MICROANATOMY AND OSTEOHISTOLOGY OF A JUVENILE ELASMOSAURID PLESIOSAUR FROM THE UPPER MAASTRICHTIAN OF MARAMBIO (=SEYMOUR) ISLAND, ANTARTICA

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ABSTRACT

We describe here the microanatomy and histology of several bones from a juvenile elasmosaurid from upper Maastrichtian beds of Marambio (=Seymour) Island, Antarctica. Transversal thin sections were obtained from the diaphyseal region of a propodial bone and from a scapula fragment. Microanatomical analyses were conducted using Bone Profiler© software to measure bone compactness in two propodials, a vertebra and scapula and pubis fragments. Our results show the following: (1) at the micro-anatomical level, we found bone matrix filling almost completely the medullar region of the analyzed bones with compactness index values between 0.858 – 0.993 (values from 0 to 1) suggesting a dense osteosclerotic-like skeleton. (2) At the histological level, we didn´t found any sign of growth zones or annuli/LAGS in the cortex region. Instead of that, we found a thin cortex composed by vascularized primary woven-fibered bone tissue with anastomosed radial vascular canals and a medullar region composed by few bony trabeculae filled with calcified cartilage with some resorption cavities and Howship´s lacunae (=evidence of chondroclastic activity). These osteo-histological features confirm the presence of osteosclerosis (probably having a "manatee-like" mode of life of a slow swimmer) and firmly suggesting that this specimen was a very juvenile at the moment of death.

Keywords: Elasmosaurid, Cretaceous, microanatomy, osteohistology, ontogeny, calcified cartilage, osteosclerosis, compactness, pachyostosis.

RESUMEN

Microanatomía y osteohistología de un plesiosaurio elasmosáurido juvenil del Maastrichtiano superior de Isla Seymour, Antártica. En este trabajo describimos la microanatomía e histología de varios huesos de un elasmosáurido juvenil de niveles del Maastrichtiano Superior de Isla Marambio (=Seymour), Antártica. Secciones finas fueron obtenidas a partir de la diáfisis de un propodio y de un fragmento de escápula. El análisis de la microanatomía fue realizado utilizando el software Bone Profiler© para medir la compactación del tejido óseo en dos propodios, una vértebra y fragmentos de escápula y pubis. Nuestros resultados muestran lo siguiente: (1) a nivel microanatómico, encontramos una alta densidad de matriz ósea en donde casi no habían espacios en la zona medular. Los valores del índice de compactación oscilaban entre 0,858-0,993 (valores de 0 a 1). Estos resultados sugieren un esqueleto denso parecido a un caso de osteosclerosis. (2) a nivel de la histología, no encontramos presencia de zonas de crecimiento ni anillos/LAGS en la zona cortical. En vez de eso, encontramos una corteza muy delgada compuesta por tejido primario entretejido altamente vascularizado con canales vasculares de disposición radial anastomosados, y una región medular compuesta por pocas trabéculas óseas, llena de de cartílago calcificado con cavidades de reabsorción y lagunas de Howship (=evidencia de actividad condroclástica). Estas características osteo-histológicas confirman la presencia de osteosclerosis (probablemente reflejando un modo de vida tipo-manatí/nadador pasivo) y sugiere fuertemente que este espécimen fue un juvenil de edad muy temprana al momento de morir.

Palabras clave: Elasmosáurido, Cretácico, microanatomía, osteohistología, ontogenia, cartílago calcificado, osteosclerosis, compactación, paquiostosis.

INTRODUCTION

The internal structure of fossil bones can provide us information about ontogenetic stages, growth rates, biomechanics, physiology and ecology. These biological variables can nurture a proper reconstruction of how an animal lived millions of years ago (de Ricqlès, 2011; Francillon-Vieillot *et al.* 1990).

During the evolutionary history of tetrapods some derived groups returned to an aquatic mode of life (de Ricqlès 1989; de Ricqlès 2001; Houssaye 2012). In this process, convergent skeletal specializations occurred and generally were presented in two structural patterns: (1) increasing the skeleton mass/density creating natural ballast and (2) decreasing the skeleton mass/density (Taylor 2000). In the first case we noted the appearance of three histological features: pachyostosis, osteosclerosis and pachyosteosclerosis. Pachyostosis can be defined as an increase in bone mass (*sensu-lato* criterion) or hyperplasia of the sub-periosteal cortices (*sensu-stricto* criterion, Houssaye 2009; Francillon-Vieillot *et al*. 1990). Osteosclerosis is defined as lack of medullary cavity with bone tissue and calcified cartilage occupying this place instead (Francillon-Vieillot *et al.* 1990) or in other cases occupying with endosteal lamellar bone (like in the basal sauropterygian *Claudiosaurus germani,* de Buffrénil and Mazin (1989)). Pachyosteosclerosis is defined as the combination of pachyostosis *sensu-stricto* with osteosclerosis (Francillon-Vieillot *et al*. 1990; Houssaye 2009). These features had the functional consequence of create a natural ballast of the skeleton allowing a passive control of the body trim in water (Domming and de Buffrénil 1991) and it has be described that this structural condition could be typical of slow moving animals that feed of non-mobile food or non-elusive preys and live in shallow waters (Webb and de Buffrénil 1990; Wiffen *et al.* 1995).

In the second case, we observed the lightening of the skeleton with the occurrence of a non-pathological osteoporosis in which the bone tissue presents several and large internal resorption cavities (Francillon-Vieillot *et al.* 1990). This osteoporotic-like state has been proposed to be related with predatory forms adapted to rapid swimming and open sea habitats because having a light skeleton would reduce the inertia of the body and improve the capability to have a high degree of maneuverability in the water column (Webb 1988; Taylor 2000).

The ontogenetic trajectory (Alberch *et al.* 1979) of the skeleton microanatomy in elasmosaurids is very interesting. So far we know, in early juvenile individuals of this clade the skeleton is compact and dense with recurrence of osteosclerosis and paquiosteosclerosis. Then in adults the bones decrease in mass and density resulting in an osteoporotic-like skeleton (Wiffen *et al*. 1995). Recent studies partially confirmed this facts in plesiosaurs from the Late Jurassic from Svalbard (Liebe and Hurum 2012). With juveniles presented dense skeletons but interestingly not all adult forms underwent a transition to a very porous bone structure through ontogeny. Suggesting an "ancestral-type" of ontogenetic trajectory with the conservation of the dense skeleton through the developmental history of these animals.

Taking into account these antecedents, we described here the microanatomy and histology of several bones of a single elasmosaurid plesiosaurian from Late Cretaceous levels of Marambio Island in Antarctica. Further discussion in this contribution is made in the context of the evolution and development of the skeleton of secondarily aquatic adapted tetrapods.

Institutional abbreviation. SGO.PV, Paleontología de Vertebrados, Museo Nacional de Historia Natural, Chile.

METHODS

The specimen was recovered by the Paleontology team of the Proyecto Anillo Antártico 2010-2013 (ACT-105, CONICYT-Chile). Prospective fieldwork on Marambio Island was conducted by DRR. The specimen studied here was recovered by RAO from recent mud deposits partially covering rocks of the Klb9 unit of the López de Bertodano Formation. The specimen was eroded from the latter and it was retained as scattered elements within the mud, in an area between 0.5 to 1 m^2. All the elements show a similar macroscopic bone texture and compactness, as well as identical macroscopical mineralization of the bones. In addition, there is no anatomical overlapping of the recovered elements. Also, all of them have a consistent relative size without disparate elements. These facts suggest that all the material belongs to a single individual.

Petrological slices of 10 μm thickness were made at the Laboratorio del Servicio Nacional de Geología y Minería (SERNAGEOMIN, Chile). Further histological descriptions were conducted by LOF. Microanatomical analyses of bone compactness were conducted by LOF utilizing the software Bone Profiler© following the protocol of Girondot and Laurin (2003). All the microphotographs under bright-field microscopy and polarized light were obtained using the Leica DM750P polarizing microscope in the Laboratorio de Geología de la Universidad del Desarrollo, Chile. For histological descriptions, we follow the concepts and structural criteria proposed by Francillon-Vieillot *et al*. (1990).

Systematic Paleontology

Sauropterygia Owen, 1860
      Plesiosauria de Blainville, 1835
            Plesiosauroidea Welles, 1943
                  Elasmosauridae Cope, 1869 (*sensu* Ketchum and Benson, 2010)
                        Elasmosauridae indet.
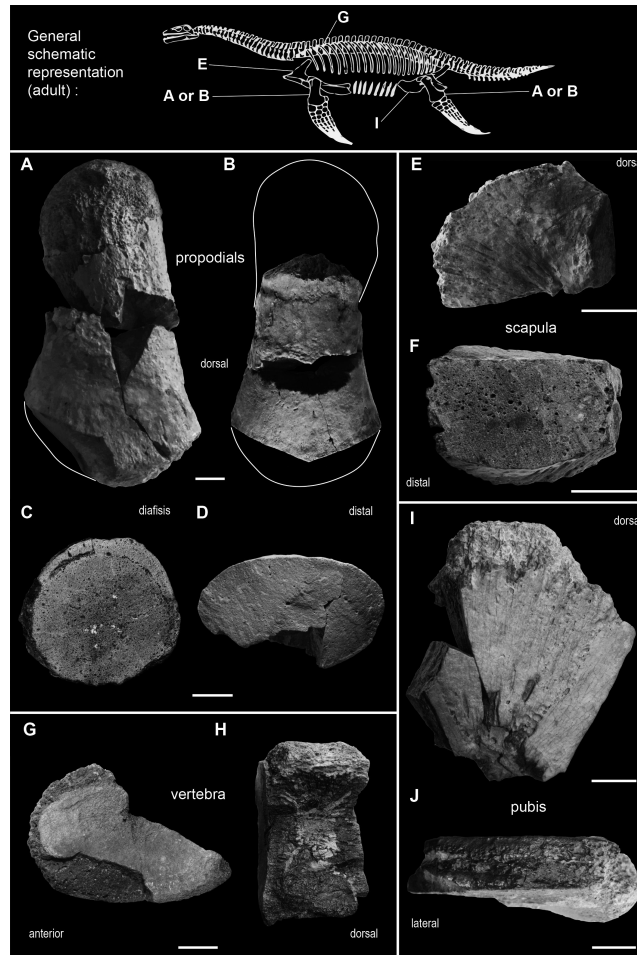   (See Figure 1)

FIGURE 1. Overview of the fossil material studied from SGO.PV.6666. A-B, dorsal view of two propodials. C, diaphyseal region of A. D, epiphyseal region of B. E-F, dorsal and distal views of a scapula fragment. G-H, anterior and dorsal views of a dorsal vertebra fragment. I-J, dorsal and lateral views of a pubis fragment. Scale bar = 2 cm

Material—SGO.PV.6666, two propodials, three dorsal vertebral centra, several pectoral/pelvic girdle fragments (more than 10) and a mesopodial element.

Locality, horizon and age—Seymour (=Marambio) Island, Antarctica. Upper levels of the López de Bertodano Formation (Klb9 molluscan unit), Upper Maastrichtian.

Description of SGO.PV.6666. The associated materials are: 1. two propodials (Figure 1A-B), one almost complete with proximal and distal articular surfaces partially eroded and not completely developed, suggesting a juvenile age (Figure 1A; Brown 1981). The second propodial is much eroded in its distal and proximal ends (Figure 1B). The shaft is diaclased, allowing the observation of natural cross-sections. In these, the propodials present a dense bone matrix, and it is not visible a central medullar cavity. 2. Three fragmentary vertebral centra (one showed in Figure 1G-H) highly eroded in the dorsal/ventral, lateral surfaces and anterior/posterior articular surfaces. Two vascular foramina at the ventral side of each centrum are preserved (typical of plesiosaurians) and the articular facets of ribs and neural arches are eroded (likely, these were poorly developed in the axial skeleton). The preserved vertebrae belong to the posterodorsal region of the animal. 3. An eroded mesopodial element with dorsal and ventral surfaces partially preserved. Its anatomical placement cannot be determined. 4. Finally, various (+10) fragments of pelvic/pectoral girdles. Two large fragments are identified as part of a scapula, based on the presence of a medial constriction consistent with the medial part of the scapula, from where the three main processes (anterior, dorsal and medial) rise (Figure1E-F) and a pubis (Fig.1I-J).

Remarks—The high degree of erosion in all the bones studied here, plus the lack of better defined characters due to the young ontogenetic stage of the specimen, preclude a better taxonomic designation. Instead, these materials are ideal for a microanatomical and histological approach to have a paleobiological reconstruction of the type of skeleton this animal could have at the moment of death. Which contribute to the understanding of the grade of compactness that the skeleton would have had, the definition of it ontogenetic stage and it paleo-ecological significance.

RESULTS

Bone microanatomy of SGO.PV.6666

The results are showed in Figure 2. An examination of the broken surfaces of the elements reveals that all the bones were very dense and had highly amounts of mineralized matrix filling the interior cavities. Only in the central part of the vertebral centrum and the pubis we observed some medullar cavities. To quantify this observation we used Bone Profiler© software and we find that all the bone analyzed presented a high value of bone compactness BC (or compactness index). This value is calculated as BC = [area occupied by bone matrix] / [total area of de section]. All the values of BC in SGO.PV.6666 are near one. Propodial transversal sections from diaphysis and epiphysis had 0.956 and 0.993 respectively. The other values are: scapula = 0.982, vertebral centrum = 0.906; and pubis 0,858. Following the study of de Buffrénil *et al.* (2010) in bones of sirenians, they established that if BC increase the 0.817 value, it indicate an osteosclerotic bone. If we extrapolate the values we previously obtained we can suggest that SGO.PV.6666 could have had an osteosclerotic skeleton.

Bone histology of SGO.PV.6666

PROPODIAL DIAPHYSIS: In Figure 3 we showed the histological thin section obtained from the propodial in Figure 1A. We observed that the medullar region occupies almost the entire area of the section, leaving just a thin cortex at the periphery, near the sub-periosteal surface (Figure 3A). Under bright-field microscopy at the cortex radial and oblique anastomosed vascular canals with a plexiform-like arrangement are observed (Figure 3B). The osteocyte lacunae are displayed in a woven pattern between the vascular canals (Figure 3D). *Globulli ossei* are observed in the areas in which calcified cartilage is preserved (Figure 3F). At the medullary region, we find bony trabeculae and small resorption cavities (Figure 3C). Extended areas with calcified cartilage and *globulli ossei* occur between the trabeculae (Figure 3E). We find Howship´s lacunae at the border of all the resorption cavities at the section indicating a possible chondroclast activity
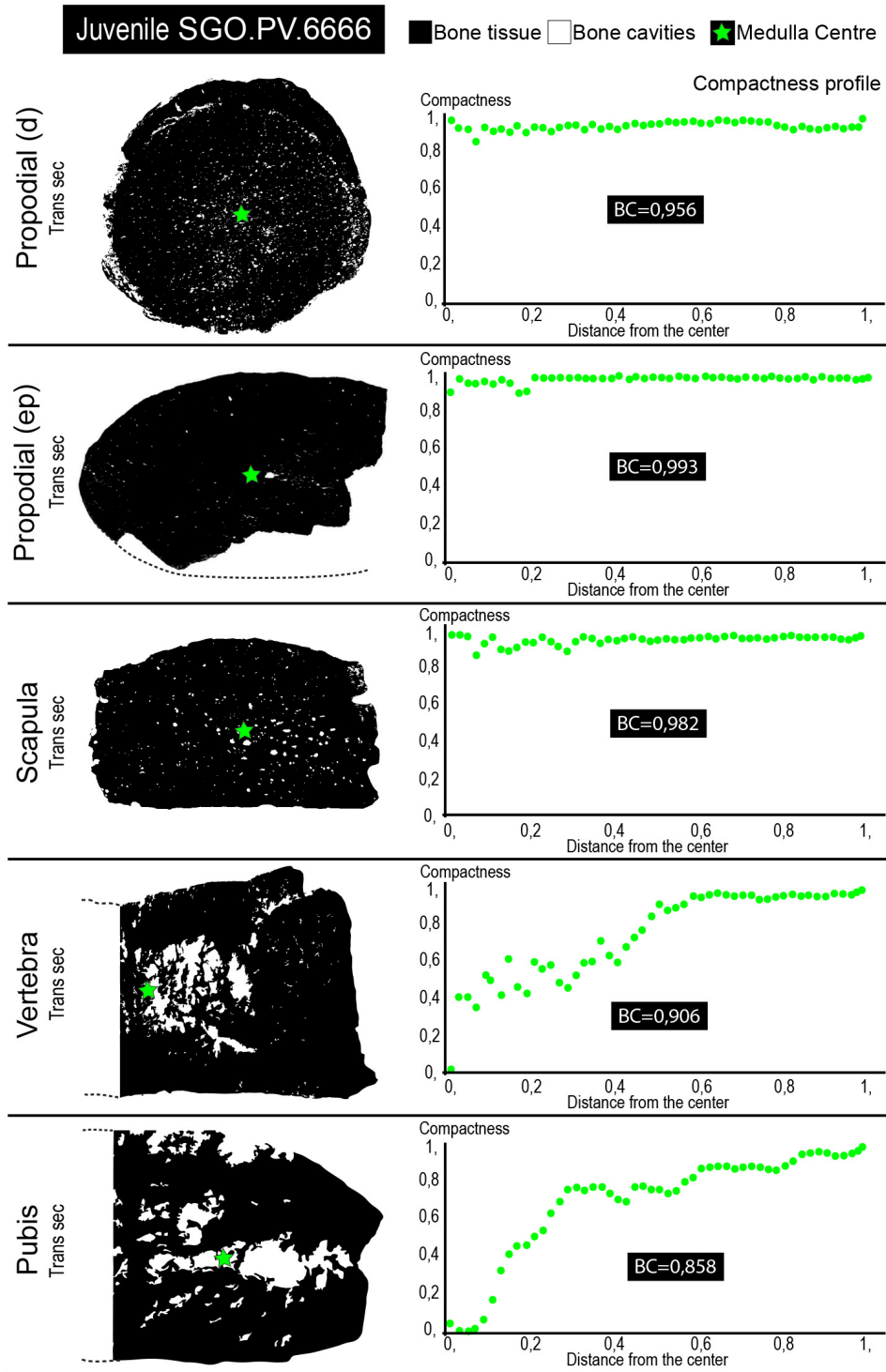
FIGURE 2. Bone Profile© analyses of bone compactness in SGO.PV.6666. It is displayed the compactness profile of transversal sections of propodials (diaphyseal and epiphyseal regions), a scapula fragment, a vertebra and a pubis fragment. Bone compactness (BC) values are attached in the figure
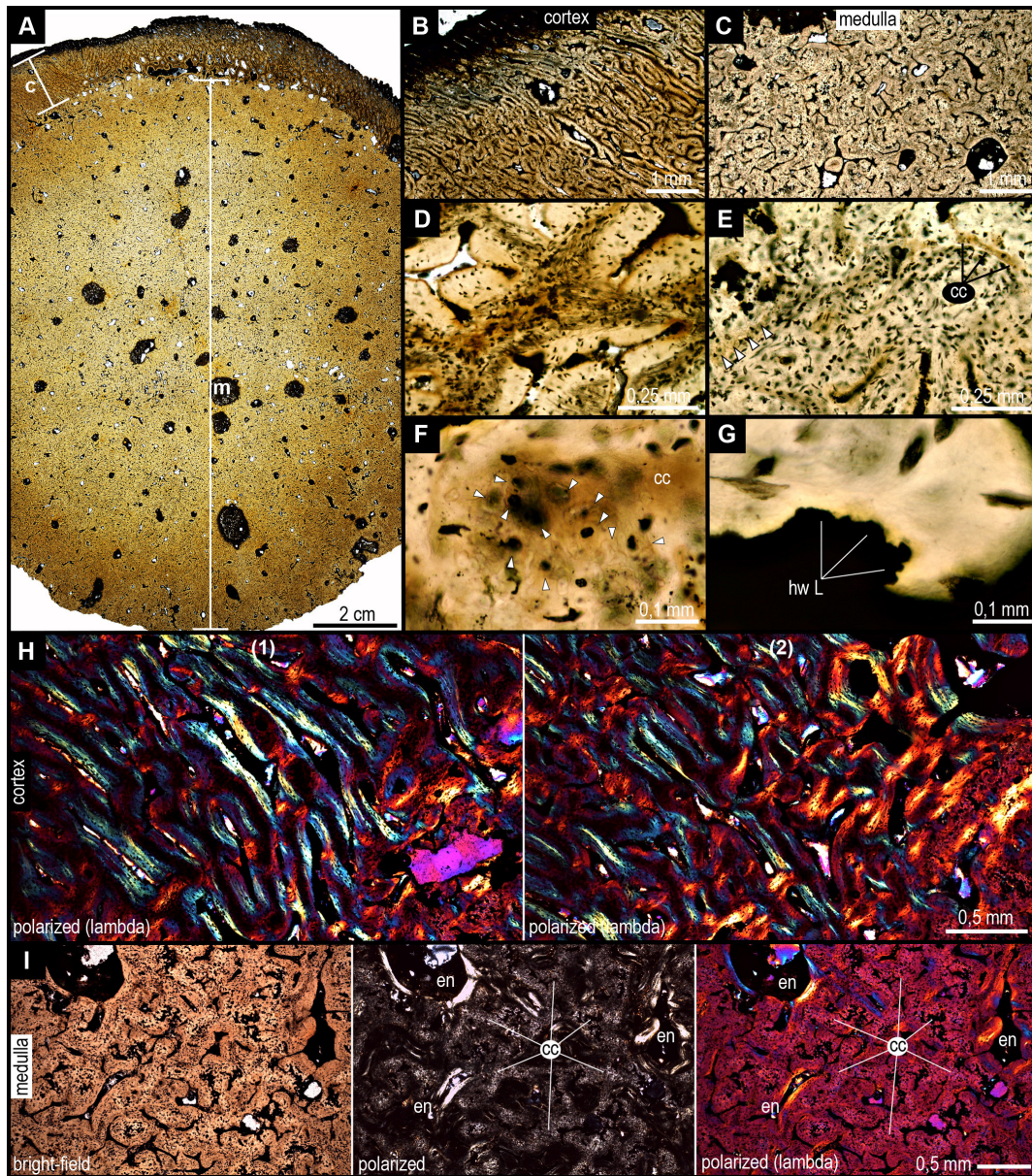
FIGURE 3. Histological section obtained from a propodial diaphysis. A, overview of the section showing that the medullar region occupied almost the entire area of the section with a very thin cortical region at the periphery. B, D, F, bright-field microphotographs of the cortex showing radial anastomosed vascular canals with a plexiform-like arrangement (B), Zoom-in of bone matrix between vascular channels showing the woven arrangement of osteocyte´s lacunae (D), and an area with globulli ossei (white arrow heads) and calcified cartilage (F). C, E, G, bright-field microphotographs of the medulla showing bony trabeculae (or endosteal trabeculae) and small resorption cavities (C), extended areas with calcified cartilage and globulli ossei (white arrow heads) between the trabeculae (E), and Howship´s lacunae in the border of resorption cavities indicating possible chondroclast activity (G). H, it shows two cortex areas (1, 2) under polarized light with woven-fibered primary tissue (primary osteons). I, medulla area under polarized light showing separated endosteal trabeculae with lamellar primary tissue with large territories of calcified cartilage between them. Abbreviations: c=cortex, m=medulla, cc=calcified cartilage, hwL=Howship´s lacunae, en=endosteal trabecular bone. Scale bars in figures
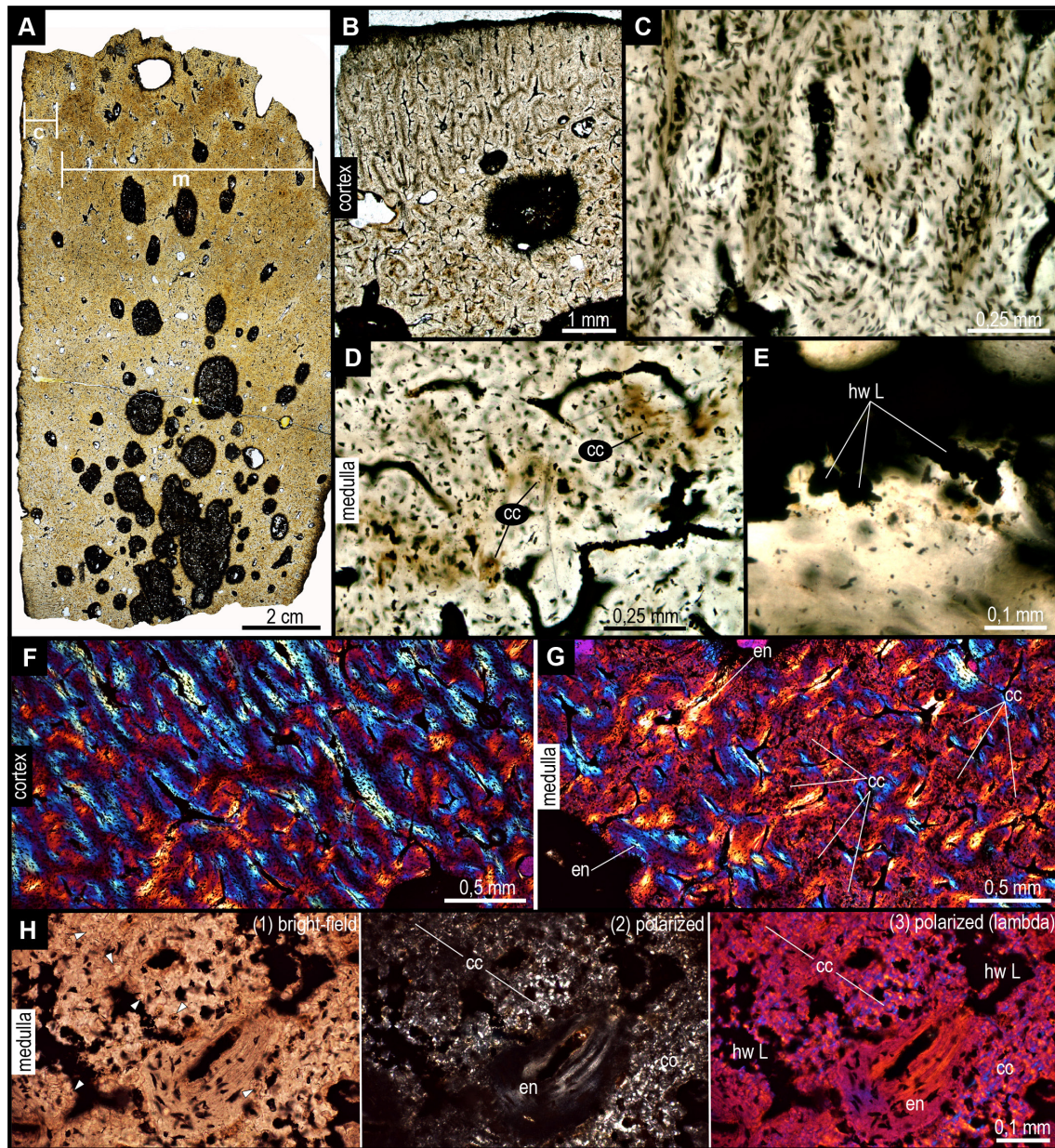
FIGURE 4. Histological section obtained from a scapula fragment. A, overview of the transversal section showing cortex and medullar regions, with the latter more extensive occupying almost all the area of the section. Note the appearance of more numerous and larger resorption cavities. B, C, bright-field microphotographs of a cortex area showing the arrangement of radial vascular canals and also the transition to the medullar region with a large resorption cavity (B), and a zoom-in of the radial canals showing the bone matrix and osteocytes lacunae in a woven arrangement. D, E, bright-field microphotographs of a medulla region showing bounded areas with calcified cartilage (D), and a detail of Howship´s lacunae (E). F, a cortex area under polarized light showing woven-fibered primary tissue. G, a medulla area under polarized light showing bony trabeculae with endosteal lamellar primary tissue separated by wide territories of calcified cartilage. H, detail of a bony trabecula and its surrounding area showing in bright-field (1) some globbuli ossei (white arrow heads) and under polarized light (2, 3) the endosteal lamellar tissue surrounded by calcified cartilage and some Howship´s lacunae

(Figure 3G). Under polarized light we observe that the cortex is composed of woven-fibered tissue (Fig 3H). Finally, in the medulla we find bony trabeculae with endosteal primary bone, separated by wide territories of calcified cartilage (Figure 3I).

SCAPULA FRAGMENT: In Figure 4 we showed the histological thin section obtained from a scapula fragment (Figure 1E-F). We find the same general pattern with mineralized matrix filling almost all the area of the section with an extensive medullary region and a relatively thin peripheral cortex. Numerous and large resorption cavities are present at the central part of the medullar region (Figure 4A). Under bright-field microscopy we find in the cortex once again radial vascular canals with a large resorption cavity at the beginning of the medullar region (Figure 4B). The bone matrix exhibits osteocyte lacunae in a disorganized arrangement (Figure 4 C). Areas of calcified cartilage (Figure 4D) are distinct in the medullary region. Howship´s lacunae are also present in this part of the element (Figure 4E). We find in the cortex woven-fibered primary tissue with primary osteons disposed radially around the radial vascular canals (Figure 4F). At the medullary region we found bony trabeculae with endosteal lamellar primary tissue separated by large areas of calcified cartilage (Figure 4G). More in detail if we approach to a particular isolated trabecula we find a vast surrounding area filled calcified cartilage and some *globulli ossei* with resorption cavities and Howship lacunae within their borders suggesting chrondroblastic activity (Figure 4H).

DISCUSSION AND CONCLUSION

Skeleton evolution of tetrapods secondary adapted to life in water
To discuss our results first we have to try see the 'whole picture' of what happened in the skeleton evolution of tetrapods secondary adapted to life in water (we also recommend consulting the works of Ricqlès y de Buffrénil 2001; Taylor 2000; Talevi 2012; Ricqlès *et al.* 2001 and Houssaye 2009 for more details). In Figure 5 we summarized the following:

(1) The first evolutionary stage corresponded to an ancestral land animal that changes its mode of life to an amphibious-like form and presented an ancestral 'tubular' stage in the transversal section of several bones of the skeleton (Figure 5, orange zone) like long bones, ribs, phalanx or metacarpals/tarsals. This ancestral skeletal stage did not present any ballast function in aquatic environments.

(2) A derived primitive-aquatic form inhabited estuarine or coastal environments. Pachyostosis (*sensu lato*) appeared in two forms, pachyostosis (*sensu stricto*) in the cortex region or osteosclerosis in the medullar region (Figure 5, light blue zone). In this stage the skeleton had a ballasting function participating in the passive control of buoyancy of the aquatic animal (Houssaye 2009).

(2.1) An extreme-derived stage can be reached (Figure 5, green zone) when pachyostosis and osteosclerosis combined resulting in pachyosteosclerosis with an even more heavy skeleton and high bone ballast. These two "pachyostotic" forms are correlated with animals that have a slow-swimming mode of life.

(3) A more derived structural transformation in the skeleton is reached when osteoporotic-like tissue emerged, lightening the skeleton in mass and density (Figure 5, dark blue zone). This structural transformation is associated with an active swimmer mode of life.

In our microanatomical/histological analyses we can suggest that the skeleton of SGO.PV.6666 have the condition (2) showed in the Figure 5. Also in our histological descriptions we did not find any sign of zones of growth nor annuli or LAG´s (lines of arrested growth). All the bone tissue we encountered was primary, which suggests an early ontogenetic stage of the specimen. We did not find secondary osteons or secondary lamellar endosteal tissue in the medullar region. The predominant tissue in our sample corresponds with calcified cartilage but with signs of chondroclast activity. In one side we had signs of a high deposition rate of primary bone tissue and in the other we had an accumulation of calcified cartilage, suggesting a heterochronical process (Alberch *et al*. 1979; Alberch 1980), resulting in delayed synthesis of bone matrix and an arrested resorption process. The chondroclast activity that we find could be reflection of a basal metabolic process (de Ricqlès 1989; Francillon-Vieillot *et al.* 1990).
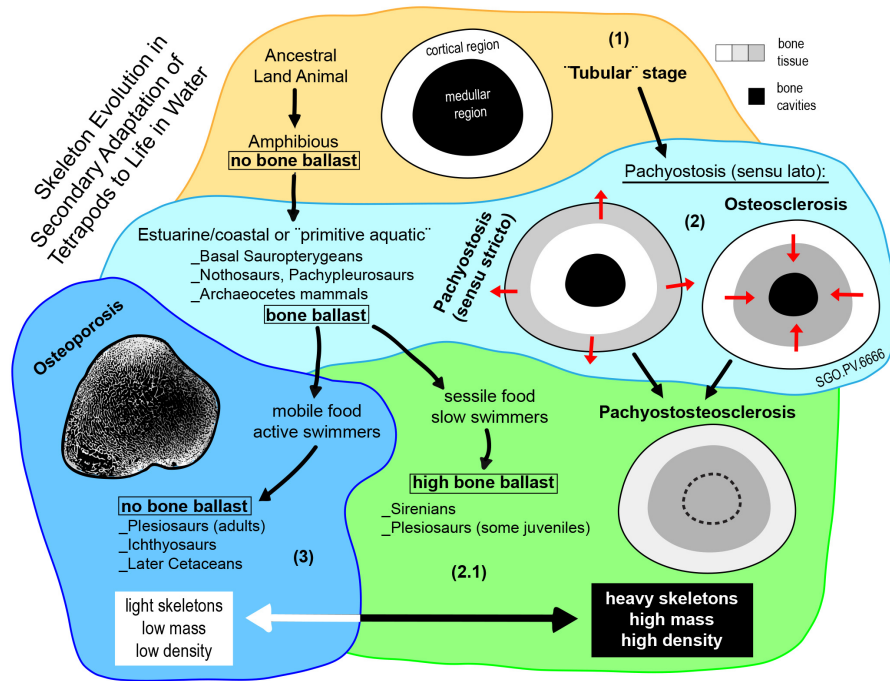
FIGURE 5. General summary of the evolution of the skeleton of tetrapods secondary adapted to life in water. Obtained and modified from Taylor (2000), Talevi (2012), Ricqlès et al. (2001), and Houssaye (2009). See details in the figure and discussion

Mode-of-life and paleo-environmental reconstructions

With the limited data we have, so far we can correlate environmental reconstructions with fossil vertebrates and then compare it with living representatives. Good comparative examples to do that are the modern sirenians and cetaceans. These two groups of aquatic mammals are the best physiological/structural analogies to infer what could occur in the skeleton of an elasmosaur regarding it ontogenetic development when was alive millions of years ago.

Sirenians like the manatee (*Trichechus*) have a dense skeleton with osteosclerosis and paquiosteosclerosis taking place in several bones of their skeleton (Houssaye 2009). Modern cetaceans like dolphins or whales in contrast have light bones with low mass and density, presenting a characteristic osteoporotic skeleton (de Buffrénil and Schoevaert 1988; Taylor 2000). Sirenians live in shallow waters, are slow swimmers and prefer sessile food to eat. Instead, modern cetaceans live in open-sea waters, they are active swimmers and they chase very mobile food (fishes, other aquatic mammals, etcetera) and also someones dive to high deep waters.

Physiologically speaking both modes of life are correlated with "*ad hoc*" skeletons in these groups of mammals: sirenians swim passively with slow metabolisms and few physical forces interacting with their muscular-skeletal system (Taylor 2000; de Ricqlès 1989; Houssaye 2009; Domming and de Buffrénil 1991), therefore the synthesis/resorption of bone matrix balance are endocrinological modified (de Ricqlès 1989; de Buffrénil *et al.* 1990; Dommnig and de Buffrénil 1991) with the resorption activity being decreased resulting in the accumulation of bone matrix and creating a natural ballasting skeleton (de Ricqlès 1989). On the other hand, modern cetaceans as active swimmers and have comparatively high metabolism with high amount of physical forces interacting with their muscular-skeletal system (de Buffrénil and Schoevaert 1988; de Ricqlès 1989; Webb and de Buffrénil 1990). Their balance between synthesis/resorption of bone matrix is very dynamic, presenting constantly high synthesis and high resorption values. Also in deep-

diving representatives, the increase of osteoporotic-like skeleton enhance it physiology to confront high pressures and avoid massive skeletal fractures(Taylor 2000; Moore and Early 2004).

As Wiffen *et al*. (1995) did, we can correlate these modern examples with the two ontogenetic stages described in elasmosaurs. First, we could have had slow swimmers juveniles with dense skeletons swimming in shallow waters or shoreface zones. Then when adulthood came across a structural/ physiological/ behavioral change occurs transforming the animal in an active-swimmer-predator that lives in open-sea waters with the lightening of the skeleton as a result.

Our data provide more evidence about the appearance of juvenile and adult specimens at high latitudes near Patagonia and Antarctica. Recent paleobiological studies reports the presence of sub-adults and adults assigned to the family Elasmosauridae from the Upper Cretaceous of Rio Negro in Argentinian Patagonia (Salgado *et al*. 2007), Elasmosauridae and the subfamily Aristonectinae from the Upper Cretaceous of Magallanes Basin in Chilean Patagonia (Otero *et al*. 2014) and a perinatal (early juvenile) Aristonectinae specimen from the Upper Cretaceous of Marambio(=Seymour) Island (O´Gorman *et al*. 2017).

Salgado *et al*. (2007) conducted microanatomical and histological analyses in bones from sub-adult and adult elasmosaurid specimens. They informed the same microanatomical pattern that Wiffen *et al*. (1995) suggested in New Zealand elasmosaurs. Finding in the sub-adult osteosclerosis, and in the adult a more osteoporotic skeleton.

O´Gorman *et al.* (2017) analyzed the histology of a right humerus from a "very early juvenile" finding a similar histological pattern of what we found in the unidentified propodial of SGO.PV.6666 (Figure 3). With high bone compactness and no open medullary cavity filled with calcified cartilage. In addition it is described the presence of the Kastschenko's line (Francillon-Vieillot *et al*. 1990) that separated the medullar and cortical tissues. The Kastschenko's line is a thin coat of embryonic cartilage matrix that persists around the diaphysis of a growing long bone not being destroyed by the condroclasts during the production of the marrow cavity (Francillon-Vieillot *et al*. 1990). The presence of this line indicated that the ontogenetic age of the studied specimen was perinatal. In SGO.PV.6666 we did not find a Kastschenko's line. Therefore, comparatively we can suggest that SGO.PV.6666 represented a slightly older ontogenetic stage than MLP 14-I-20-8 being an early juvenile with a dense/ballasting skeleton.

Finally if we included the geological information obtained by previous studies at the López de Bertodano Formation (Macellari 1988; Otero *et al.* 2014), we can suggest that SGO.PV.6666 inhabited a shallow marine water environment and was part like other elasmosaurs and aristonectines juveniles of a "breeding area" for marine reptiles (hypothesis suggested by Martin *et al*. 2007) at the end of the Cretaceous period in the Antartic Peninsula (Weddellian Province).

ACKNOWLEDGEMENTS

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