CrossMark

ORIGINAL PAPER

Shell bone histology of the long-necked chelid *Yaminuechelys* (Testudines: Pleurodira) from the late Cretaceous—early Palaeocene of Patagonia with comments on the histogenesis of bone ornamentation

Juan Marcos Jannello [1,2] ⬤ · **Ignacio A. Cerda** [3,4] · **Marcelo S. de la Fuente** [1,2]

Abstract *Yaminuechelys* is a long-necked chelid turtle whose remains have been recovered from outcrops of the Santonian-Maastrichtian and Danian of South America. With the purpose of providing data about shell sculpturing origin and palaeoecology, the bone histology of several shell elements (including neural, costal, peripheral and plastral plates) of *Yaminuechelys* is described herein. Histological analysis reveals that *Yaminuechelys* shares with Chelidae the presence of interwoven structural fibre bundles in the external cortex, and parallel-fibred bone of the internal cortex. The presence of resorption lines in several samples indicates that the particular ornamentation of the external surfaces originated, at least in part, by focalized resorption and new bone deposition. This mechanism for ornamentation origin and maintenance is here described for the first time in a turtle. Compactness of the shell bones is consistent with an aquatic habitat, which supports previous hypothesis based on palaeoenvironmental and morphological data.

Abbreviations

MPEF-PV	Paleovertebrate collection of the Museo Paleontológico "Egidio Feruglio", Trelew, Chubut, Argentina
MAU-Ph	Paleohistological collection of the Museo Municipal "Argentino Urquiza", Rincón de los Sauces, Neuquén, Argentina
N	Narambuena collection
CO	Cerro Overo collection
PR	Puesto La Rinconada collection
LI	La Invernada collection

✉ Juan Marcos Jannello
jjannello@mendoza-conicet.gob.ar; marcosjannello@hotmail.com

Ignacio A. Cerda
nachocerda6@yahoo.com.ar

Marcelo S. de la Fuente
mdelafuente@mendoza-conicet.gob.ar;
mdelafuente1910@gmail.com

1   Grupo vinculado al IANIGLA-CONICET, San Rafael, Argentina

2   Present address: Museo de Historia Natural de San Rafael, Av. Ballofet s/n, frente al parque Mariano Moreno, CP5600 San Rafael, Mendoza, Argentina

3   CONICET, Instituto de Investigaciones en Paleobiología y Geología, Universidad Nacional de Río Negro, Belgrano 1700, Paraje Pichi Ruca (predio Marabunta), CP8300 Cipolletti, Río Negro, Argentina

4   Present address: Museo Carlos Ameghino, Belgrano 1700, Paraje Pichi Ruca (predio Marabunta), CP8300 Cipolletti, Río Negro, Argentina

Introduction

Yaminuechelys is the oldest known long-necked chelid (de la Fuente et al. 2015). This taxon is named on the basis of a complete specimen from the Upper Cretaceous of northern Patagonia (de la Fuente et al. 2001, 2015). The chelid turtles are freshwater aquatic to semiaquatic pleurodires, characterized by two main synapomorphies also present in *Yaminuechelys*: i.e. the formula of articulations of the cervical vertebrae, with a second, third and fourth opisthocoelous

Springer

cervical, fifth and eighth biconvex, sixth procoelous and seventh biconcave cervical vertebrae; and the development of the lateral cheek emargination of the skull nearly up or up to the back of the dorsal face of the skull that preclude the development of a quadratojugal bone (Gaffney 1977). Two basic morphotypes have been recognized in chelid turtles (Boulenger 1889): chelids with a neck shorter than the dorsal vertebrae (i.e. *Pseudemydura*, *Emydura*, *Phrynops*), and chelids with a neck longer than the dorsal vertebrae, according to the lengthening of each vertebra (*Chelus*, *Chelodina* and *Hydromedusa*). *Yaminuechelys* is clearly a member of the long-necked clade of chelids including *Chelus*, *Chelodina* and *Hydromedusa* in accordance with morphological topologies (see Gaffney 1977). This relationship could be expressed in parenthetic notation as follows: (*Chelus fimbriatus* (*Chelodina oblonga* ((*Hydromedusa tectifera* + *Hydromedusa maximiliani*) (*Yaminuechelys maior* + *Yaminuechelys gasparinii*)))) (Bona and de la Fuente 2005). On the other hand, it is noteworthy that different phylogenetic signals are recognized by molecular phylogenies (i.e. Seddon et al. 1997; Georges et al. 1998; Guillon et al. 2012) recovering *Chelodina* species as closely related to Australasian short-necked chelids instead of long-necked South American ones. A possible phylogenetic signal is the strong polygonal decorations shared not only by the three taxa but also by other fossil and extant chelid taxa (e.g. *Pseudemydura umbrina*, *Emydura victoriae*). A homoplastic development from a common moderately decorated ancestor is one of the possible hypotheses to explain the strong decoration in some taxa of both short- and long-necked chelids.

In recent years, the bone microstructure of dermal bones in fossil tetrapods has increasingly attracted the interest of the palaeobiologists, for example, in temnospondyls amphibians (e.g. Witzmann and Soler-Gijon 2008, placodonts (Scheyer 2007), pareiasaurian parareptiles (Scheyer and Sander 2009), turtles (e.g. Scheyer and Sanchez-Villagra 2007; Scheyer 2009), archosaurs (e.g. de Buffrénil et al. 1986; Main et al. 2005; Hayashi et al. 2009), squamates (de Buffrénil et al. 1986) and xenarthran mammals (e.g. Hill 2006). In turtles, most of the current studies are based on groups from the northern hemisphere (e.g. Scheyer 2008; Pérez-García et al. 2013; Cadena et al. 2013; Scheyer et al. 2014a) and a few from the southern hemisphere (e.g. Scheyer 2009; Sterli et al. 2013; Jannello et al. 2014; de la Fuente et al. 2015).

Among the different issues addressed by the turtle shell microstructure, published studies have mostly focused their discussion on the histogenesis of the different types of plates, the relationship between the shell microanatomy and the palaeoecology, and the systematic value of the histology (e.g. Scheyer and Sanchez-Villagra 2007; Pérez-Garcia et al. 2012; Pérez-García et al. 2013; Cadena et al. 2013; Scheyer et al. 2014a, b; Cerda et al. 2015; Vlachos et al. 2015). A less discussed topic is the origin and maintaining of the superficial ornamentation of the shell bones. On this topic, a

recent publication of Scheyer et al. (2014b) has studied the particular ornamentation of the shell bones in Solemydidae, a stem Testudines.

In the present work, we describe the shell bone microstructure of *Yaminuechelys*. The major aims of the present contribution are to characterize the bone shell microstructure of *Yaminuechelys*, to recognize the presence of histological characters with systematic value, to compare the shell histology with other close related chelids and to infer the lifestyle of *Yaminuechelys* from its shell bone microstructure. Since the carapace bones of the different species of *Yaminuechelys* exhibit a characteristic sculpturing which consist of a combination of small and large polygons, we also attempt to establish the origin and maintaining of this ornamentation pattern.

Materials and methods

The shell bones used in this study (Table 1) include materials from *Yaminuechelys maior* (Staesche 1929), *Yaminuechelys* indet., *Yaminuechelys* aff. *Y. maior* and *Yaminuechelys* cf. *Y. maior*. Although most of the studied specimens have been collected from Upper Cretaceous outcrops from different Argentinean localities (Table 1), we also include one specimen from the Paleocene. For the microstructural analysis, 19 thin sections from 14 shell bones have been sampled, including one neural, nine costals, two peripherals, one hypoplastron and one xiphiplastron (Fig. 1). Planes of sectioning varied between the different shell bone elements: Neurals were cut perpendicular to their anteroposterior axis (perpendicular to the vertebrae, Fig. 1b); costals were cut parallel to the anteroposterior axis (perpendicular to the incorporated ribs, Fig. 1f); peripherals were cut perpendicular to the anteroposterior axis of the turtle shell (Fig. 1l); and plastron elements were mainly cut parallel or slightly oblique to the anteroposterior axis of the turtle shell. Two consecutive cuts were made parallel to the anteroposterior axis in two costal plates (MAU-Ph-N-001 and MAU-Ph-PR-005), one cut in the medial region and the other in the marginal region. Last, four cuts were made in the costal plate MAU-Ph-LI-007, three parallel to the anteroposterior axis and one in the marginal region perpendicular to the anteroposterior axis.

Specimens were prepared into thin sections based on the methodology outlined in Chinsamy and Raath (1992). The preparation of the histological sections was carried out in the Museo Paleontológico Egidio Feruglio (Trelew, Argentina). The slides were studied using petrographic polarizing microscope (Nikon ECLIPSE E200). 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

Table 1 List of specimens sectioned in this study, including collection number, plate element, horizon and locality

Taxon	Collection number	Plate	Horizon and locality
Yaminuechelys maior	MPEF-PV-10953	Costal	Fm. Salamanca, Lower Paleocene, Cerro Castillo, Chubut.
Yaminuechelys indet.	MPEF-PV-10958	Costal	Fm. La colonia, Campaniano–Maastrichtiano, Upper
	MPEF-PV-10959	Peripheric	Cretaceous, Cerro Bosta, Chubut.
	MPEF-PV-10960	Neural	
Yaminuequelys cf. *Y. maior*	MAU-Ph-N-001	Costal	Fm. Anacleto, Campaniano, Upper Cretaceous,
		Costal	Narambuena, Rincón de los Sauces, Neuquén.
		Costal	
		Costal	
Yaminuequelys cf. *Y. maior*	MAU-Ph-N-002	Costal	Fm. Anacleto, Campaniano, Upper Cretaceous,
		Xiphiplastron	Narambuena, Rincón de los Sauces, Neuquén.
		Hypoplastron	
		Peripheric	
Yaminuechelys cf. *Y. maior*	MAU-Ph-CO-003	Costal	Fm. Anacleto, Campaniano, Upper Cretaceous, Cerro Overo, Rincón de los Sauces, Neuquén.
Yaminuechelys aff. *Y. maior*	MAU-Ph-PR-005	Costal	Fm. Anacleto, Campaniano, Upper Cretaceous, Puesto la Rinconada, Rincón de los Sauces, Neuquén.
Yaminuechelys cf. *Y. maior*	MAU-Ph-LI-007	Costal	Fm. Bajo de la Carpa, Santoniano, Upper Cretaceous, La Invernada, Rincón de los Sauces, Neuquén.

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, towards the core or towards the surface of the plate, respectively.

To infer the lifestyle of *Yaminuechelys* specimens 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 Software (Girondot and Laurin 2003). To be used with the program, images of the sectioned bones were transferred into black and white (black for bone and white for vascular spaces, Fig. 2). Since several of the sampled plates are incomplete or crushed, we performed the compactness analysis only in four elements, including two costal bones (Fig. 2a, b), one peripheral bone (Fig. 2c) and one hypoplastral bone (Fig. 2d). The obtained results (Table 2) were compared with those published previously (Pérez-Garcia et al. 2012, 2013; Scheyer et al. 2014a, b; Cerda et al. 2015).

Results

The studied plates of *Yaminuechelys* show some degree of variability with regard to their relative size and thickness, especially in costal plates. All shell bones of *Yaminuechelys* show a diploë structure with external cortex (ECO) and internal cortex (ICO) framing an interior area of cancellous bone (CB). In peripheral plates, the ECO can be divided in dorsal and ventral portions, which merge on the lateral side of the

plate. The CB is well developed in most of the samples and commonly occupies the main proportion of the total area in section. Since the sampled shell elements share several histological features, we describe them together. Histological variation among samples is mentioned if applicable.

External cortex

The ECO consists of a compact bone tissue which is thicker in comparison with the ICO. The matrix consists entirely of a compact bone tissue of thick fibres (structural fibre bundles) that extend parallel, sub-parallel and transversely to the outer surface, which commonly exhibits two main arrangements. The first arrangement, observed in the inner portion of the cortex, consists of fibres loosely grouped and oriented principally in two directions, longitudinally and transversely to the element main axis (Fig. 3a). The second type of arrangement is observed in the external portions of the cortex, in which the fibres are principally organized in parallel bundles (Fig. 3b). In the marginal section of costal plate (MAU-Ph-CO-003), the fibres correspond to bundles that are clearly identified when they are transversely sectioned. Some degree of stratification is observed in several costal plates (MAU-Ph-N-001, MAU-Ph-LI-007).

The degree of vascularization of the ECO is variable, even within a single type of plate (MAU-Ph-N-001, MAU-Ph-PR-005). In general terms, all the elements are well vascularized with small simple canals (SC), primary osteons and secondary osteons (Fig. 3c, d). "Vertical" canals opened to the outer surface are abundant in some specimens (MAU-Ph-N-002,

Fig. 1 Selected material used in this study. **a–c** *Yaminuechelys* indet., **a** peripheral (MPEF-PV-10959), **b** neural (MPEF-PV-10960) and **c** costal (MPEF-PV-10958). **d** *Yaminuechelys maior*, costal (MPEF-PV-10953). **e, h, j, k** *Yaminuechelys* cf. *Y. maior*, costals (MAU-Ph-N-001). **f** *Yaminuechelys* cf. *Y. maior*, costal (MAU-Ph-LI-007). **g** *Yaminuechelys* cf. *Y.* maior, costal (MAU-Ph-CO-003). **i, l–n** *Yaminuechelys* cf. *Y. maior* (MAU-Ph-N-002), **i** costal, **l** peripheral, **m** xiphiplastron and **n** hypoplastron. **o** *Yaminuechelys* aff. *Y. maior*, costal (MAU-Ph-PR-005). The dashed lines in **b, f, l** show the planes of sectioning. *Scale bar* equals 5 mm
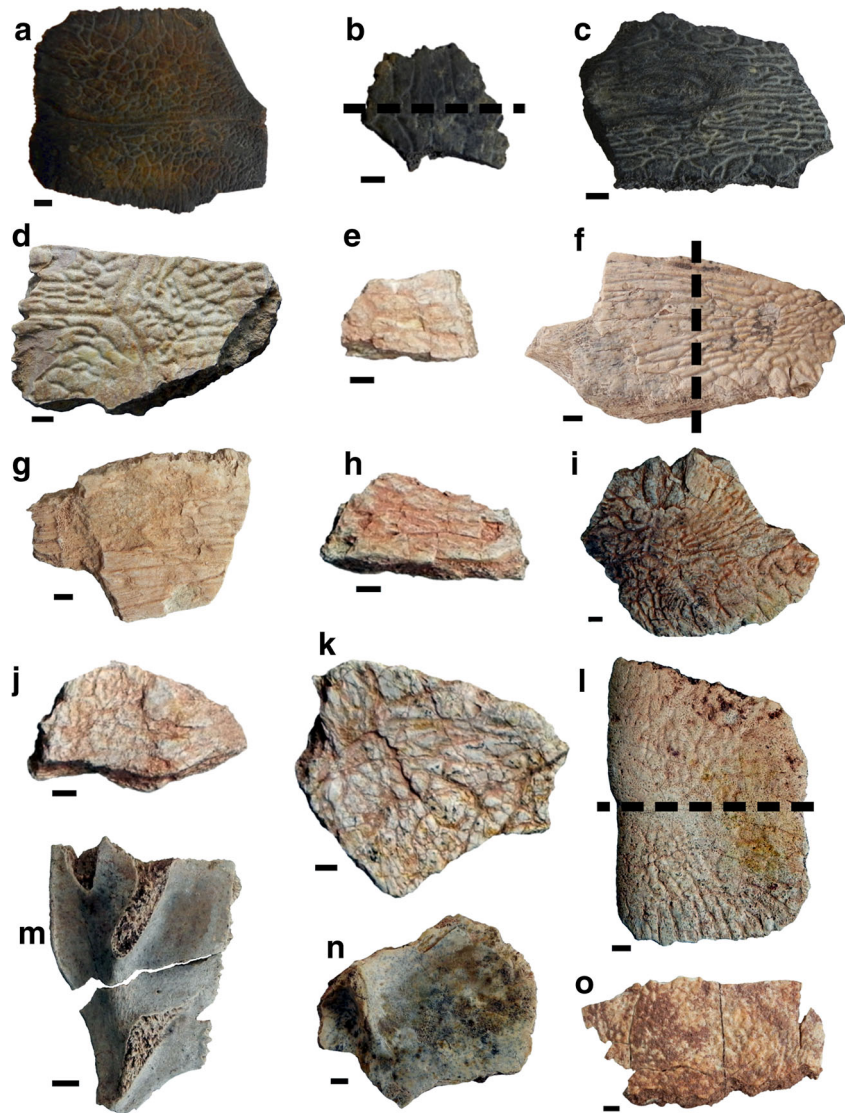
MAU-Ph-CO-003, MAU-Ph-LI-007, MPEF-PV-10953, MPEF-PV-10958, MPEF-PV-10959).

Sharpey's fibres are present in several samples (e.g. MPEF-PV-10953, MAU-Ph-N-001). These extrinsic fibres are short, and they are oriented mostly perpendicularly or slightly oblique to the outer surface (Fig. 3c, d). In some samples, they extend until the inner portion of the cortex (e.g. MPEF-PV-10953). Growth marks are rather common in the ECO of some samples (e.g. MPEF-PV-10953, MAU-Ph-N-001); they resemble typical lines of arrested growth (LAGs). The ornamentation has a regular pattern of valleys and crests formed by lamellar and parallel fibred bone, which contains fusiform bone cell lacunae. Whereas the valleys are always formed of lamellar bone, the crests can have parallel fibred bone. Resorption lines (RL) are present in the ornamentation in some specimens (MAU-Ph-N-001, MAU-Ph-LI-007) (Figs. 3e, f and 4), but in other samples, these lines are not visible (MPEF-PV-10953, MPEF-PV-10958).

Variations on the general histological pattern of the ECO described above are observed in some samples. In this regard, in some specimens (MPEF-PV-10958, MAU-Ph-N-001), the intrinsic fibres exhibit just diffuse variation in their arrangement and they are organized as a typical parallel fibred bone (PFB). This bone appears to be formed during successive episodes of cortical erosion and redeposition, which is evidenced by the presence of resorption lines. In other cases (costal plate MAU-Ph-N-002 and MAU-Ph-CO-003), the cortical tissue varies between lamellar and parallel fibred bone, but resorption lines are not evident. In costal MAU-PV-N-001, the resorption line is followed by the deposition of highly vascularized fibro-lamellar bone (FLB) in the middle region on the cortex (Fig. 4a, b).

Variation of the vascular arrangement was observed among different plates and even within single elements. In this sense, the vascular canal arrangement and the degree of vascularization of the costal plates change if the section is obtained from
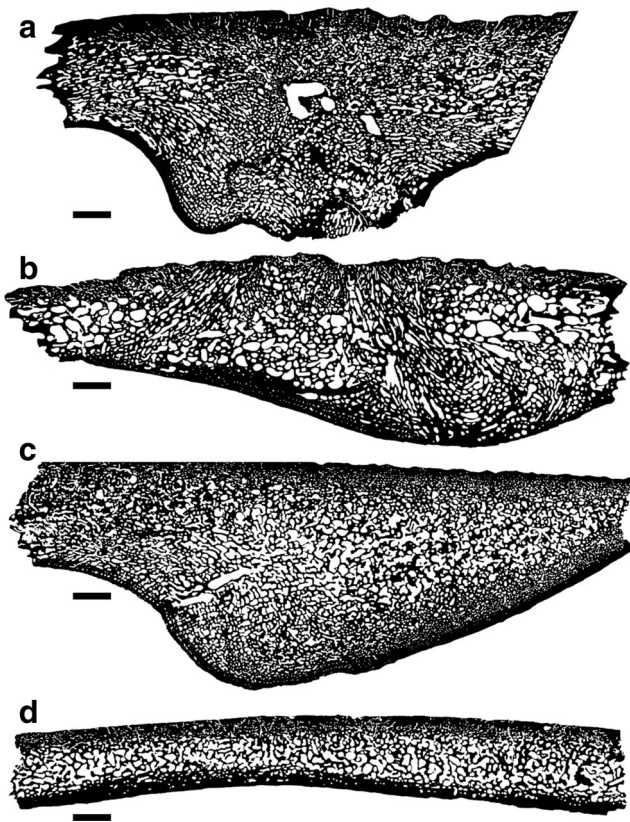
Fig. 2 Resulting binary images of sectioned specimens used for compactness analysis BONE PROFILER. **a** *Yaminuechelys maior* costal (MPEF-PV-10953). **b** *Yaminuechelys* indet. costal (MPEF-PV-10958). **c** *Yaminuechelys* indet. peripheral (MPEF-PV-10959). **d** *Yaminuechelys* cf. *Y. maior* hypoplastron (MAU-Ph-N-002). *Scale bar equals 3 mm*

the medial, middle, or marginal (lateral) region. In the medial and middle region, vascular canal density varies from scarce to high and its orientation is predominantly radial and longitudinal (Fig. 5a). Local anastomoses are also common in these areas. Conversely, the vascularization at the marginal region consists predominately of longitudinal canals, which exhibit scarce anastomosis. Regarding inter-elemental variation, peripheral plates exhibit a plexiform or reticular vascular pattern in some areas.

Cancellous bone

The CB is well developed in most of the samples and commonly occupies the main portion of the total area in section. It comprises principally both short and long, slender trabeculae. Inter-trabecular spaces are circular and sub-circular 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. 5b, c). Flattened bone cell lacunae follow the centripetally deposited lamellar bone linings. There is variation in the fibre 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 ECO. Histological variation between the sampled specimens is mostly related to the length and width of the bony trabeculae and also with the size of the inter-trabecular spaces. In some region of the first costal (MPEF-PV-10953) and peripheral elements (MAU-Ph-N-002, MPEF-PV-10959), trabeculae and inter-trabecular spaces are oriented parallel and sub-parallel to the internal and external cortex. The specimen MPEF-PV-10958 exhibits a particular orientation in the cancellous bone (Fig. 2b). In this element, there are two regions in which the trabeculae are mostly oriented in parallel to each other and perpendicular to the ECO forming two "column-like" structures that deviate towards the ICO, one at 30° and the other at 50° with regard to the surface.

Internal cortex

The ICO is reduced in comparison to the ECO. The maximum thickness of the ICO is observed in the plastral elements. In costal plates, the ICO is usually thicker in the portion where the rib is incorporated and thinner towards the sutured margins

Table 2 Mean global values of bone compactness parameters as calculated in Bone Profiler

Bone specimen	Min (SE)	Max (SE)	s:slope (SE)	p:transition (SE)	Compactness (%)	Compactness at the centre (%)	Compactness at the periphery (%)
Costal MPEF-PV-10953	0.6269529 (0.0003377)	0.999999 (0)	0.0394243 (0.000238)	0.9281332 (0.0002373)	**68**	62.7	94.2
Costal MPEF-PV-10958	0.5574728 (0.0004824)	0.999999 (0)	0.0614388 (0.0004389)	0.9215636 (0.0003421)	**63.2**	55.7	89.7
Peripheral MPEF-PV-10959	0.5575535 (0.000482)	0.999999 (0)	0.0613347 (0.0004387)	0.9217116 (0.0003417)	**63.2**	55.8	89.7
Hypoplastron MAU-Ph-N-002	0.339197 (0.0031223)	0.999999 (0)	0.2867314 (0.0022919)	0.7310307 (0.0031633)	**64**	38.9	81.2

SE standard errors

Fig. 3 Histology of external cortex of *Yaminuechelys* cf. *Y. maior* shell bones. **a, b** General view of the cortex in a costal plate (MAU-Ph-LI-007) viewed under cross-polarized light using a lambda compensator. The figures show interwoven structural fibre bundles in the inner portion of the cortex (**a**) and parallel fibred bone tissue in the outer region (**b**). **c, d** External cortex of a peripheral plate (MAU-Ph-N-002) viewed under normal transmitted light (**c**) and cross-polarized light (**d**). **e, f** general view of the external cortex of *Yaminuechelys* cf. *Y. maior* costal plate (MAU-Ph-LI-007) under cross-polarized light using a lambda compensator (**a**) and detail of the same image (**f**) showing a resorption line (*arrowhead*). *ISF* interwoven structural fibres, *PFB* parallel fibred bone, *SC* small simple canals, *ShF* Sharpey's Fibres, *SO* secondary osteon. *Scale bar* equals 0.2 mm
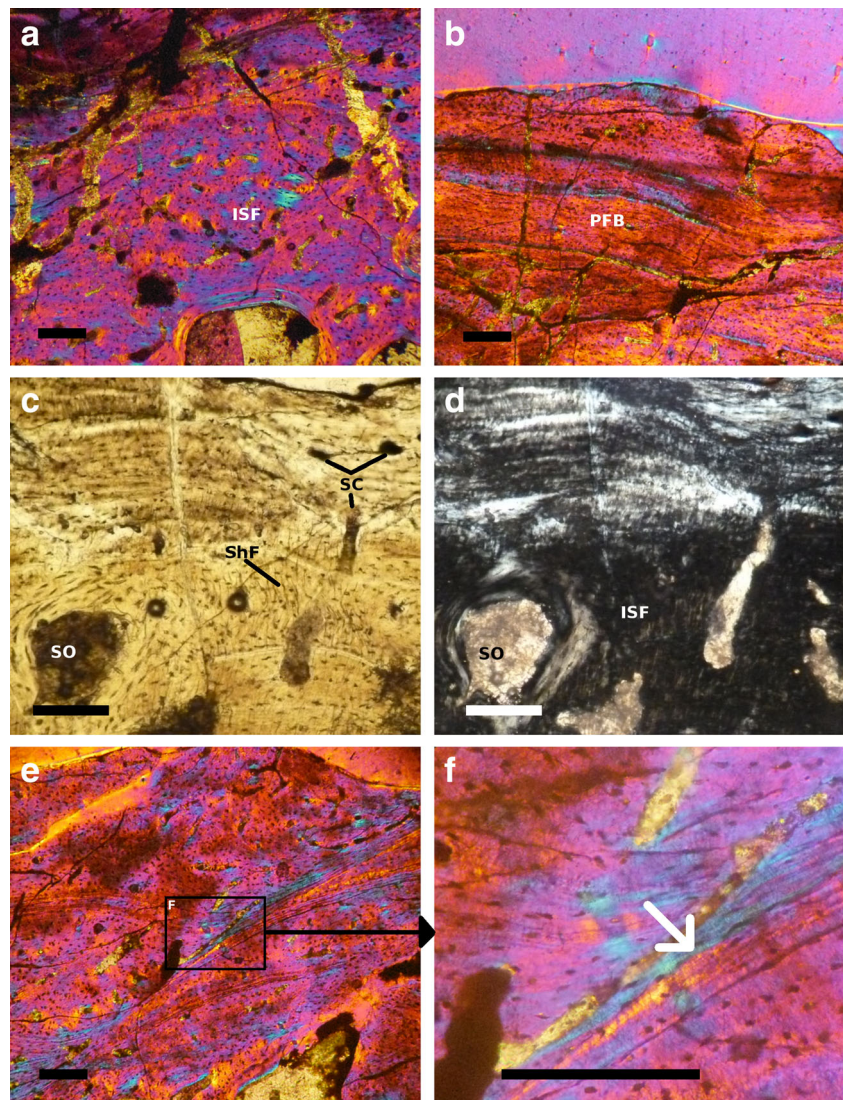

of the bones. The ICO is moderately vascularized (avascular in several instances). The matrix is composed principally of lamellar bone in plastral elements (Fig. 4a), although parallel fibred bone can be present in costals, neurals and peripherals. The intrinsic fibres of costal bones are parallel to the latero-medial axis in the central area (which correspond to the rib) but gradually change their arrangement towards the suture margins, where they become parallel to the antero-posterior axis. This change is evidenced not only by a variation in the optic properties of the bone (monorefringent in the centre, birefringent towards the margins) but also by a change in the shapes of the bone cell lacunae. These lacunae are circular/sub-circular in section in the centre and strongly elongated towards the suture margins. Long canaliculi radiate from each cell lacuna. Sharpey's fibres are present in several samples, whereas in some samples (MPEF-PV-10958, MAU-PV-Li-007), they are short and oriented transversally to the cortex, and in others (MAU-PV-N-001, MPEF-PV-10953), they are

relatively long and embedded in the cancellous bone directly adjacent to the rib of the costal plate. Lines of arrested growth (LAGs) are present in plastral plate MAU-PV-N-002 and costal plates MAU-PV-N-002, MAU-PV-N-001 (Fig. 6).

Other variations of the general histological pattern of the ICO described above are also observed in some samples. Costals MAU-PV-N-001 and MAU-PV-N-002 have a strong stratification, in which three different regions (inner, middle and outer) can be distinguished (Fig. 4d). The inner region is avascular and consists of lamellar tissue. In the middle region, the matrix is composed of parallel fibred bone in which the intrinsic fibres are parallel to the latero-medial axis. Longitudinally oriented primary osteons are present in this area. The outer zone is characterized by a mixture of lamellar and parallel fibred bone with scattered primary osteons longitudinally oriented. Secondary osteons are observed between the inner and middle region of the ICO forming a layer (Fig. 5d).

Fig. 4 General view (**a**, **c**) and detail (**b**, **d**) of the external cortex of *Yaminuechelys* cf. *Y. maior* costal plate (MAU-PV-N-001). Note the presence of a distinct resorption line (arrowhead) in the cortex. **a**, **b** Normal light. **c** Cross-polarized light using lambda compensator. **d** Cross-polarized light. *FLB* fibro lamellar bone, *ISF* interwoven structural fibres, *PO* primary osteon. *Scale bar* equals 0.2 mm
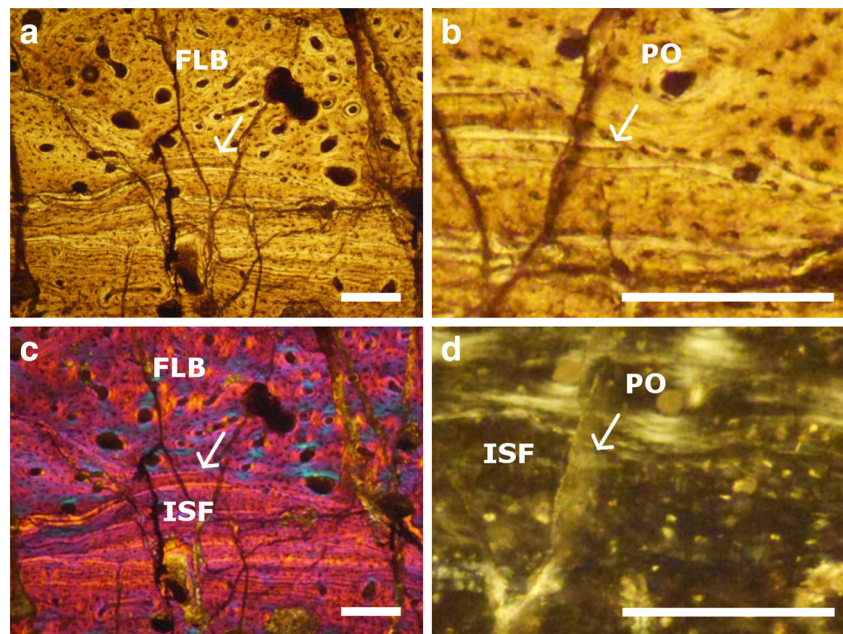
Sutures

The internal structure of sutural areas could only be studied in some costal plates (MPEF-PV-10953, MAU-Ph-N-001, MAU-Ph-LI-007) and in a plastral plate (MAU-Ph-N-002). The bone tissue adjacent to the sutures is similar to the tissue described for the ECO but shows more signs of remodelling (Fig. 7). The margins show sutures with short protrusions and sockets. Both protrusions and sockets are commonly lined by a thin layer of birefringent tissue. 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 fibres are present in several samples, but not in all. When present, these fibres are commonly oriented parallel to the protrusions.

Discussion

Bone ornamentation origin and maintenance

The external surface of the tetrapod dermal bones is mostly ornamented by pits, ridges and/or tubercles. This superficial ornamentation is mainly originated by two different mechanisms. First, local resorption and partial redeposition of the cortical bone have been reported in sculptured dermal skull bones and osteoderms from several archosaurian taxa (e.g. de Buffrénil 1982; Hua and de Buffrénil 1996; Scheyer and Sander 2004; Cerda and Desojo 2011; de Buffrénil et al. 2014). The second mechanism, proposed for osteoderms and dermal bones of basal tetrapods (Witzmann and Soler-Gijon 2008; Witzmann 2009), is preferential deposition of the bony areas that correspond with ridges or tubercles, without resorptive processes. This last mechanism has been recently proposed for the origin of the particular ornamentation in solemydid turtles (Scheyer et al. 2014b), and also appears to be involved in the formation of soft shelled turtle (Trionychidae) ornamentation (Scheyer et al. 2007). Our sample clearly reveals that the sculptured pattern of *Yaminuechelys* shell bones actually originated from a combination of the main mechanisms mentioned above, whereas in some samples, resorption lines are well marked (Fig. 3 e, d), indicating a resorptive process followed by new bone tissue deposition, and in others, bone resorption is clearly absent. The sculpturing in these last samples is formed by local changes in the rates of bone deposition, which is also evidenced by the differences in the bone tissues (while valleys are mostly formed by a lamellar bone tissue, the ridges are formed by parallel-fibred bone tissue). Resorptive processes are only evident in those samples in which the sculpturing is strongly pronounced. It is interesting to note that in *Hydromedusa tectifera* (a chelid turtle closely related to *Yaminuechelys* in which the ornamentation pattern is similar but less pronounced), previous histological descriptions do not report the presence of resorption lines in the external cortex (Scheyer et al. 2007). The pattern of sculpturing of *Yaminuechelys* differs from that reported in other ornamented turtles as Solemydidae and Trionychidae (Scheyer et al. 2007, 2014b). Furthermore, there are no previous reports of such patterns in extant or fossil Testudines, suggesting a derived feature in *Yaminuechelys*. Although Cerda et al. (2015) reported resorptive processes in few plates of *Condorchelys antiqua*, they interpret that such pattern is originated from a pathological condition.

Fig. 5 Shell histology of *Yaminuechelys*. **a** Thin-section of *Yaminuechelys* cf. *Y. maior* xiphiplastron (MAU-Ph-N-002) showed in normal transmitted light (*left half*) and cross-polarized light (*right half*). **b** Detail of a large internal cavity in the cancellous bone of *Yaminuechelys maior* costal plate (MPEF-PV-10953). **c** Cancellous bone of *Yaminuechelys* cf. *Y. maior* costals (MAU-Ph-N-001). **d** Detailed view of the stratified internal cortex in a *Yaminuechelys* cf. *Y. maior* peripheral plate (MAU-Ph-N-002). *CB* cancellous bone, *ECO* external cortex, *ICO* internal cortex, *LB* lamellar bone, *PFB* parallel-fibred bone, *PO* primary osteon, *SO* secondary osteon, *TR* trabeculae. *Scale bar* equals **a** 0.5, **b** 0.3 and **c**, **d** 0.2 mm
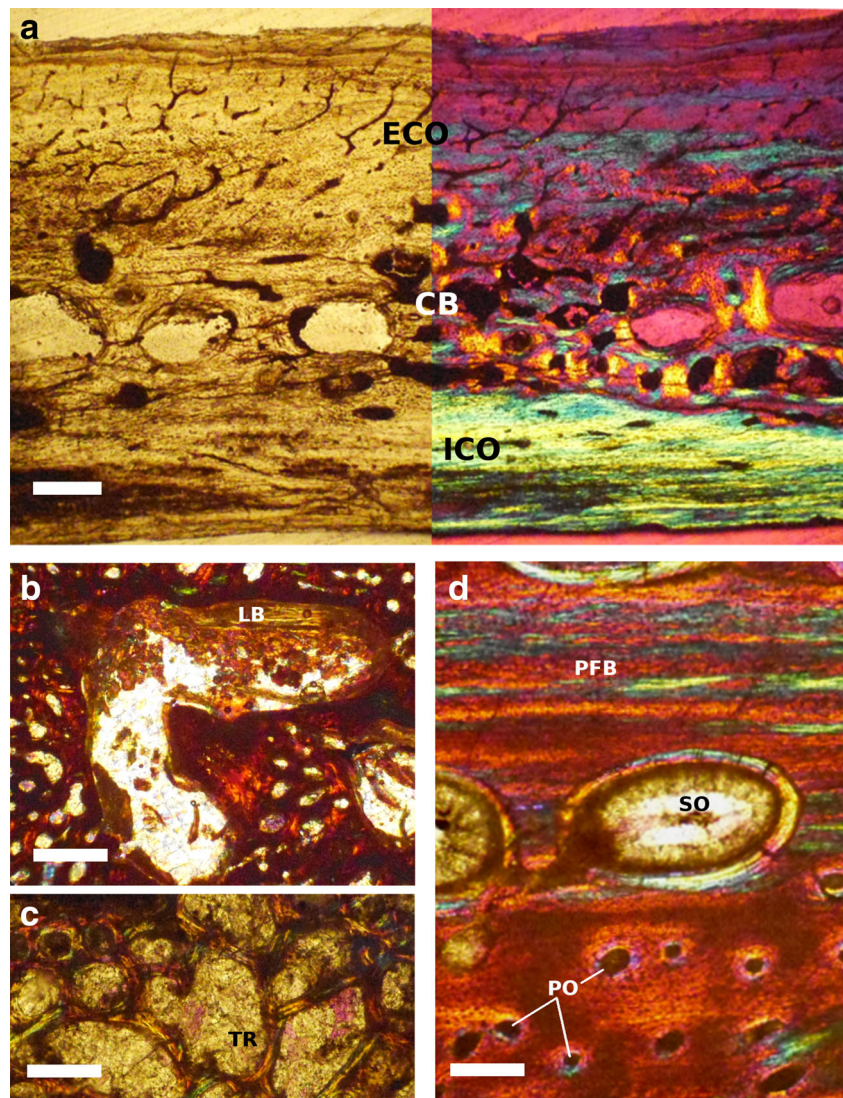
Comparison with other Chelidae

General histology of *Yaminuechelys* shows some similar characteristics to other studied chelids (Scheyer 2009; de la Fuente et al. 2015). Based on some comparative studies on pelomedusoid taxa, such as *Stupendemys geographicus*, *Podocnemis erythrocephala* (Scheyer and Sanchez-Villagra 2007), the primitive pan-pleurodire *Dortoka vasconica* (Pérez-Garcia et al. 2012), the pan-pleurodiran like *Platychelys oberndorferi*, and with stem turtles (Scheyer and Sander 2007), several histological features in chelid shells (i.e. the diploë structure of the shell, the metaplastic incorporation of dermal interwoven structural fibre bundles in the external cortex, the parallel-fibred bone of the internal cortex and the presence of Sharpey's fibres) are plesiomorphic for all turtle shell bones (Scheyer et al. 2007). Whereas cortices of the shell bones of *Platemys platycephala*, *Phrynops geoffroanus* and *Hydromedusa tectifera* were of similar thickness, in

Emydura sp., *Chelodina longicollis*, *Chelus fimbriatus* and *Yaminuechelys*, the ICO is reduced in thickness compared to the ECO (Scheyer 2009). Nevertheless, the presence of resorption lines associated with the maintenance of the ornamentation is a particular feature reported only in *Yaminuechelys*. The canals opening up into small foramina on the surface is a character shared only with *P. geoffroanus* (Scheyer 2009).

Palaeoecology

Chelidae is a clade of turtles characterized by living in freshwater aquatic to semiaquatic environment (de la Fuente et al. 2014). However, some specimens of the extant *Hydromedusa tectifera* have been recovered in estuarial environments of Rio de la Plata with barnacles on the carapace (Frazer 1986; de la Fuente 1999), indicating that these turtles could venture large distances into estuarine-marine environments. A similar
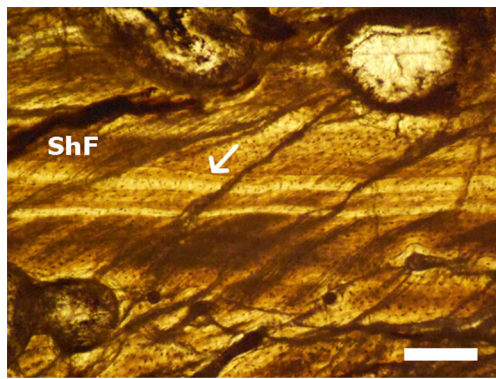
Fig. 6 Line of arrested growth (*arrowhead*) in the internal cortex of *Yaminuechelys* cf. *Y. maior* costal plate MAU-PV-N-001. *ShF* Sharpey's Fibres. *Scale bar* equals 0.2 mm

estuarine palaeoenvironment has been suggested for the Danian levels of the Salamanca Formation, where the remains of *Yaminuechelys maior* were found (Bona et al. 1998, and references therein). In contrast, fluvial environments have been inferred for the sediments of different outcrops of the Anacleto Formation (Filippi et al. 2011; Filippi and Garrido 2012) that yielded *Yaminuechelys* aff. *Y. maior* (de la Fuente et al. 2015) and *Y.* cf. *Y. gasparinii* (de la Fuente et al. 2010, and references therein). Nevertheless, as Joyce and Gauthier (2004) pointed out, it is plausible to infer that a turtle discovered in terrestrial sediments is not marine, but it does not follow that a turtle found in fluvial or marine sediments cannot be of terrestrial origins, because rivers can bury terrestrial faunas or transport them to marine environments. For this reason, palaeoenvironmental information does not appear to yield conclusive evidence for the habitat selection of *Yaminuechelys*.

Another method used to infer the habitat preference of extinct turtles is based on the relative proportions of the stylopodium, zeugopodium and autopodium of the forelimb (Joyce and Gauthier 2004). Zangerl (1953) made a disquisition on limbs of the fresh water turtles and sea turtles. In fresh-water chelonians, the forelimbs and humeri are shorter than hind limbs and femora, respectively. This condition can be recognized in extant and extinct chelids such as *Yaminuechelys gasparinii*, *Linderochelys rinconensis* or *Hydromedusa casamayorensis* (de la Fuente et al. 2001, 2007; Maniel et al. 2012). We test this aquatic lifestyle hypothesis for *Yaminuechelys* using shell bone microanatomy.

Fig. 7 Histology of suture margins of *Yaminuechelys* plates. **a**, **b** ▶ Xiphiplastron of *Yaminuechelys* cf. *Y. maior* (MAU-Ph-N-002) viewed under normal transmitted light (**a**) and cross-polarized light using a lambda compensator (**b**). **c**, **d** Costal plate of *Yaminuechelys* cf. *Y. maior* (MAU-Ph-LI-007) viewed under normal transmitted light (**c**) and cross-polarized light using a lambda compensator (**d**). *ISF* interwoven structural fibres, *ShF* Sharpey's Fibres. *Scale bar* equals 0.3 mm

The carapace microstructure has been used as a valuable tool in palaeoecological studies of fossil turtles (Pérez-Garcia et al. 2012; Scheyer 2007; Scheyer and
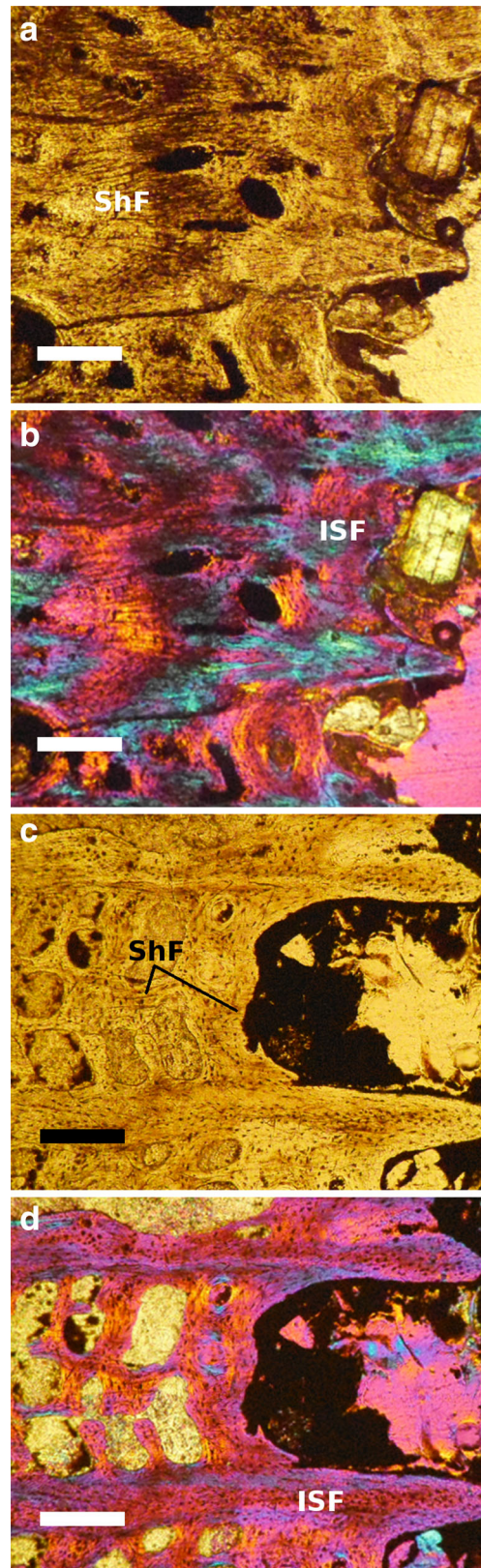
Sander 2007; Scheyer et al. 2014a; Sterli et al. 2013; Cerda et al. 2015). These studies are based on the degree of development of compact and cancellous bone and the degree of cortical vascularization. Scheyer (2007) proposed four categories according to their degree of aquatic adaptation (I: terrestrial; II: semiaquatic to mainly aquatic; III: fully aquatic; and IV: extreme aquatic/marine adaptation). More recently, quantitative approaches using BONE PROFILER Software (Fig 2, Table 2) have been proposed for the determination of palaeoecological traits in extinct turtles (Scheyer et al. 2014a). In this regard, the elements with a low degree of compactness are linked with aquatic habits. Using both approaches, the microstructure of the shell bone of *Yaminuechelys* sample fits with the fully aquatic condition (i.e. reduction of compact bone layers, especially the internal cortex, high degree of vascularization in the cortical bone). Compactness values of *Yaminuechelys* (63.2-68) also fit the range of aquatic turtles (Scheyer et al. 2014a). In this case, both gross anatomical and histological data support an aquatic lifestyle for *Yaminuechelys*. A more precise habitat preference (fluvial or marine) cannot be discerned based on the current data.

Conclusions

Many aspects of *Yaminuechelys* histology are evidenced. The results indicate that the particular ornamentation of the external plate surfaces is originated by differential rates of bone apposition in the bones and, in some cases, also by focalized resorption and new bone deposition. This last feature, which is evidenced by the presence of resorption lines in the cortex, appears to be a characteristic feature of this genus and could serve as a taxonomic signal for *Yaminuechelys*. Compactness of the shell bones is consistent with aquatic habitat, which coincides with palaeoenvironmental/morphological data collected for this genus. It remains to be discovered if these turtles could expand their habitat into marine environments, given its similarity to its sister taxon *Hydromedusa* and sedimentological record in which they find themselves.

References

Bona P, de la Fuente MS (2005) Phylogenetic and paleobiogeographic implications of *Yaminuechelys maior* (Staesche, 1929) new comb., a large long-necked chelid turtle from the early Paleocene of Patagonia, Argentina. J Vertebr Paleont 25:569–582

Bona P, Cladera G, de la Fuente MS (1998) Las tortugas pleurodiras de la Formación Salamanca (Paleoceno inferior) en el área de Cerro Hansen, Provincia de Chubut, Argentina. X Congr Latinoamer Geología VI Congr Naci Geol Económica, Actas 1:268–273

Boulenger C (1889) Catalogue of the Chelonians, Rhynchocephalians and Crocodiles in the British Museum (Natural History). Trustees British Museum (Natural History). pp 311

Cadena AE, Ksepka DT, Norell MA (2013) New material of *Mongolemys elegans* Khosatzky and Mlynarski, 1971 (Testudines: Lindholmemydidae), from the Late Cretaceous of Mongolia with comments on bone histology and phylogeny. Am Mus Nov 3766: 1–27

Cerda IA, Desojo JB (2011) Dermal armour histology of aetosaurs (Archosauria: Pseudosuchia), from the Upper Triassic of Argentina and Brazil. Lethaia 44:417–428

Cerda IA, Sterli J, Scheyer T (2015) Bone shell microstructure of the *Condorchelys antiqua* Sterli 2008, a stem turtle from the Jurassic of Patagonia. C R Palevol 821:1–14. doi:10.1016/j.crpv.2015.01.004

Chinsamy A, Raath MA (1992) Preparation of fossil bone for histological examination. Palaeontol Afr 29:39–44

de Buffrénil V (1982) Morphogenesis of bone ornamentation in extant and extinct crocodilians. Zoomorphology 99:155–166

de Buffrénil V, Farlow JO, de Ricqlès A (1986) Growth and function of *Stegosaurus* plates: evidence from bone histology. Paleobiology 12: 459–473

de Buffrénil V, Clarac F, Fau M, Martin S, Martin B, Pellé E, Laurin M (2014) Differentiation and growth of bone ornamentation in vertebrates: a comparative histological study among the Crocodylomorpha. J Morphol 276:425–445

de la Fuente MS (1999) A review of the Pleistocene reptiles of Argentina: taxonomic and palaeoenvironmental considerations. Quat South Am Antar Penin 12:111–138

de la Fuente MS, Lapparent de Broin F, Manera de Bianco T (2001) The oldest and first nearly complete skeleton of a chelid, of the Hydromedusa sub-group (Chelidae, Pleurodira), from the Upper Cretaceous of Patagonia. Bull Soc Géol France 172:237–244

de la Fuente MS, Salgado L, Albino A, Báez AM, Bonaparte JF, Calvo JO, Chiappe LM, Codorniú LS, Coria RA, Gasparini Z, González Riga BJ, Novas FE, Pol D (2007) Tetrápodos continentales del Cretácico de la Argentina: una síntesis actualizada. Asoc Paleontol Arg Publ Esp 11:137–153

de la Fuente MS, Barbieri R, Chafrat P (2010) Una tortuga Chelidae (Testudines: Pleurodira) de cuello largo en el Grupo Neuquén, Río Negro, Argentina. Significado cronológico y paleobiogeográfico. Andean Geol 37:399–413

de la Fuente MS, Sterli J, Maniel IJ (2014) Origin, Evolution and Biogeographic History of South American Turtles. Springer Earth System Sciences, Heidelberg

de la Fuente MS, Maniel IJ, Jannello JM, Filippi LS, Cerda I (2015) Long-necked chelid turtles from the Campanian of northwestern Patagonia with comments on K/P survivorship of the genus *Yaminuechelys*. C R Palevol 14:563–576

Filippi LS, Garrido AC (2012) Nuevo registro del género *Dinilysia* (Squamata, Serpentes) para la Formación Anacleto (Campaniano Inferior-Medio), Rincón de Los Sauces, Neuquén, Argentina. Ameghiniana 49:132–136

Filippi LS, García RA, Garrido AC (2011) A new titanosaur sauropod dinosaur from the Upper Cretaceous of North Patagonia, Argentina. Acta Palaeontol Pol 56:505–520

Francillon-Vieillot H, de Buffrénil V, Castanet J, Géraudie J, Meunier FJ, Sire JY, Zylberberg L, de Ricqlès A (1990) Microstructure and mineralization of vertebrate skeletal tissues. In: Carter JG (ed) Skeletal biomineralization: patterns, processes and evolutionary trends. Van Nostrand Reinhold, New York, pp 471–548

Frazer J (1986) Epizoic barnacles on pleurodiran turtles: is the relation rare? Proc Biol Soc Washington 99:472–477

Gaffney ES (1977) The side-necked turtle family Chelidae: a theory of relationship using shared derived characters. Amer Mus Nov 2681: 1–23

Georges A, Birrell J, Saint KM, McCord W, Donnellan SC (1998) A phylogeny for side-necked turtle (Chelonia: Pleurodira) based on mitochondrial and nuclear gene sequence variation. Biol J Linn Soc 67:213–246

Girondot M, Laurin M (2003) Bone profiler: a tool to quantify, model, and statistically compare bone-section compactness profiles. J Vertebr Paleont 23:458–461

Guillon JM, Guéry L, Hulin V, Girondot M (2012) A large phylogeny of turtles (Testudines) using molecular data. Contrib Zool 81:147–158

Hayashi S, Carpenter K, Suzuki D (2009) Different growth patterns between the skeleton and osteoderms of *Stegosaurus* (Ornithischia: Thyreophora). J Vertebr Paleont 29:123–131

Hill RV (2006) Comparative anatomy and histology of xenarthran osteoderms. J Morphol 267:1441–1460

Hua S, de Buffrénil V (1996) Bone histology as a clue in the interpretation of functional adaptations in the thalattosuchia (Reptilia, Crocodylia). J Vertebr Paleont 16:703–717

Jannello JM, Cerda IA, de la Fuente MS (2014) Estudio histológico comparativo del caparazón óseo de quelidos de cuello largo (Testudines: Pleurodira) del Paléogeno de Patagonia: Datos preliminares. Ameghiniana 51:14

Joyce WG, Gauthier JA (2004) Palaeoecology of Triassic stem turtles sheds new light on turtle origins. Proc R Soc London B 271:1–5

Main RP, de Ricqlès A, Horner J, Padian K (2005) The evolution and function of thyreophoran dinosaur scutes: implications for plate function in stegosaurs. Paleobiology 31:291–314

Maniel IJ, de la Fuente MS, Sterli J (2012) New material of *Hydromedusa casamayorensis* from the Middle Eocene of Patagonia and its phylogenetic implications. Symposium on turtle evolution, Tübingen, Germany. Programe and Abstract 29

Pérez-Garcia A, Scheyer TM, Murelaga X (2012) New interpretations of *Dortoka vasconica* Lapparent de Broin and Murelaga, a freshwater turtle with an unusual caparace. Cret Res 36:151–161

Pérez-García A, Scheyer TM, Murelaga X (2013) The turtles from the uppermost Jurassic and Early Cretaceous of Galve (Iberian Range, Spain): anatomical, systematic, biostratigraphic and palaeobiogeographical implications. Cret Res 44:64–82

Scheyer TM (2007) Skeletal histology of the dermal armor of Placodontia: the occurrence of postcranial fibro-cartilaginous bone and its developmental implications. J Anat 211:737–753

Scheyer TM (2008) Aging the oldest turtles: the placodont affinities of *Priscochelys hegnabrunnensis*. Naturwissenschaften 95:803–810

Scheyer TM (2009) Conserved bone microstructure in the shells of long-necked and short-necked chelid turtles (Testudinata, Pleurodira). Fossil Rec 12:47–57

Scheyer TM, Sanchez-Villagra MR (2007) Carapace bone histology in the giant pleurodiran turtle *Stupendemys geographicus*: phylogeny and function. Acta Pal Pol 52:137–154

Scheyer TM, Sander PM (2004) Histology of ankylosaur osteoderms: implications for systematics and function. J Vertebr Paleont 24: 874–893

Scheyer TM, Sander PM (2007) Shell bone histology indicates terrestrial palaeoecology of basal turtles. Proc Royal Soc B 274:1885–1893

Scheyer TM, Sander PM (2009) Bone microstructures and mode of skeletogenesis in osteoderms of three pareiasaurs taxa from the Permian of South Africa. J Evol Biol 22:1153–1162

Scheyer TM, Sander PM, Joyce WG, Bohme W, Witzel U (2007) Unique plywood structure in the shell of fossil and recent soft shelled turtles (Trionychidae) revealed by soft tissue and bone histology: a key adaptation? Org Divers Evol 7:136–144

Scheyer TM, Danilov IG, Sukhanov VB, Syromyatnikova EV (2014a) The shell bone histology of fossil and extant marine turtles revisited. Biol J Linnean Soc 112:701–718

Scheyer TM, Pérez-García A, Murelaga X (2014b) Shell bone histology of solemydid turtles (stem Testudines): palaeoecological implications. Org Divers Evol 15:199–212

Seddon JM, Georges A, Baverstock PR, McCord W (1997) Phylogenetic relationship of chelid turtles (Pleurodira: Chelidae) based on mitochondrial 12S rRNA sequence variation. Mol Phyl Evol 7:55–61

Staesche K (1929) Schildkrötenreste aus der oberen Kreide Patagoniens. Palaeontographica A 72:103–112

Sterli J, Pol D, Laurin M (2013) Incorporating phylogenetic uncertainty on phylogeny-based palaeontological dating and the timing of turtle diversification. Cladistics 29:233–246

Vlachos E, Cerda IA, Tsoukala E (2015) The morphology and bone histology of the first soft–shelled turtle from the Pliocene of Greece. Sci Nat. doi:10.1007/s00114-015-1295-2

Witzmann F (2009) Comparative histology of sculptured dermal bones in basal tetrapods, and the implications for the soft tissue dermis. Palaeodiv 2:233–270

Witzmann F, Soler-Gijon R (2008) The bone histology of osteoderms in temnospondyl amphibians and in the chroniosuchian *Bystrowiella*. Acta Zool (Stockholm) 89:1–19

Zangerl R (1953) The vertebrate fauna of the Selma Formation of Alabama. Part IV. The turtles of the family Toxochelyidae. Field Geol Mem 3:137–277