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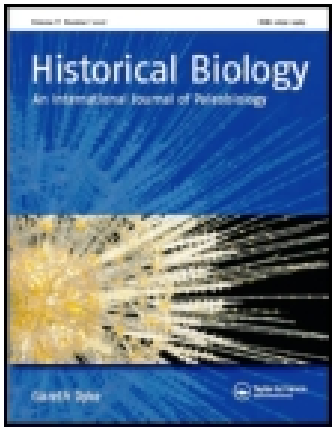

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Unexpected microanatomical variation among Eocene Antarctic stem penguins (Aves: Sphenisciformes)

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The microanatomical and histological structure of Eocene Antarctic stem penguin tarsometatarsi is examined in order to characterise the bone microstructure. Eight adult tarsometatarsi belonging to eight fossil species (*Palaeeudyptes gunnari*, *Palaeeudyptes klekowskii*, *Anthropornis grandis*, *Anthropornis nordenskjoeldi*, *Archaeospheniscus wimani*, *Marambiornis exilis*, *Delphinornis arctowskii* and *Delphinornis larseni*) collected from the Antarctic *A. nordenskjoeldi* Biozone (La Meseta Formation, ~34.2 Ma) were examined. The thin sections revealed a distinctive microanatomical variation among taxa. Whereas *Anthropornis* spp., *A. wimani* and *P. gunnari* possess massive, clearly osteosclerotic bones (medullary cavities absent or strongly reduced), the bones of *Delphinornis* spp., *P. klekowski* and *M. exilis* exhibit well-developed medullary cavities. The cortical bone in all the specimens consists of primary, well-vascularised fibro-lamellar bone and variable amounts of secondary bone. Medullary cavities are coated by a thick layer of lamellar bone tissue and coarse compacted cancellous bone. Although several causes can explain the striking microanatomical variation (e.g. ontogeny), we interpret that such variation is related to differential adaptations to the aquatic life, for which taxa with more massive bones were possibly adapted to deeper and more prolonged diving excursions.

Keywords: Aves; Sphenisciformes; microanatomy; histology; skeletal adaptations

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1. Introduction

The skeletons of tetrapods secondarily adapted to aquatic life, as compared with those of terrestrial forms, exhibit numerous modifications, often leading to a complete reshaping of the body (Houssaye 2013). These changes are more evident in the limbs and bear on both the external morphology of the bones (transformation of weight-bearing limbs into hydrofoil-like fins or flippers) and their internal organisation. Among these modifications, secondary adaptation to an aquatic life always induced modifications of the inner architecture (microanatomy) and histological characteristics of bones (Houssaye 2009; Canoville and Laurin 2010). Depending on the general adaptations of the animals, extant and fossil vertebrates secondarily adapted to aquatic life show four major specialised, non-pathological, bone typologies: osteoporotic-like, pachyostotic, osteosclerotic and pachy-osteosclerotic (de Ricqlès and de Buffrénil 2001; Houssaye 2009). Osteosporotic-like type consists of a lightening of the bones by decreasing the thickness of the compact cortical bone and increasing the osseous porosity. Conversely, pachyostosis (*sensu stricto*) corresponds to a hyperplasy (increase of deposit) of periosteal cortices that leads to an alteration of the bone morphology by increasing its volume (de Ricqlès and de Buffrénil 2001; Houssaye 2009). Osteosclerosis is an increase of bone inner compactness either as a result of incomplete endochondral ossification, inhibition of secondary remodelling and/or the filling of inner cavities, with no effect on the external dimensions of the bone. Finally, pachy-osteosclerosis corresponds to the combination of the pachyostotic and osteosclerotic states in the same bone (de Ricqlès and de Buffrénil 2001; Houssaye 2009). The good preservation of the histological details of calcified tissues during fossilisation makes it possible to study the progressive changes of bone structural specialisations in aquatic tetrapods during geological time.

The Sphenisciformes (penguins) are one of the most highly derived clades of extant birds and they are among the best examples of the morphological modifications induced by a secondary adaptation to marine life. Extensive morphological, physiological and behavioural modifications from the typical avian condition have allowed penguins to achieve a largely aquatic lifestyle and invade some of the most inhospitable environments in the world. Penguins are wing-propelled diving birds that seem poorly fitted to locomotion. They are morphologically characterised by a complete transformation of the forelimbs into flippers, an increased volume of the rib cage and a very posterior relocation of the short legs that oblige the birds, when standing on land or ice, to adopt an upright position

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(Hémery 2001). The anatomical characters of their apneumatic skeleton, as well as the biomechanical aspect of swimming in these animals, have been extensively described in numerous publications and theses (Pycraft 1899; Shufeldt 1901; Simpson 1946; Acosta Hospitaleche 2004; Jadwiszczak 2006, 2012; Ksepka 2007; Triche 2007). However, the peculiarities of the inner structure of bone in penguins, which are another significant aspect of their adaptation to the aquatic life, remain poorly documented.

The first study in this field was provided by Meister (1962), who analysed long bones (femora, humeri and tibiotarsi) of extant penguin taxa (*Aptenodytes patagonica*, *Aptenodytes forsteri* and *Pygoscelis adeliae*). In the work of Meister, penguin long bones were characterised as 'typically pachyostotic', massive elements, dominated by a thick cortex of compact bone. Such microanatomical pattern was directly attributed to their diving lifestyles. Based on the same data from Meister, de Ricqlès and de Buffrénil (2001) considered that penguin long bones fitted in the osteosclerotic category. Sclafani et al. 2013 studying humeri and femora of divers applied the same category for penguins. In a recent review of the pachyostosis *sensu lato* state (which includes pachyostosis, osteosclerosis and pachy-osteosclerosis), Houssaye (2009) (using the original data of Meister 1962) defined the bone of the penguins as pachy-osteosclerotic.

This study documents the bone microanatomy and histology of a group of fossil penguin taxa from La Meseta Formation (Eocene) of Antarctica. The outcrops of La Meseta Formation contain the major taxonomic and body-size diversity of stem Sphenisciformes, including 10–14 species (Myrcha et al. 2002; Jadwiszczak 2006; Tambussi et al. 2006; Jadwiszczak and Mörs 2011; Reguero et al. 2013). This diversity remains impressive even if it is partly based on sexual size dimorphism, supposedly well marked in the huge-bodied penguins (Jadwiszczak and Mörs 2011, but see Jadwiszczak and Acosta Hospitaleche 2013). The high penguin diversity of the La Meseta Formation (regarding number of species and variation in body size) and the abundance of materials provide an excellent opportunity to study the bone histology of these aquatic birds.

The aim of the present contribution is to characterise the bone microstructure of fossil penguins from La Meseta Formation and to determine what this can tell us about the process of secondary adaptation to their aquatic life. Given that we performed a comparative study between different taxa, we also established inter-specific histological variation among the studied taxa. Likewise, we discussed and compared our results with published data on penguin bone histology (Meister 1962; Chinsamy et al. 1998; de Margerie et al. 2004).

2. Materials and methods

Histological analysis was used to examine the bone tissue structure of eight species of stem Sphenisciformes from the Late Eocene of Antarctica (Table 1 and Figure 1). All skeletal elements examined were excavated from the *Anthropornis nordenskjoeldi* Biozone (Tambussi et al. 2006), Submeseta Allomember, Facies Association III, La Meseta Formation, Priabonian, ~34.2 Ma based on $^{87/86}$Sr dates (Marenssi et al. 1998). Eight tarsometatarsi, positively identified at species level, were selected for histological analysis (Table 1). We chose tarsometatarsi on the basis of their value for taxonomical identification (Myrcha et al. 2002).

Specimens were prepared for thin sectioning using the methods outlined in Chinsamy and Raath (1992). The preparation of the histological sections was carried out in Departamento de Geología de la Universidad Nacional de San Luis (Argentina). Because this is a destructive method, all specimens were photographed and standard measurements were taken before sectioning.

We calculate and compare the proportion of osseous tissue in each bone. For this, we obtained the area occupied by osseous tissue in each transversal section, and then we multiplied this value by 100 and divided by the total sectional area (de Buffrénil and Francillon-Vieillot 2001; Talevi et al. 2011). This measurement was calculated by means of the software ImageJ (Abramoff et al. 2004). Nomenclature and definitions of structures used in this study are derived from Francillont-Vieillot et al. (1990) and Chinsamy Turan (2005). Anatomical nomenclature follows Baumel et al. (1993).

Table 1. Estimated body mass, height and proportion of osseous tissue of the stem penguins sample.

Taxon	Collection number	Body mass[a]	Height[a]	Proportion of osseous tissue
A. grandis (Wiman, 1905)	MLP 93-X-1-149	44.2	137	97.7
A. nordenskjoeldi Wiman, 1905	MLP 84-II-1-19	81.7	165.6	94.7
P. gunnari (Wiman, 1905)	MLP 82-IV-23-6	41.1	133	96.9
P. klekowskii Myrcha et al., 1990	MLP 83-V-30-17	56	147	73.6
M. exilis (Myrcha et al., 2002)	MLP 93-X-1-111	6.1	74.7	66.9
D. arctowskii Myrcha et al., 2002	MLP 93-X-1-92	11.5	90.7	69.7
D. larseni Wiman, 1905	MLP 83-V-20-5	14.1	95.8	76.2
A. wimani (Marples, 1953)	MLP 91-II-4-173	13	94.1	94.4

Note: Body mass is expressed in kg, height in cm and proportion of osseous tissue in %.
[a] Taken from Jadwiszczak (2001) and Jadwiszczak and Mörs (2011).

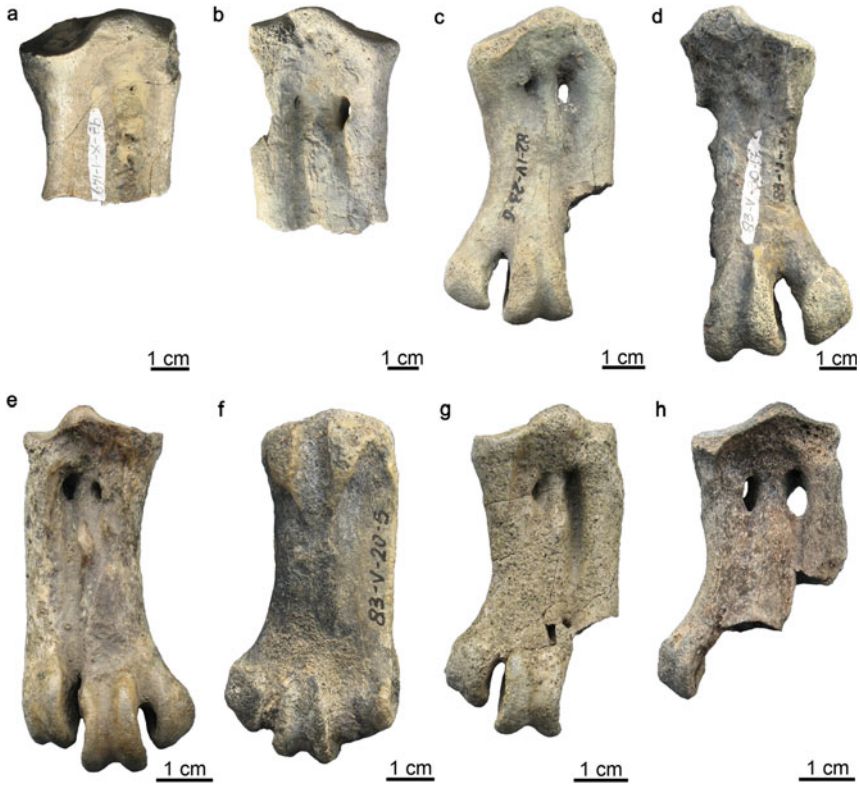

Figure 1. (Colour online) Tarsometatarsi of stem Sphenisciformes from La Meseta Formation sampled in this study. (a) *A. grandis*, (b) *A. nordenskjoeldi*, (c) *P. gunnari*, (d) *P. klekowskii*, (e) *M. exilis*, (f) *D. arctowskii*, (g) *D. larseni* and (h) *A. wimani*.

3. Results

3.1 General histological features

The transverse sections of all the elements revealed an important degree of variation with regard to the bone microanatomy of the specimens (Figure 2). Whereas some taxa (e.g. *Anthropornis* spp.) are composed almost entirely of compact bone (the medullary cavities occupy less than 6% of the area in section), others possess well-developed medullary cavities (*Delphinornis* spp.), which occupy more than 20% of the total area. The primary bone consists of well-vascularised fibro-lamellar bone. The channels in the bone (in which vascular canals and other soft tissue are located, Starck and Chinsamy 2002) are organised as primary osteons, which are mostly longitudinally and radially oriented. Secondary bone reconstruction is usually high and several specimens exhibit dense Haversian bone

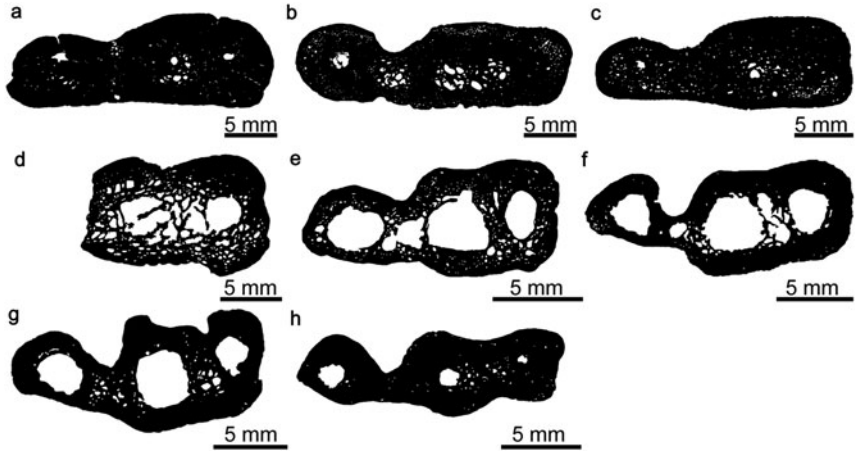

Figure 2. General view of the tarsometatarsi microanatomy. (a) *A. grandis*, (b) *A. nordenskjoeldi*, (c) *P. gunnari*, (d) *P. klekowskii*, (e) *M. exilis*, (f) *D. arctowskii*, (g) *D. larseni* and (h) *A. wimani*. In all the images the lateral side is located towards the left of the figure.

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tissue in their cortices (mostly at the mid and perimedullary regions of the compacta). The medullar cavities are entirely or partially coated by a thick layer of endosteally deposited lamellar bone tissue (inner circumferential layer (ICL)).

Since some taxa share some microanatomical and/or histological traits (e.g. *Anthropornis* spp., *P. gunnari*), we first describe their general features and then mention individual differences if applicable.

3.2 *Anthropornis grandis, A. nordenskjoeldi and Palaeeudyptes gunnari*

These species possess massive bones for which the compact tissue occupies between 94.7% and 97.7% of the total area in transverse section. Large medullary cavities or trabecular bone is not well developed. Some large cavities are scattered at the inner regions that correspond to the os metatarsale III and IV. In *A. nordenskjoeldi*, partially filled resorption cavities are also located at the inner region below the *sulcus longitudinalis dorsalis lateralis* (SLDL). In this specimen, the larger vascular spaces are slightly more abundant at the inner region.

The cortical tissue in the specimens consists of both primary and secondary bone. The primary bone is best preserved in *P. gunnari* and *A. grandis* materials. This bone is essentially composed of fibro-lamellar bone tissue (primary osteons embedded in a woven-fibred matrix). The woven-fibred matrix exhibits a high density of rounded osteocyte lacunae. Vascularisation is variable in terms of spatial arrangement, even in a single specimen. For example, while vascular spaces in *P. gunnari* show a rather plexiform arrangement towards the medial cortex, they are mostly radially oriented in the lateral cortex. In *A. grandis*, although the vascular pattern is variable, radially oriented primary osteons predominate (Figure 3(a),(b)). Vascular canals in the fibro-lamellar bone of the specimen of *A. nordenskjoeldi* are mostly longitudinally oriented. Sharpey's fibres are imbedded in the primary bone tissue in different regions of the compacta (plantar region of the os metatarsale III in *A. grandis* and lateral region in *P. gunnari*) (Figure 4(c)). There is no evidence for the development of an outer circumferential layer (OCL) in the outer cortex of the specimens.

Secondary bone is evident in the compacta of the three specimens, but the degree of secondary remodelling is variable. Whereas secondary osteons are mainly developed at the inner and mid cortex in *P. gunnari* and *A. grandis*, they occupy almost the whole compacta in *A. nordenskjoeldi* (Figure 3(d)). The degree of secondary remodelling is also variable in single element. For example, in *P. gunnari*, secondary osteons are more densely distributed at the plantar region.

At the medullary region, the largest internal cavities are entirely or partially surrounded by a thick layer of lamellar bone. Coarse compacted cancellous bone, composed of thick and convoluted lamellae of secondary bone, is well developed in some areas of inner compacta (Figure 3(e)–(g)). This process of secondary compactation is clearly observed in the specimen *P. gunnari*, in which abundant layers of lamellar bone tissue are deposited around thin bony trabeculae at the inner region of the os metatarsale IV and II (Figure 3(h)).

3.3 *Palaeeudyptes klekowskii*

Only the portions that correspond to the os metatarsale II and III have been preserved. The element is composed of a thick cortex that surrounds two medullary cavities. The boundaries between the medullary cavities are not well marked and are partially divided by thin bony trabeculae composed of lamellar bone deposited during different generations of secondary reconstruction (Figure 4(a)). The margins of the medullary cavities are distinctly resorptive in nature. The cortex is almost entirely composed of dense secondary bone tissue. Partially filled secondary osteons and resorption cavities are observed towards the perimedullary region of the compacta (Figure 4(b)). The resorption cavities are particularly important at the plantar region, where they reach the outer cortex. Remains of primary bone tissue are preserved at the sub-periosteal cortex. This primary bone consists of fibro-lamellar tissue with longitudinally oriented primary osteons. Sharpey's fibres are observed at the medial portion of the cortex in the plantar region. There is no evidence for periosteal deposition of OCL.

3.4 *Marambiornis exilis, Delphinornis larseni and Delphinornis arctowskii*

Cross section reveals that these elements are composed of a compact cortex that surrounds three distinct medullary cavities. These cavities are developed in the internal regions that correspond to the os metatarsale II, III and IV. In *M. exilis* and *D. arctowskii*, a fourth cavity is observed in the medullary region below the SLDL. Although the diameter of the internal cavities is variable, the one that belongs to the os metatarsale III is always proportionally larger than the others. The largest medullary cavity in *D. arctowskii* is partially divided by bony trabeculae of irregular shape.

The thickness of the compact bone is rather uniform in the whole cortex of each specimen, slightly decreasing at the plantar region of the os metatarsale IV. The cortical bone of specimens of *M. exilis* and *D. larseni* is composed almost entirely of dense Haversian bone tissue with several superposed generations of secondary osteons (Figure 4(c), (d)). Secondary osteons are also well developed in the cortex of *D. arctowskii*, but they are less abundant than in the other two specimens. Secondary osteons contain a large number of centripetally deposited layers of new

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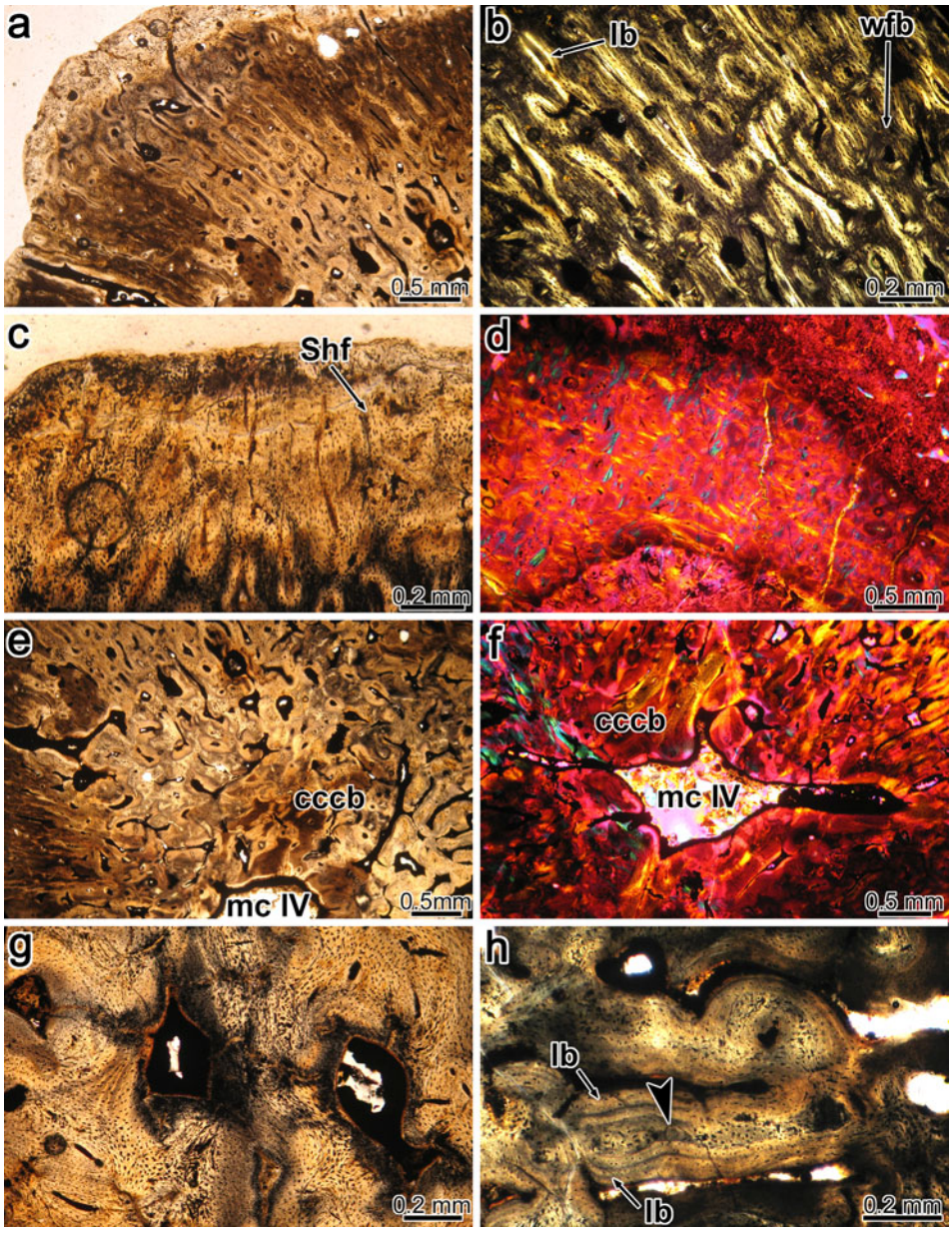

Figure 3. Bone histology of *A. grandis* (a, b, e and f), *P. gunnari* (c and h) and *A. nordenskjoeldi* (d and g). (a) Fibro-lamellar bone tissue with predominance of radial vascular spaces (normal light). (b) Detail of the same tissue under polarised light. Note the general isotropy of the woven-fibred bone (wfb) matrix and the birefringence of the lamellar bone (lb) surrounding the vascular spaces. (c) Enlarged view of the fibro-lamellar bone tissue at the external cortex. Sharpey's fibres (Shf) are oriented towards the external surface of the bone (normal light). (d) Detail of compact bone composed almost entirely of dense Haversian bone tissue (polarised light with lambda compensator). (e) Inner cortex around the reduced medullary cavity (mc) of the os tarsometatarsale IV (normal light). The compact bone is composed of remains of primary bone, secondary osteons and coarse cancellous compacted bone (cccb). (f) Coarse compacted cancellous bones surrounding the medullary cavity of os tarsometatarsale IV (polarised light with lambda compensator). (g) Detailed view of the coarse compacted cancellous bone at the inner core of the element (normal light). (h) Detail of the compacted cancellous bone at the inner core of the element (normal light). Note the thick layer of lamellar bone tissue surrounding the previously formed bony trabeculae (arrowheads).

lamellar bone and they are delimited by conspicuous cementing lines. Haversian canals are mostly longitudinally oriented. Secondary osteons located in the inner cortex are larger than those observed in the mid and outer cortex and they also possess a more irregular shape.

In *D. larseni*, Haversian systems are interconnected by abundant Volkman's canals. In this specimen, several secondary vascular spaces are also opened to the medullary cavity. Coarse compacted cancellous bone is developed in some areas of perimedullary cortex. The

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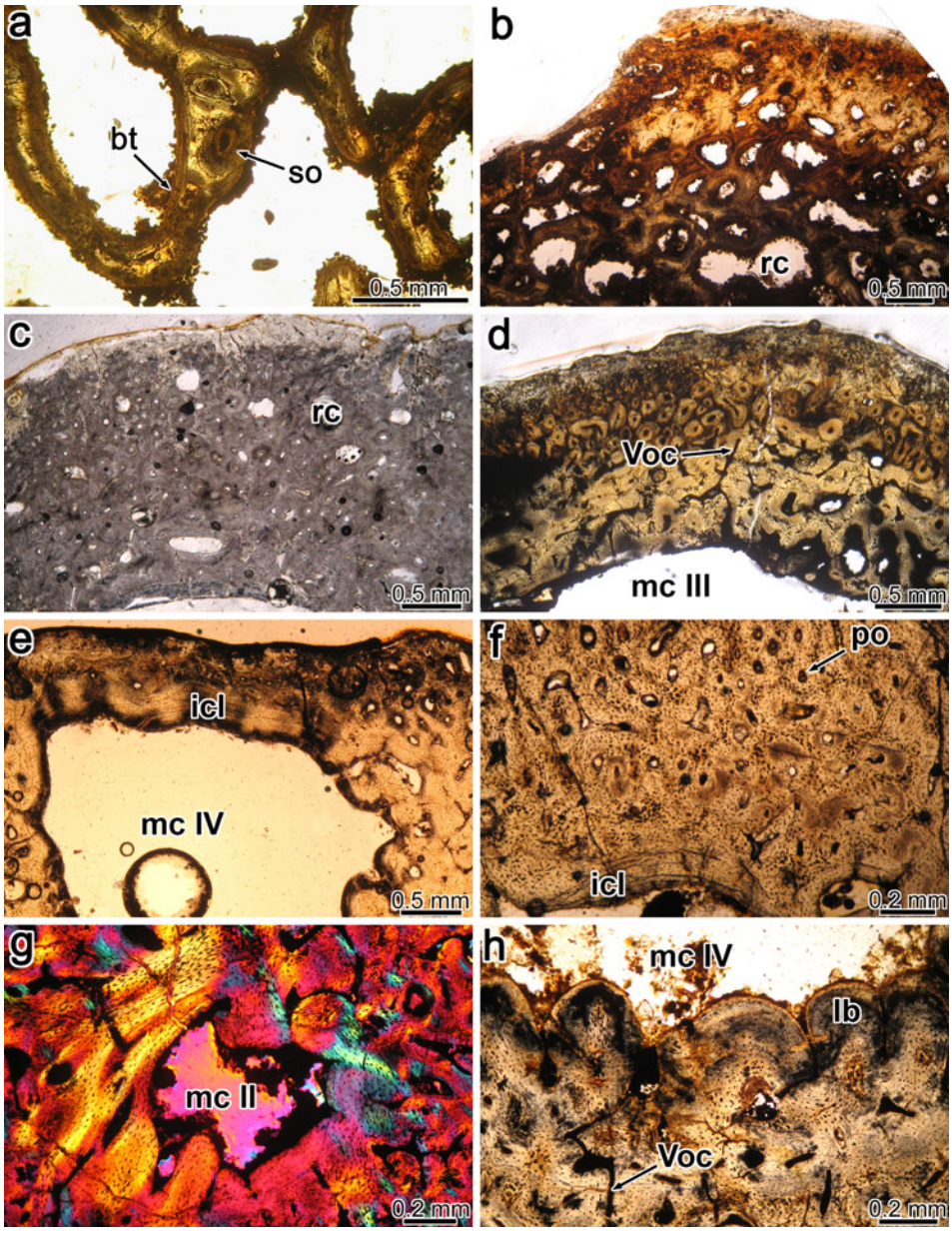

Figure 4. Bone histology of *P. klekowskii* (a and b), *M. exilis* (c), *D. larseni* (d), *D. arctowskii* (e and f) and *A. wimani* (g and h). (a) Detailed view of the cancellous bone of the internal core (normal light). The bony trabeculae (bt) are composed of secondary lamellar bone tissue. Remains of secondary osteons (so) of previous generations of remodelling are observable. (b) General view of the compact bone showing the abundance of resorption cavities (rc) in the whole cortex (normal light). (c) Compact bone composed almost entirely of dense Haversian tissue (normal light). (d) Cortical bone around the reduced medullary cavity (mc) of the os tarsometatarsale III (normal light). Volkman's canal (Voc) connects the Haversian systems at the perimedullary cortex. (e) Cortical bone around the reduced medullary cavity of the os tarsometatarsale IV (normal light). A think ICL of lamellar bone surrounds the medullary cavity. (f) Primary bone composed of fibro-lamellar tissue (normal light). Vascularisation consists of abundant longitudinally oriented primary osteons (po). (g) Coarse compacted cancellous bones surrounding the medullary cavity of os tarsometatarsale IV (polarised light with lambda compensator). (h) Detailed view of the perimedullary cortex around the medullary cavity of os tarsometatarsale IV (normal light). Note the undulated pattern of the thick layer of perimedullar lamellar bone.

internal cavities in all the specimens are partially filled with endosteally deposited lamellar bone tissue (ICL) (Figure 4(e)–(h)). This layer shows a rather undulated pattern in *D. arctowskii*, which is particularly thick at the inner region of the os metatarsale IV (Figure 4(h)).

Non-reconstructed (primary) bone tissue is restricted to the outer cortex. In *D. arctowskii*, it is also observed at the mid cortex in the plantar region. The primary bone tissue in the three specimens mostly consists of well-vascularised fibro-lamellar bone. Primary osteons are

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mainly longitudinally arranged (Figure 4(f)). In *D. larseni*, the outermost cortical bone is formed by a thin layer of parallel fibred bone tissue.

3.5 *Archaeospheniscus wimani*

The section of the bone displays a thick, dense compact bone wall surrounding three small but distinct medullary cavities. The larger medullary space corresponds to the os metatarsale IV and the smaller one to the os metatarsale II. The thickness of the cortical bone is rather homogeneous in the whole element. The cortex contains abundant secondary osteons, which are more densely developed in the perimedullary regions. The density of secondary osteons decreases at the mid region of the dorsal cortex. A large secondary vascular space is located at the plantar cortex, between the os metatarsale II and III. The perimedullary cortex contains secondary osteons and remains of coarse compacted cancellous bone (Figure 4(g)). Also, the medullary cavities are surrounded by a distinctive, thick layer of lamellar bone tissue (ICL). The margin of the ICL possesses an undulated shape, with vascular spaces (Volkman's canals) that extend radially from the medullary cavity into the compacta (Figure 4 (h)). The primary bone is mostly preserved at the outer cortex and consists of fibro-lamellar tissue, which contains mostly longitudinally oriented primary osteons. Abundant Sharpey's fibres are observed in the fibro-lamellar bone. There is no evidence of an OCL.

4. Discussion

The bone microstructure in Eocene Antarctic penguins is, in general terms, consistent with previously published histological data of extant Sphenisciformes (Meister 1962; Chinsamy et al. 1998; de Margerie et al. 2004). The primary bone tissue is composed of well-vascularised, azonal fibro-lamellar bone tissue, which indicates high and sustained growth rates (de Margerie et al. 2004). Secondary remodelation around the medullary cavity and the development of an ICL in this region were also described in extant penguin long bones (Meister 1962; Chinsamy et al. 1998). Regarding the bone microanatomy of the sampled bones, our data partly coincide with the pachyostotic (*sensu lato*) state found by Meister (1962) in the long bones of *Aptenodytes* spp. and *P. adeliae*. However, some differences were found on this regard in our sample, which shows a clear inter-specific micro-anatomical variation. Our data indicate that the bone microstructure of *P. gunnari*, *A. grandis*, *A. nordenskjoeldi* and *A. wimani* tarsometatarsi corresponds to an osteo-sclerotic condition, given that the medullary cavity is strongly reduced (absent in some specimens) and no distinct hyperplasia can be observed in the cortical bone. These taxa show the most compact internal architecture, in which the osseous tissue occupies the most of the total volume of the bone. Conversely, *M. exilis* and the two species of *Delphinornis* possess well-developed medullary cavities and their bone microanatomy does not appear to correspond to an osteosclerotic state or any pachyostotic (*sensu lato*) type. In the case of *P. klekowskii*, despite its cortical bone exhibiting a more porous aspect, this is clearly non-osteoporotic-type. Given that Sphenisciformes bones have been always considered as pachyostotic (*sensu lato*) (Meister 1962; de Ricqlès and de Buffrénil 2001; Houssaye 2009), the particular variation reported here is rather unexpected. This variation is actually the most noticeable result from our data and could be related to different causes, several of them not mutually exclusive.

A possible source of variation could be related to the ontogenetic stages of the sampled specimens. As observed in the humeral and femoral mid-shaft of *A. patagonicus* (Meister 1962; de Margerie et al. 2004), the medullary cavity in chick is actually more developed than in the adult specimen. However, this explanation appears to be improbable, since all the studied specimens correspond to adult individuals. Moreover, the cortical bone of *M. exilis* and *D. larseni* exhibits a high degree of secondary reconstruction, which is generally correlated with age. Also, an OCL is formed in the sub-periosteal margin of *D. larseni*, indicating that somatic growth reaches a plateau in this individual.

Mechanical factors related with the body size may be other possible causes of microanatomical variation. This hypothesis is supported by the fact that the most massive bones were found in three of the four largest taxa (body mass and body height larger than 40 kg and 130 cm, respectively) and, conversely, the bones with large and well-developed medullary cavities correspond to three of the four smallest taxa (body mass and body height lesser than 15 kg and 100 cm, respectively) (Jadwiszczak 2001; Jadwiszczak and Mörs 2011; Table 1).

Gender-specific variation originated during egg-shell formation could be another explanation for the micro-anatomical variation reported here. As noted by Wilson and Thorp (1998), egg-producing female fowls lose cancellous bone in the metaphyses of long bones. In the females of the semi-aquatic Nile monitors *Varanus niloticus*, the medullary cavity of the femora becomes relatively larger as they undergo more egg-laying cycles (de Buffrénil and Francillon-Vieillot 2001). Also, in some extant crocodylians and turtles, osseous porosity is more developed in ovulating females (Schweitzer et al. 2007 and references therein). If the same pattern occurs in penguins, the sample specimens of *M. exilis*, *D. arctowskii* and *D. larseni* were actually females which have gone through several egg-laying cycles. Nevertheless, a study of the extant penguin *Spheniscus magellanicus* has demonstrated that the calcium for the egg formation in this species is not obtained from the bones, but from selective ingestion of

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mollusc shells (Boersma et al. 2004). We considered this explanation rather improbable, only plausible for the specimen of *P. klekowskii*, which shows a relatively higher degree of porosity than the other specimens. Interestingly, Jadwiszczak and Mörs (2011) suggested that *Palaeeudyptes klekowskii* and *Palaeeudyptes gunnari* were synonyms, in which the morphological differences between both taxa are product of sexual dimorphism (see also Jadwiszczak 2013; Jadwiszczak and Acosta Hospitaleche 2013). This hypothesis could be tested by increasing the number of samples for each species.

Finally, differential adaptations to the aquatic life in the different species can be reflected by their bone microanatomy. Increase in the bone mass (osteosclerosis) has been considered to represent an adaptation for hydrostatic regulation of body trim (Taylor 2000; de Ricqlès and de Buffrénil 2001; Houssaye 2009; Hayashi et al. 2013). The thick-walled appendicular bones of the penguins act as ballast to weight the skeleton and counteract the animal's buoyancy when in water (Chinsamy et al. 1998; Habib and Ruff 2008; Houssaye 2009). Consequently, the differences in the cortical bone thickness observed in our sample could be related to variations in the diving behaviour in the different taxa. Therefore, taxa with more massive bones were possibly adapted to deeper and more prolonged diving. Studies on extant penguin taxa have demonstrated that different taxa exhibit different diving behaviours and such variation is correlated with the body size of the species (larger species spend a greater proportion of time in deeper water than the smaller species) (Prince and Harris 1988; Wilson et al. 1991; Kokubun et al. 2010). Interestingly, three of the four largest species included in our sample (*P. gunnari*, *A. nordenskjoeldi* and *A. grandis*) whose estimated body mass varies between 41 and 82 kg (Jadwiszczak 2001) are those with more cortical thickness.

The microanatomy observed in *P. klekowskii* deserves further discussion. Contrary to the other samples, the cortical bone of *P. klekowskii* reveals a high degree of porosity. As we previously mentioned, this pattern could be related to an enhanced cortical resorption in ovulating females. Another possible source for the high porosity of the cortex may be related to the moulting cycles. The compact bone tissue of the long bones of birds undergoes a series of transformations during the moult cycle, apparently correlated to the increased need for minerals (chiefly calcium) during this period (Meister 1951). These transformations include an increasing of the whole cortical porosity, which becomes again compact by the infill of the resorption cavities after the moulting season (Meister 1951). In most species of extant penguins, moulting occurs once a year, usually after breeding and, contrary to other birds, the moulting period is fast (from 13 to 34 days depending on the species) (Adams and Brown 1990). We considered that the enhanced porosity observed in the sample of *P. klekowskii* is possibly related to the high degree of compact bone resorption during the moulting season (an enhanced rate of bone resorption increases the whole cortical porosity).

5. Conclusions and perspectives

Histological data concerning penguins have been so far too scarce to perform precise homologous comparisons at a large scale. Despite these restrictions, the histological data described above enable some trends to be observed.

Not all morphological modifications at the skeleton level are obviously related to the aquatic condition of the animal, and bone microanatomy may provide additional ecological information to morphology (Kriloff et al. 2008; Canoville and Laurin 2010). In this regard, our work reveals an unexpected microanatomical variation which seems to be linked to diving habits of each taxon. Our prediction is that species with more massive bones would dive deeper and longer whereas penguins with less massive bones would have shorter and reduced vertical displacement. This and other possible causes (e.g. biomechanical ones) for the microanatomical variation observed in fossil taxa can be actually tested in extant forms. The high diversity in sizes and diving behaviour in living penguin taxa provides a valuable source of information to test our hypothesis, and we encourage future studies on this regard, which are clearly necessaries to understand the pattern observed in extinct forms.

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