due to diagenetic alteration of the bone tissues). When present, these fibers are commonly oriented parallel to the protrusions.

3.5. Compactness

Compactness values for the four analyzed bones of *C. antiqua* (Fig. 7) using Bone Profiler (Girondot and Laurin, 2003) are: 54.8% (peripheral MPEF-PV 10854), 77.5% (costal MPEF-PV 10851), 78.9% (costal MPEF-PV 10847), 67.1 and 61.8% (costal MPEF-PV 10849). Global compactness in costal sections (61.8 to 78.9%) is higher compared with the single peripheral sampled (54.8%). Nevertheless, given the small number of sampled plates, it is not possible to determine whether the obtained values reflect a true variation. No important variation occurs in the compactness values within a single element (67.1 and 61.8% in costal MPEF-PV 10849).

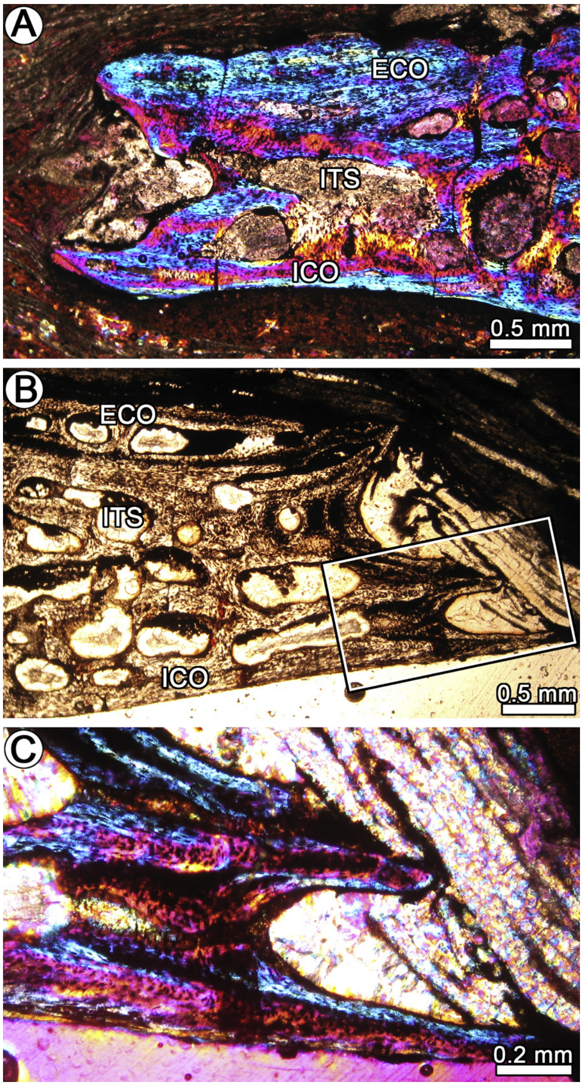

Fig. 6. (Color online.) Histology of the suture margins of *Condorchelys antiqua* shell bones. A. Neural plate MPEF-PV 10883. B. Costal plate MPEF-PV 10851A. C. Enlarged view of the same specimen (box inset in B). A, C. Cross-polarized light with lambda compensator. B. Normal transmitted light.

Fig. 6. (Couleur en ligne.) Histologie au niveau d'une zone de suture entre des os de la carapace de *Condorchelys antiqua*. A. Écaille neurale MPEF-PV 10883. B. Écaille costale MPEF-PV 10851A. C. Vue rapprochée du même spécimen (provenant de I B). A, C. Lumière polarisée avec compensateur lambda. B. Lumière transmise naturelle.

4. Discussion

4.1. Shell origin and development

Several studies on extant taxa have provided information about the embryological origin of the different bones of the turtle shell and some generalizations can be made on this regard. First, general consensus exists regarding the exoskeletal origin of the nuchal, peripheral, suprapygal, pygal and plastral plates (Gilbert et al., 2001). For the origin of the neural and costal plates, however, two main hypotheses have been invoked. The costals and neurals have been interpreted either as separate dermal elements that secondarily fuse with the underlying endoskeletal elements (e.g., ribs and vertebrae/neural arches) or as solely derived from these endoskeletal elements (see review in Rieppel, 2013). Recent studies (Gilbert et al., 2001; Hirasawa et al., 2013; Lima et al., 2011; Scheyer et al., 2008) have provided support for the second hypothesis. In this sense, ontogenetic data of extant turtles reveal that the costal and neural plates start their development as initial outgrowths of the periosteum of the ribs and vertebral arches, respectively, before the mode of mineralization switches to metaplastic ossification (i.e., transformation of pre-existing, non-osseous tissue into bone) of the dermis (Lima et al., 2011; Scheyer et al., 2008). Metaplastic tissue has been inferred by the

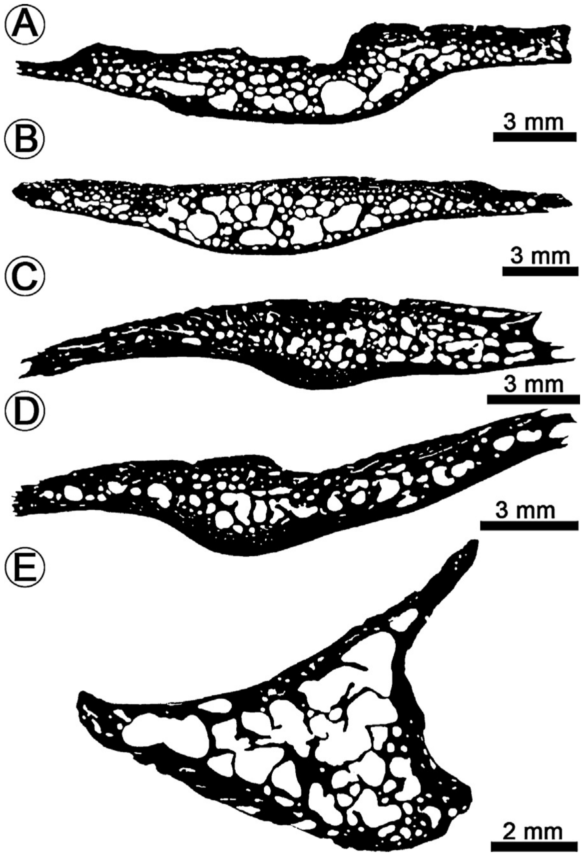

Fig. 7. Schematic black and white drawings of the four plates of *Condorchelys antiqua* prepared for use with Bone Profiler. Bone tissue is shown in black, vascularization and resorption spaces in white. A, B. MPEF-PV 10849, costal plate. C. MPEF-PV 10851A, costal plate. D. MPEF-PV 10847, costal. E. MPEF-PV 10854, peripheral plate.

Fig. 7. Schéma en noir et blanc des quatre plaques osseuses de *Condorchelys antiqua* préparées pour Bone Profiler. Le tissu osseux est illustré en noir, la vascularisation et les espaces de résorption en blanc. A, B. MPEF-PV 10849, écaille costale. C. MPEF-PV 10851, écaille costale. D. MPEF-PV 10847, écaille costale. E. MPEF-PV 10854, écaille périphérique.

presence of interwoven structural fibers in both extant and extinct turtles (Scheyer et al., 2007, 2008; Sterli et al., 2013a). In this sense, the structural fibers correspond with coarse collagenous fiber bundles located in the dermis, which are mineralized without true periosteal activity. The presence of structural fibers in the external cortex of both neural and costal plates in *Condorchelys antiqua* is congruent with the model that implies an incorporation of dermal structures via metaplasia during the growth of the plate. Moreover, the structural fibers formed in the external cortex of peripheral and plastral bones suggest that metaplastic ossification was also (at least in part) performed during the formation of these elements.

4.2. Histological variation

The inclusion of several samples of different types of plates allows comparing the degree of variation among different elements of the shell and even among the same element in different individuals. In general terms, there is not important variation with regard to the histological nature of the external cortex in the sampled bones. In the same way, cancellous bone structure also exhibits a low degree of variation. Where present, variation is related to the relative proportion occupied by the cancellous bone within the plates and the relative size and shape of the inter-trabecular spaces. In some costal plates, an enlarged, roughly circular cavity is present in the inner core of the elements. This central cavity has been previously described in costal plates of fossil and extant turtles (Lima et al., 2011; Lyson et al., 2013; Scheyer et al., 2014a). Scheyer et al. (2014a) interpreted this tubular structure as representing the original location of the embryonic rod-like cartilaginous rib early in costal growth. The internal diameter of this structure (1,45 mm in MPEF-PV 10849) is, however, relatively larger than the diameter of the costal growth center of turtle embryos (Hirasawa et al., 2013; Lima et al., 2011; Scheyer et al., 2008, 2014a). As pointed out by Scheyer et al. (2014a), the size of the primordial ribs varies among embryos of extant turtles, which appears to be linked to the individual size of the specimen sampled, as well as the final adult size of the respective species. Nevertheless, the absence of a typical "Kastchenko's line", which marks the site of the initial perichondral ossification (Francillon-Viellot et al., 1990), indicates that at least the internal surface of these cavities in *C. antiqua* does not correspond with the site of initial growth of the cartilaginous rib.

The most important degree of variation between different types of plates corresponds with the microstructural organization of the primary bone of the internal cortex. In both costal and plastral plates the cortical tissue is composed of parallel fibered bone, which exhibits changes in fiber arrangement (within successive layers and even within a single layer). Conversely, the internal cortex of neural plates is formed by structural fibers. Finally, peripheral plates are formed by both structural fibers and parallel fibered bone. This variation among different elements from the same species has not been previously reported in other turtles (extant or fossil) and could be related to variations in the developmental pattern of the plates. Structural fibers in the internal cortex of the neural and peripheral elements indicate that, as in the external cortex, the plate formation includes incorporation of soft tissue from the dermis, which appears not to occur in the costal and plastral bones. Moreover, the presence of structural fibers occupying partially (peripherals) or entirely (neurals) the bone tissue in the internal cortex suggests that the visceral portion of these plates were partially or entirely imbedded within the dermis. Interestingly, this position coincides with that observed in the early stages of development of several extant taxa, including *Podocnemis unifilis*, *Emydura subglobosa* and *Pelomedusa subfrula* (Lima et al., 2011; Scheyer et al., 2008), but differs from *Pelodiscus sinensis*, in which neural plates develop entirely outside of the dermis (Hirasawa et al., 2013).

Histological variation is not only present among different types of plates, but also in the same element from different individuals. The most important variation in this regard is observed in costal MPEF-PV 10859 and hyo-hypoplastron MPEF-PV 10844. As previously mentioned, the outer portion of the external cortex in these elements is not entirely composed of structural fibers as in other plates. Instead, it is in part formed by parallel fibered bone deposited during different stages of bone erosion and bone deposition. This pattern, which indicates a high degree of secondary remodeling, has been commonly reported in strongly ornamented dermal bones (de Buffrénil, 1982; Cerda and Desojo, 2011; Scheyer et al., 2014c), but is rather uncommon in turtles. Two main hypotheses can be invoked to explain this particular variability:

- more than one species is represented by the material identified as *C. antiqua*;
- the "anomalous" material represents a pathological condition.

The first hypothesis is poorly supported with the available evidence because all the repeated elements belonging to a turtle found in Queso Rallado locality (e.g., carapaces, skulls) show the same general size, ornamentation, external morphology, among others. Up to now, all the turtle remains found in Queso Rallado do not show the expected variation to claim that more than one species was present. Besides, a new, not yet described, articulated specimen (MPEF-PV 10884) of *C. antiqua* containing shell remains and postcranial remains allowed us (JS, pers. obs.) to confirm that previously isolated postcranial remains surely belong to *C. antiqua*.

Regarding the pathological hypothesis, this source of variation is supported by the fact that the inner portion of the external cortex of MPEF-PV 10859 is actually composed of structural fibers, as commonly occurs in the other samples as well. Therefore, at least before the secondary remodeling, the microstructure of the external cortex was the same as the one observed in other samples of *C. antiqua*. Another line of evidence comes from recent investigations on shell diseases in turtles (Gardner et al., 1997; Hernandez-Divers et al., 2009). These studies show that disfiguring shell diseases are associated with dermal bone remodeling. Histological descriptions of pathological shell bones have been published for example by Scheyer and Sánchez-Villagra (2007), and Scheyer et al. (2014a). In these cases, however, the osseous microstructure is clearly different from that observed in specimens MPEF-PV 10859 and MPEF-PV 10844. Abnormal shell morphology and microstructure could be caused by diet deficiencies (Gerlach, 2004), but such changes (e.g., increase in the cancellous bone) also differ from that reported in this study. Although it is not possible to accept or reject any hypothesis on the basis of the current data, a pathological origin appears to be a more reliable source of variation for specimens MPEF-PV 10859 and MPEF-PV 10844.

4.3. Comparison with stem Testudines

The shell microstructure of other stem Testudines, including *Proganochelys quenstedti*, *Proterochersis robusta*, *Kayentachelys* sp., *Eileanchelys waldmani*, and *Heckerochelys romani* have been described by Scheyer (2009), Scheyer and Sander (2007) and Scheyer et al. (2014a). As in *C. antiqua*, the shell bones in these taxa are composed of a diploe structure. The diploe structure of the shell bones has been considered a plesiomorphic character for all turtles (Scheyer et al., 2007). Except for the peripheral bones of *E. waldmani*, in which the internal cortex is strongly reduced, there is not distinct variation in the thickness of both external and internal cortices. The external cortex of stem Testudines consists of an interwoven meshwork of structural fibers, which is considered a plesiomorphic feature of turtles. In *P. quenstedti*, *Pr. robusta* and *Kayentachelys* sp. the structural fibers extend perpendicular or at high angles to the surface of the bone (Scheyer, 2009; Scheyer and Sander, 2007). Conversely, in *C. antiqua*, *E. waldmani* and *H. romani* the structural fibers are mostly arranged subparallel to the surface (Scheyer et al., 2014a), which could be interpreted as a derived feature. The external cortex in all the taxa is vascularized by scattered primary osteons and simple primary vascular canals. Secondary osteons occur at the transition to the interior cancellous bone. In stem Testudines it is composed of trabeculae formed by secondary lamellar bone and remains of primary tissue. The internal cortex of all taxa is composed of parallel fibered bone. In *P. quenstedti* and *Pr. robusta*, the parallel fibered bone can locally reach the organization grade of lamellar bone. The degree of vascularization in the internal cortex exhibits some degree of variation. While the internal cortex in *P. quenstedti* is mainly avascular, a fine reticular vascularization pattern of thin primary vascular canals is locally developed in the plastral fragment of *Pr. robusta*. *Kayentachelys* sp., *E. waldmani*, and *H. romani* share a rather poorly vascularized internal cortex. Sharpey's fibers are commonly well developed in the internal cortex of stem Testudines.

In general terms, the shell microstructure of *C. antiqua* appears to be more similar to those described for *E. waldmani* and *H. romani* than to the other stem taxa. The shared features are the arrangement of the structural fibers in the external cortex and the general microanatomy of the shell. The low compactness shared by *C. antiqua*, *E. waldmani* and *H. romani* is possibly related to the influence of the aquatic environment on the shell bone microstructure in these taxa (Scheyer and Sander, 2007; Scheyer et al., 2014a, see below). The structural fiber arrangement in the internal cortex, however, is possibly linked to other causes than the ecology of these taxa (e.g., interspecific variations in the dermal fiber arrangement, changes in the relative position of the plate within the dermis). This character appears to be a derived feature of *C. antiqua*, *E. waldmani* and *H. romani*. Despite the phylogenetic position of *Kayentachelys* sp. (more derived than *P. quenstedti* and *Pr. robusta* and closely related to *C. antiqua* and *H. romani*, Sterli, 2010), this taxon retains the plesiomorfic condition of the character (e.g., structural fibers extend perpendicular or at high angles to the surface of the bone). The only histological characters observed only in *C. antiqua* are the total absence of Sharpey's fibers in the internal cortex of all sampled plates and the organization of the parallel fibered bone in the internal cortex of the costal and plastral plates. The absence of Sharpey's fibers is rather unexpected, because these fibers are commonly present in the internal cortex not only in stem Testudines, but also in several other turtles. Because the particular organization of the parallel fibered bone in the internal cortex of plastral and costal plates of *C. antiqua* has been only described for this taxon, these features could be valuable for systematics in future studies.

4.4. Paleoecology

The paleoecology of extinct turtles has been inferred using different methods, some of them more accurate or more controlled than others. One of the most common trends in general paleontology is to infer the lifestyle of vertebrates based on the depositional environment where the fossils have been found. However, this evidence should not be used alone. Joyce and Gauthier (2004) noticed that in general descriptions the convexity of the carapace has been used to infer the lifestyle of a turtle. For example, those turtles with flat carapaces would be more aquatic and those turtles with domed carapaces would be terrestrial. This characteristic could be useful in some cases, but it is usually problematic. There are examples of highly terrestrial extant turtles with very fat shells (e.g., *Malacochersus tornieri*) and some aquatic species with domed shells (e.g., kinosternids). And this character is even more difficult to evaluate in extinct turtles, where the shells could be compacted during fossilization. The general shape of the limbs has also been used to infer the lifestyle of extinct turtles. However, the use of this evidence alone could also lead to wrong conclusions. For example although the presence of flippers is characteristic of marine turtles, flippers are also present in the fresh water turtle *Carettochelys insculpta*. Another useful and reliable method is the one suggested by Joyce and Gauthier (2004) where the relationship among the stylopodium, the zeugopodium, and the autopodium of the forelimb are used to establish the paleoecology of turtles. The disadvantage of this method is that complete forelimbs (or their natural casts) need to be available to establish the lifestyle for extinct taxa. Unfortunately, due to the nature of the fossil record, finding a complete forelimb is not a usual discovery, as is the case for *C. antiqua*. It is in this context that the microanatomy becomes a highly valuable tool to explore the paleoecology of extinct taxa.

Regarding the microanatomy of amniote bones, two main techniques have been applied so far to infer the paleoecology of extinct taxa. One technique is related to the compactness of the long bones (e.g., humerus), whereas the other is related to the compactness of the bony plates of the shell. The first technique is based on the relationship between the compactness of the long bones and the known lifestyle of extant species. Although this methodology has been applied to different groups of amniotes, its reliability is still under debate (e.g., Canoville and Laurin, 2010; Laurin et al., 2004; Nakajima et al., 2014). For example in turtles, recent studies have concluded that the humeral compactness cannot be used alone to indicate the lifestyle of extinct turtles (e.g., Canoville and Laurin, 2010; Nakajima et al., 2014). On the other hand, Scheyer and colleagues (e.g., Scheyer and Sander, 2007; Scheyer et al., 2014a) have been exploring the correlation between the shell microstructure and the lifestyle of turtles and they concluded that it is a valuable tool to infer different ecological habits in the group. In general terms, those bones with a low degree of compactness and homogenization of compact cortical and cancellous shell areas are correlated with aquatic specializations. The compactness analysis of the three shell bones of *C. antiqua* with Bone Profiler (Girondot and Laurin, 2003) revealed overall observed compactness values between 61.8% and 78.9% for costal elements and 54.8% for the peripherals. In comparison to stem aquatic turtles, overall compactness values for the costal of *H. romani* ranged between 56.3% and 60.3%, whereas the section of the peripheral of *E. waldmani* yielded 39.5% (Scheyer et al., 2014a). Although the range of values found in *C. antiqua* bone sections are, with one exception (costal MPEF-PV 10849), higher than those of the comparable bone elements in *H. romani* and *E. waldmani*, they are still within the range of those of aquatic turtles. For comparison, Scheyer et al. (2014a, 2014b) reported compactness values for some terrestrial *Solemys* shell bones, ranging between 86 and 96%. The nature of the preservation of *C. antiqua* (e.g., compressed specimens, incomplete forelimbs, few humeri preserved) prevented the assessment of its lifestyle in previous works (Sterli, 2008; Sterli and de la Fuente, 2010). Consequently, the analysis of the shell microstructure is a valuable tool in these cases where the preservation of the specimens does not allow applying other methods. The shell microstructure analysis performed here presents for the first time evidence suggesting an aquatic life style for *C. antiqua*. Considering the Late Toarcian to Aalenian (–Bajocian?) age of the Cañadón Asfalto Formation (Cúneo et al., 2013), *C. antiqua* would represent the oldest known fresh water turtle found up to now. In the study of *E. waldmani*, Anquetin et al. (2009) suggested that fresh water (aquatic) turtles would have originated during, or prior to, the Middle Jurassic. Considering this study and the ghost lineages shown in the recent phylogenies (e.g., Sterli et al., 2013b), fresh water turtles would have appeared earlier than previously thought, sometimes during the Late Triassic and the Early Jurassic. The paleoecology suggested for *C. antiqua* is in accordance with the inferred depositional environment for Queso Rallado locality: "relatively small, shallow lacustrine body placed in a supralitoral environment adjacent to the main water body of the basin" (Rougier et al., 2007). Although the majority of the vertebrates found in Queso Rallado locality show a certain amount of transportation, turtles are among the most complete and articulated remains recovered there (e.g., almost complete shells).

In summary, many methods have been suggested to infer the paleoecology of extinct turtles. However, the nature of the fossils and/or the nature of the methodologies sometimes preclude the use of only one of those methods to establish the lifestyle of extinct turtles. As a consequence, when it is possible, the use of more than one method or one method complemented with other sources of evidence is recommended to explore the habitats of extinct turtles. Furthermore, more information about the anatomy of stem Testudines will help to better understand the evolution and paleoecology of basal turtles.

5. Conclusions

We study the microanatomy and histology of a large sample of shell bones of *C. antiqua* (to date, the largest sample for a single fossil turtle species). Our data allow establishing that histological variation actually exists among the different types of bones in the turtle shell. To determine whether these variations are common in Testudinata is an important issue to explore in future works. The large sample studied here also provides valuable information to differentiate those histological features that exhibit a major degree of individual variation (e.g., vascularization of the external cortex) from those more conservative (e.g., type and arrangement of the intrinsic fibers of the internal cortex). This determination is important for systematic studies, given that it allows discerning which characters have the most systematic value. In this regard, those characters less influenced by individual variation appear to be more important for systematic studies. Although bone shell microstructure of Testudinata has proven to be an important tool for systematic studies (e.g., Scheyer and Sánchez-Villagra, 2007; Scheyer et al., 2007), it is evident that more research on this field is still necessary.

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