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RESEARCH

Penguin osteohistology: the key role of the inner circumferential layer in sex determination

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Abstract

In this study, we investigate the osteohistological variation in Sphenisciformes, focussing on the microstructural patterns associated with medullary bone (MB) and the inner circumferential layer (ICL) across several species, including *Aptenodytes forsteri*, *Pygoscelis antarctica*, *P. papua* and *Spheniscus magellanicus*. Histological sections of both appendicular and axial skeletons were analysed, revealing significant variations in the presence of MB and the ICL across taxa. We aimed to expand the understanding of microanatomy and histology in Sphenisciformes by describing these variations and testing a hypothesis regarding sexual dimorphism based on ICL presence. Key findings include the identification of MB in all specimens, while *S. magellanicus* exhibited a more limited distribution of MB. Additionally, females of *P. antarctica* and *A. forsteri* had an ICL, although this layer was partially remodelled due to osteoclastic activity linked to egg laying. These findings contribute to the ongoing discussion of sex discrimination in extant and extinct penguin taxa and highlight the importance of MB and the ICL in understanding reproductive and developmental histories. Our results suggest that, although the ICL may not be an absolute indicator of sex, its absence or remodelling in adult individuals may serve as a useful tool to differentiate females, even in the fossil record. Furthermore, the variability in MB distribution in *S. magellanicus* suggests dietary influences, specifically calcium intake, which warrants further investigation under controlled conditions.

Keywords Spheniscidae · Bone microanatomy · Bone histology · Sexual differences · Eocene · Antarctic Peninsula

Introduction

At the anatomical level, the bone morphology of penguins has been well known for more than half a century, with important contributions in the 70 s (e.g. Simpson 1946, 1970, 1971, 1972, 1975, 1976). Several authors mention high bone density and the absence of pneumatisation in penguin bones (e.g. Meister 1962; Chinsamy et al. 1998; Cerda et al. 2015; Ksepka et al. 2015), characters shared with other extinct aquatic forms such as *Hesperornis*, *Ichthyornis*, *Polarornis* and *Vegavis* (Chinsamy et al. 1998; Wilson and Chin 2014; Garcia Marsà et al. 2019). These characteristics have therefore led to their recurrent use for osteohistological comparative purposes. However, histological studies within the group are still insufficient. The earliest research on Sphenisciformes histology dates back to the 60 s (Meister 1962) and only in the last 10 years has there been an increasing in this topic (Cerda et al. 2015; Ksepka et al. 2015; Acosta Hospitaleche et al. 2020; Garcia Marsà et al. 2020; Garat et al. 2023; Canoville et al. 2024).

In addition to osteohistological contributions, we can highlight the work of Cerda et al. (2015) and Ksepka et al. (2015), who discuss the microstructural patterns of medullary regions in both fossil and extant Sphenisciformes taxa. Cerda et al. (2015) identified fossil penguin taxa with either absent or greatly reduced medullary cavities, as well as taxa with well-developed medullary cavities, attributing these differences primarily to adaptations related to the aquatic habits of the species studied. In contrast, Ksepka et al. (2015) observed microstructural differences between fossil

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and extant penguins, with some taxa exhibiting well-developed medullary cavities, while others showed a trabecular ‘latticework’ at the medullary margin. However, Ksepka et al. (2015) interpret these variations as reflecting different stages in the species’ pursuit of bone compaction.

More recently, Canoville et al. (2024) describe an ontogenetic series of *Aptenodytes patagonicus* (including chicks and adults) and analyses microstructural changes in the hind limb and forelimb bones. Despite these important contributions, little is known about how these microstructural patterns respond to other parameters such as sexual differences, reproductive status, plumage moult and food supply.

Regarding histological qualities linked to sex differences, the presence of medullary bone along with microstructural patterns associated with osteoclastic activity has been recorded in *Pygoscelis adeliae* (Garat et al. 2023). This led to the formulation of a new hypothesis proposing an auxiliary criterion for sex discrimination in modern and extinct penguins, based on isolated remains, as they are often found in the fossil record. In that analysis, three tissue characteristics were highlighted: (1) differences in the degree of compactness between males and females, with females showing a lower degree of compactness and wider intertrabecular spaces; (2) the presence of medullary bone in females; and (3) the development of an inner circumferential layer (ICL), present in males and absent in females. Although, in the first instance, the presence of an ICL could be used as a reliable sex indicator, the authors suggested that female penguins should be studied outside the oviposition period in order to whether they develop an ICL, or not, given that this structure disappears by osteoclastic action during egg laying.

To broaden microanatomical and histological knowledge in Sphenisciformes and to evaluate the development of ICL, thus testing the proposed hypothesis, in this present study, histological sections of new penguin specimens corresponding to different genera and current species were described (*Aptenodytes forsteri*, *Pygoscelis antarctica*, *P. papua* and *Spheniscus magellanicus*), which allowed the establishment of reference microstructural patterns.

Table 1 Taxa of Sphenisciformes (adults). (N°?), rib number unknown; MB, medullary bone; ICL, inner circumferential layer

Collection Number	Species	Bone element	Histological Collection Number	Presence of MB/ICL status
MLP-O 38	*Pygoscelis papua*	Humerus	CHO 03–01	MB/Without ICL
		Radius	CHO 03–02	MB/Without ICL
		Ulna	CHO 03–03	MB/Without ICL
		Carpometacarpus	CHO 03–04	Without ICL
		Femur	CHO 03–05	MB/Without ICL
		Tibiotarsus	CHO 03–06	MB/Without ICL
		Tarsometatarsus	CHO 03–07	MB/Without ICL
		Vertebral rib (N°?)	CHO 03–08	MB/Without ICL
		Sternal rib N°3	CHO 03–09	MB/Without ICL
MLP-O 930	*Pygoscelis antarctica*	Humerus	CHO 04–01	MB/Without ICL
		Femur	CHO 04–02	MB/Remodelled ICL
		Tibiotarsus	CHO 04–03	MB/Remodelled ICL
		Vertebral rib (N°?)	CHO 04–04	MB/Without ICL
		Sternal rib (N°?)	CHO 04–05	MB/Without ICL
MLP-O 937	*Spheniscus magellanicus*	Humerus	CHO 11–01	Without ICL
		Radius	CHO 11–02	Without ICL
		Ulna	CHO 11–03	Without ICL
		Carpometacarpus	CHO 11–04	MB
		Femur	CHO 11–05	With ICL
		Tibiotarsus	CHO 11–06	With ICL
		Tarsometatarsus	CHO 11–07	MB/Without ICL
		Vertebral rib (N°?)	CHO 11–08	Without ICL
		Sternal rib (N°?)	CHO 11–09	With ICL
MLP-O 15192	*Aptenodytes forsteri*	Humerus	CHO 12–01	MB/Without ICL
		Femur	CHO 12–02	MB/Without ICL
		Vertebral rib D5	CHO 12–03	MB/Remodelled ICL
		Sternal rib N°3	CHO 12–04	MB/Remodelled ICL

Materials and methods

The specimens analysed, which die due to natural causes and were collected post-mortem, are housed in the osteological collection of the Ornithology Section of the Vertebrate Zoology Division at Museo de La Plata (MLP-O). Sex determination was based on the presence of medullary bone and the absence of osteopathology following the criteria #1, #4, #5f and #7 set by Canoville et al. (2020). This included the identification of medullary bone within the medullary cavity and trabecular spaces of appendicular and axial skeletal elements, the absence of osteopathological markers on the periosteal surface, the line of medullary bone along trabecular surfaces, and its presence in multiple skeletal elements, particularly the tibiotarsus. Histological sections of the appendicular and axial skeleton of *Pygoscelis papua* (MLP-O 38), *P. antarctica* (MLP-O 930), *Spheniscus magellanicus* (MLP-O 937) and *Aptenodytes forsteri* (MLP-O 15192) were prepared. A total of 27 thin sections (Table 1) were examined at the Thin Section Laboratory of Instituto de Investigación en Paleobiología y Geología (CONICET-UNRN), in the city of General Roca, Río Negro province (Argentina). The procedure outlined in Chinsamy and Raath (1992) was used to obtain sections. The slides prepared were analysed with a petrographic microscope (*ZEISS Axio Imager*) under plane- and cross-polarized light. Images of each thin section were captured by a digital camera (*ZEISS Axiocam 105*) and processed with *Adobe Photoshop 2020* and *Adobe Illustrator 2022*.

Results

Microanatomy

All the elements analysed show clear compact tissue and have a high degree of bone remodelling (Fig. 1). In wing bone sections, no medullary cavity was observed, but several medullary spaces developed over the medullary region. In sections of the hindlimb bones and ribs, there is more histological variation, including well-defined medullary cavities, medullary cavities with an irregular margin (converging with

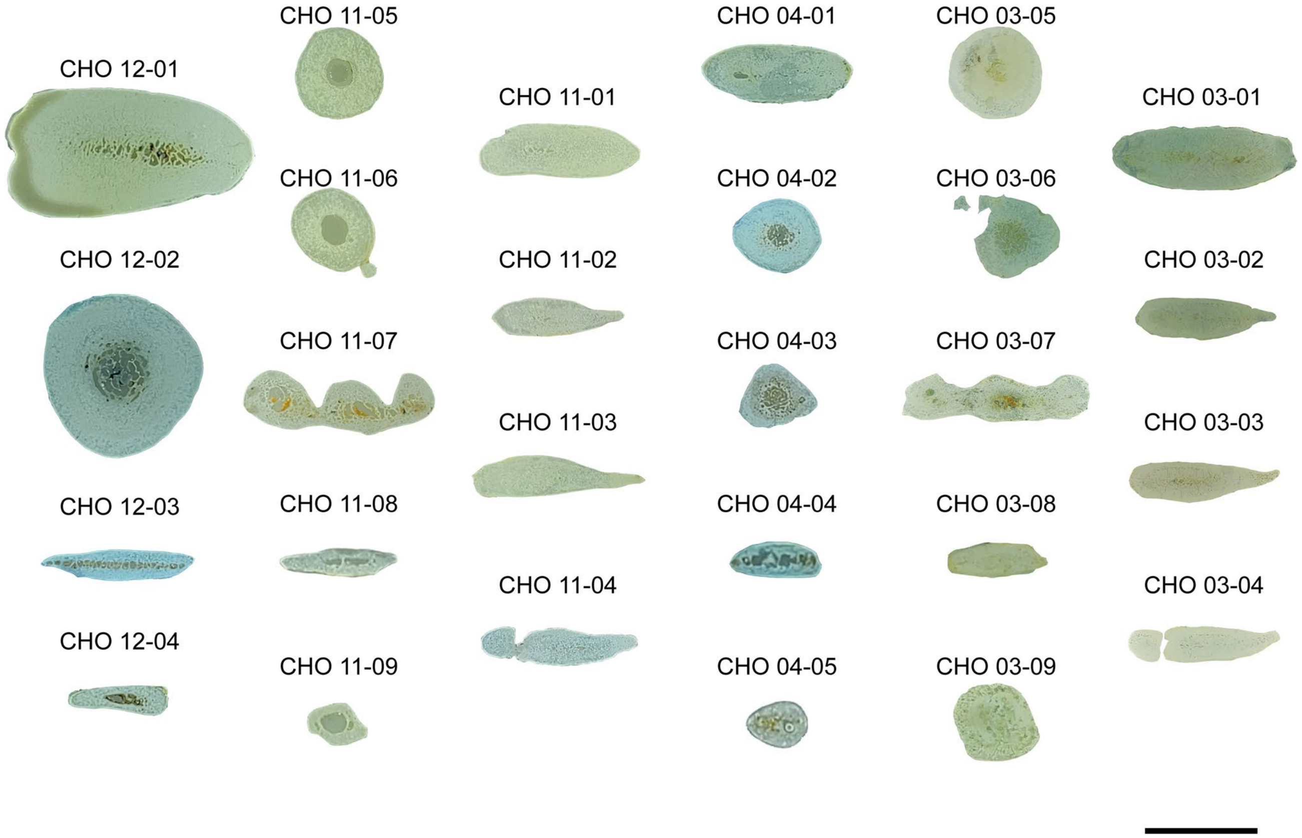
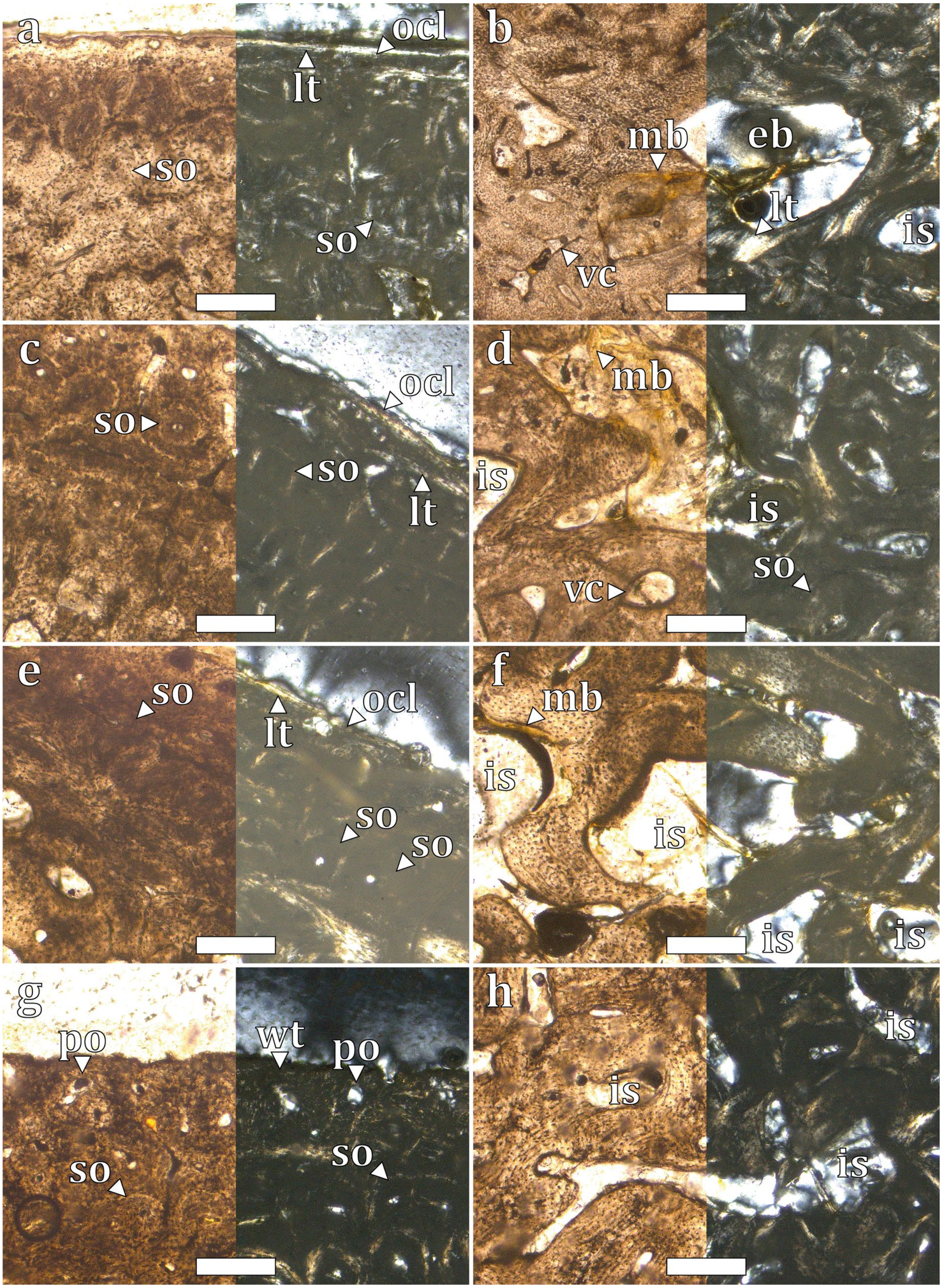


Fig. 1 Microanatomical features of *Pygoscelis papua* (Series CHO 03), *Pygoscelis antarctica* (Series CHO 04), *Spheniscus magellanicus* (Series CHO 11) and *Aptenodytes forsteri* (Series CHO 12). Humeri, radii, ulnae, carpometacarpi, femora, tibiotarsi, tarsometatarsi, vertebral (CHO 12–03) and sternal rib (CHO 12–04), scale bar 1 cm. Other vertebral (CHO 03–08, CHO 04–04, CHO 11–08) and sternal (CHO 03–09, CHO 04–05, CHO 11–09) ribs, scale 0.5 cm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◂**Fig. 2** Histological features of *Pygoscelis papua* (MLP-O 38). (**a**, **b**) humerus, (**c**, **d**) radius, (**e**, **f**) ulna, (**g**, **h**) carpometacarpus, under normal transmitted light (left) and under polarized light (right). (**a**, **c**, **e**, **g**) cortical and perimedullary region, (**b**, **d**, **f**, **h**) medullary region. (eb) erosion bay, (is) intertrabecular space, (lt) lamellar tissue, (mb) medullary bone, (po) primary osteon, (so) secondary osteon, (vc) vascular canal and (wt) woven-fibered tissue. Scale 100 µm

other intertrabecular spaces) and bone latticework over the medullary region.

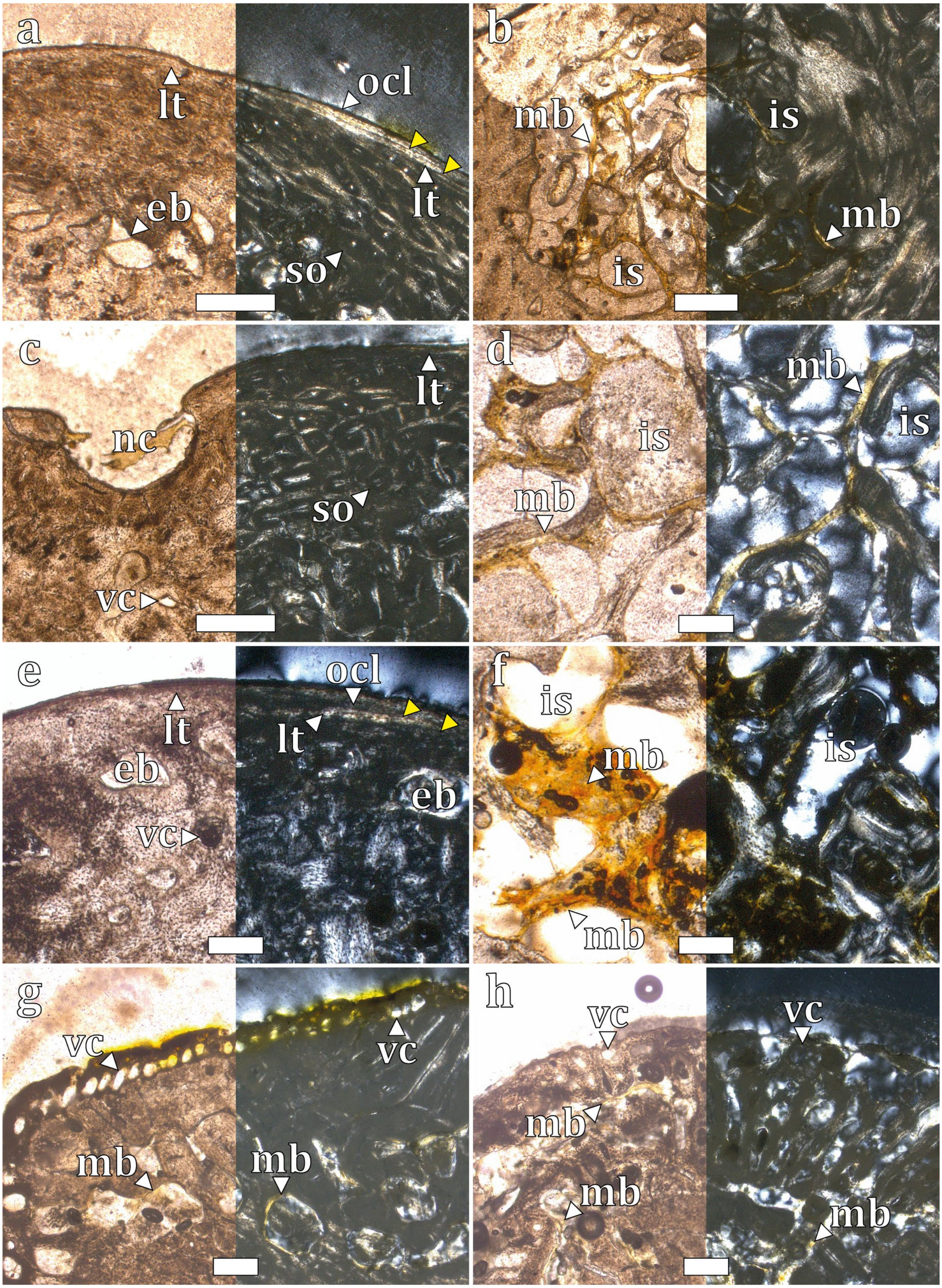
Histology

Since some taxa share certain histological traits, we first describe their general features and then mention particular characteristics of each taxon if applicable.

The cortical region (Figs. 2a, c, e and g, 3a, c, e, g and h, 4a, c, e, g and h, 5a, c, e and g, 6a, c, e, g and h and 7a, c, e and g) presents an outer circumferential layer (OCL), formed by a thin layer of avascular lamellar tissue which, in some cases, presents lines of arrested growth (LAGs). The vertebral and sternal ribs of *Pygoscelis papua* have a series of primary vascular canals distributed longitudinally in a laminar vascular pattern. In addition, several primary vascular canals with a longitudinal distribution were observed in the cortical margin of the *Spheniscus magellanicus* sections (except for the ribs) (Figs. 5 and 6). Regarding the number of LAGs, the humerus, femur, tibiotarsus, vertebral and sternal ribs of *Pygoscelis antarctica* and the humerus of *Aptenodytes forsteri* showed one LAG at the cortical margin. Two LAGs were identified in the femur and tarsometatarsus of *P. papua* and the sternal rib of *A. forsteri*. In the vertebral rib of *A. forsteri*, four LAGs were found (the highest number of LAGs recorded) (Fig. 7e).

In the perimedullary region (Figs. 2a, c, e and g, 3a, c, e, g and h, 4a, c, e, g and h, 5a, c, e and g, 6a, c, e, g and h and 7a, c, e and g), the tissue is mainly composed of secondary osteons with a longitudinal vascular distribution characterised by clear cementing lines. These osteons remodel the primary bone matrix almost completely. Although the bone matrix cannot be distinguished from the remnant of the primary tissue under polarized light, remodelling is evidenced by the morphology of the osteocyte lacunae. These lacunae, derived from static osteogenesis, are circular and larger than the lacunae derived from dynamic osteogenesis, which are smaller, discoidal and oriented according to the distribution of the fibres (e.g. D'Emic and Benson 2013; Grunmeier and D'Emic 2019). In the case of *Pygoscelis papua*, the degree of bone remodelling is lower. A bone matrix of parallel-fibered to woven-fibered bone can be distinguished (Fig. 2g). In *Spheniscus magellanicus*, the radius showed the development of a drifting osteon-like structure (Fig. 5d). Regarding vascular organisation, in the humerus and femur of *P. antarctica*, the vascular pattern becomes slightly laminar over the outer margin of the perimedullary region (Figs. 4a and c). In addition, some erosion bays were identified for the hindlimb bones of *Pygoscelis papua* (Figs. 3a and c). A nutrient channel is also observed on the perimedullary margin of the tibiotarsus of *P. antarctica* and *S. magellanicus* and of the femur of *P. papua* (Figs. 3c, 4c and 6e).

In general, in the medullary region (Figs. 2b, d, f and h, 3b, d, f, g and h, 4b, d, f, g and h, 5b, d, f and h, 6b, d, f, g and h and 7b, d, f and h), there is a secondarily compacted trabecular tissue where the medullary spaces are surrounded by lamellar tissue. However, the greatest osteohistological differences between different taxa are found in the medullary region. In the humerus of *Pygoscelis antarctica*, humerus, radius, ulna, carpometacarpus and vertebral rib of *Pygoscelis papua* and *Spheniscus magellanicus*, sternal rib of *P. papua*, and humerus, femur and vertebral rib of *Aptenodytes forsteri*, no medullary cavity was observed. Instead, several smaller medullary spaces were identified over the medullary region. In the femur of *A. forsteri*, in the femur, tibiotarsus and tarsometatarsus of *P. papua*, and the tarsometatarsus of *S. magellanicus*, a trabecular bone lattice dividing the medullary region into a number of medullary spaces was observed. On the other hand, in sections of the femur, tibiotarsus, vertebral and sternal ribs of *P. antarctica*, the medullary cavity is not well delimited and shows an irregular margin, which may converge with other adjacent medullary spaces. In sections of the sternal rib of *A. forsteri* and the femur and tibiotarsus of *S. magellanicus*, the medullary cavity is well delimited by surrounding tissue. Finally, an inner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◂**Fig. 3** Histological features of *Pygoscelis papua* (MLP-O 38). (**a**, **b**) femur, (**c**, **d**) tibiotarsus, (**e**, **f**) tarsometatarsus, (**g**) vertebral rib, (**h**) sternal rib, under normal transmitted light (left) and under polarized light (right). (**a**, **c**, **e**, **g**, **h**) cortical and perimedullary region, (**b**, **d**, **f**, **g**, **h**) medullary region. (eb) erosion bay, (is) intertrabecular space, (lt) lamellar tissue, (mb) medullary bone, (nc) nutrient canal, (so) secondary osteon, (vc) vascular canal and (yellow triangle) LAGs. Scale 200 μm

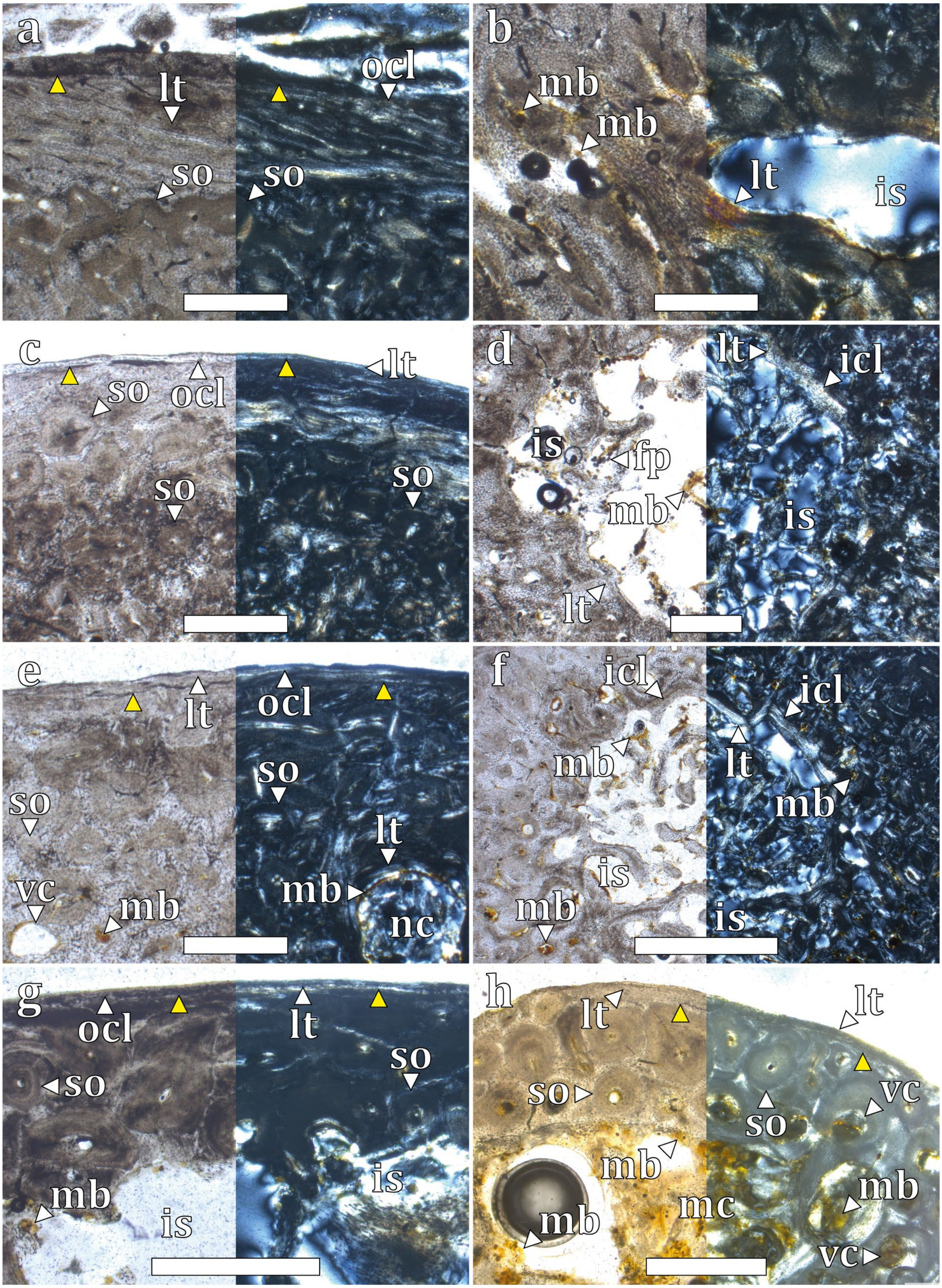
circumferential layer (ICL) was identified at the margin of the medullary cavity of some taxa. A partially remodelled ICL was identified in the femur and tibiotarsus of *P. antarctica* and in the vertebral and sternal ribs of *A. forsteri*. This remodelling involved partial resorption of the tissue that forms the ICL, along with the development of bony trabeculae invading the medullary space. Consequently, the semi-circular contour of the medullary cavity was lost and interrupted, resulting in an irregular margin that may converge with other trabecular spaces and erosion bays. On the other hand, an extensive ICL was found in the tibiotarsus of *S. magellanicus* (Fig. 6d). In all the species analysed, the presence of medullary bone was identified in the medullary cavity and spaces (Figs. 2b, d and f, 3b, d, f, g and h, 4b, d, f, g and h, 5h, 6f, 7b, d, f and h). However, in *S. magellanicus,* medullary bone was only observed in the carpometacarpus and tarsometatarsus sections (Figs. 5 and 6), while in the carpometacarpus of *P. papua,* this tissue was absent. Consequently, for *S. magellanicus* and *P. papua,* a complete skeletal distribution of this tissue is lacking. No Sharpey's fibres were found in any of these sections.

Discussion

Microstructural variations in the medullary region

Several studies have focussed on the microstructural variations in the medullary region of penguins due to their relationship with biomechanical factors, sexual differences and adaptations to the aquatic environment (Cerda et al. 2015; Ksepka et al. 2015; Garat et al. 2023). Previous histological studies have documented such variations in both extant and fossil species. Specifically, Cerda et al. (2015) observed taxa with absent or greatly reduced cavities, as well as taxa with well-developed medullary cavities in the tarsometatarsi of fossil penguins. To explain this microstructural diversity, the authors proposed three hypotheses: (1) biomechanical factors related to body size, as larger taxa have more massive bones, while smaller taxa have well-developed medullary cavities; (2) sex-specific variations related to eggshell formation, as female birds lose bone tissue in bone metaphysis during egg laying (Wilson and Throp 1998; de Buffrénil and Francillon-Vieillot 2001) and (3) different degrees of adaptation in response to aquatic habit, which means that species exhibiting more massive bones would be better adapted to deeper and longer dives compared to those with less dense bones. In this study, we identified microstructural patterns like those reported by Cerda et al. (2015) in fossil taxa (e.g. absent or greatly reduced cavities/well-developed medullary cavities). Contrary to Cerda et al. (2015), these patterns were linked to the presence of medullary bone, irrespective of the species' body size. In both large (*Aptenodytes*) and small (*Pygoscelis*, *Spheniscus*) genera, medullary bone was found to occupy medullary cavities and spaces. Likewise, when no medullary bone was identified in the sections, medullary cavities well defined by surrounding tissue were observed. This evidence would suggest that the microstructural differences in the medullary region of the taxa could be the result of sexual variations and osteoclastic activity associated with the medullary bone.

Ksepka et al. (2015) describe microstructural differences between extant and Eocene penguins, explaining variations in the medullary cavity of the humeri. These authors found taxa with cavities well delimited by an ICL and others with a trabecular bone 'latticework' that divides the medullary region into a number of medullary spaces. According to them, these patterns reflect different stages in a transition from a pneumatic to an osteosclerotic humerus. Thus, in the first instance, there is a reduction of the medullary cavity due to decreased perimedullary osteoclastic activity, followed by secondary compaction of the cortex. In the present and previous studies (Garat et al. 2023), cavities delimited by an ICL have been observed in males and females, and others with a trabecular bone 'lattice' with medullary bone development have been found in females. Perhaps these differences may be linked to bone compaction and sexual histological variations associated with the need to extract calcium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◂**Fig. 4** Histological features of *Pygoscelis antarctica* (MLP-O 930). (**a**, **b**) humerus, (**c**, **d**) femur, (**e**, **f**) tibiotarsus, (**g**) vertebral rib, (**h**) sternal rib, under normal transmitted light (left) and under polarized light (right). (**a**, **c**, **e**, **g**, **h**) cortical and perimedullary region, (**b**, **d**, **f**, **g**, **h**) medullary region. (fp) framboidal pyrite, (icl) inner circumferential layer, (is) intertrabecular space, (lt) lamellar tissue, (mb) medullary bone, (mc) medullary cavity, (nc) nutrient canal, (so) secondary osteon, (vc) vascular canal and (yellow triangle) LAGs. Scale 500 µm

for egg laying in females. The ICL appears to remain unaltered in the absence of medullary bone. Similarly, when segmentation of the medullary region into several medullary spaces or a partially remodelled ICL was found in females, these cavities showed medullary bone within them.

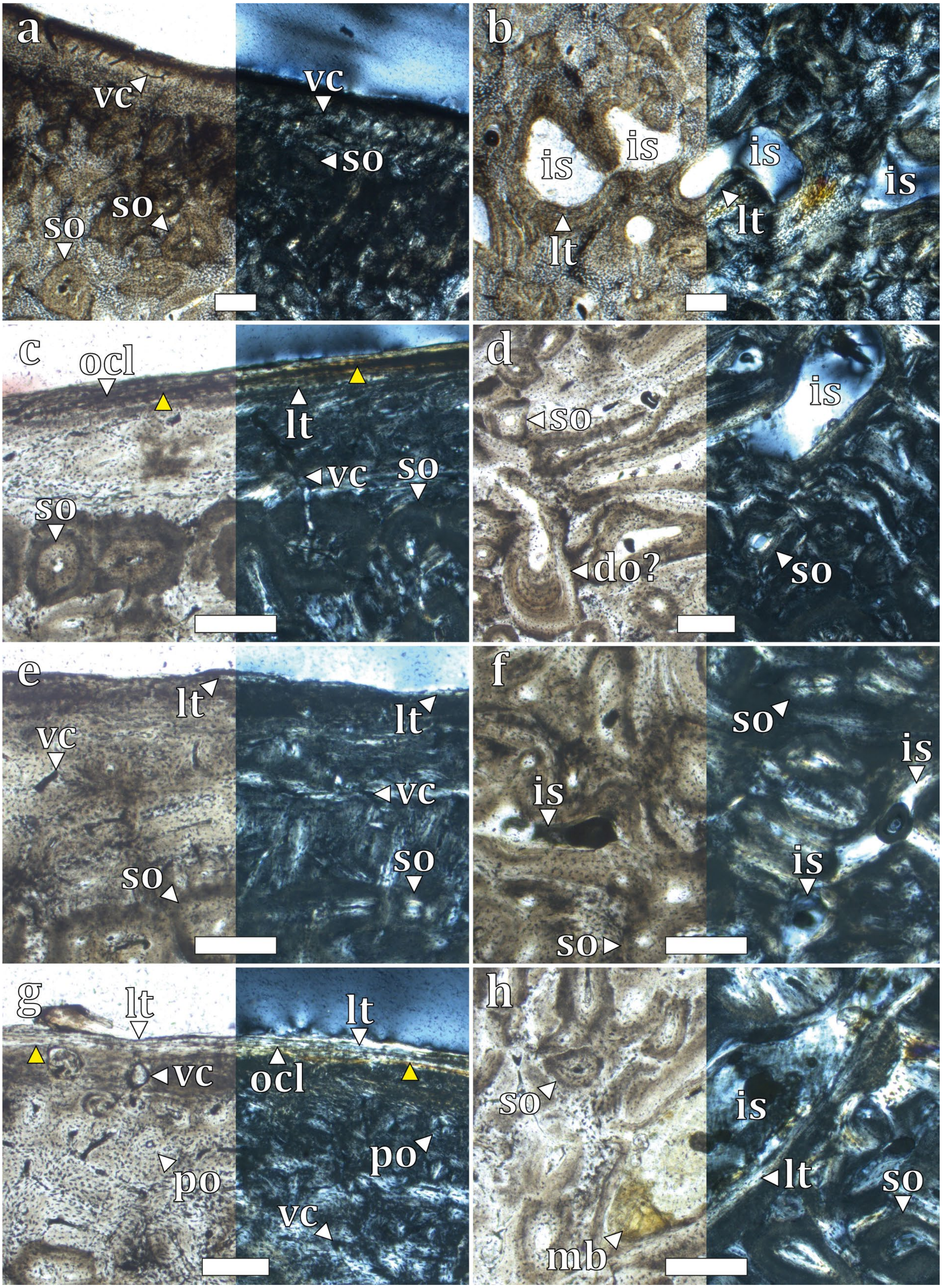
Concerning microstructural variations in the medullary region, Garat et al. (2023) identified patterns like those described by Cerda et al. (2015) and Ksepka et al. (2015) in appendicular and axial elements of the skeleton of a male and a female *Pygoscelis adeliae*. These patterns were associated with the presence of medullary bone. In the male specimen, the medullary cavity was well defined by an ICL (in the femur, tibiotarsus, tarsometatarsus, vertebral and sternal ribs), exhibited less development of intertrabecular spaces and, consequently, had a higher degree of bone compaction. In contrast, in the female specimen, the ICL was absent, and more extensive intertrabecular and medullary spaces lined with medullary bone were observed. Thus, the authors proposed evaluating females outside the oviposition period to determine if they develop an ICL that then disappears by osteoclastic action during egg laying, or if they never develop suck layers. Under this criterion, the absence of an ICL in adult females outside the oviposition period could be used as a tool to determine sex. The present study identified the presence of medullary bone in all specimens analysed and the presence of a partially remodelled ICL in *Pygoscelis antarctica* and *Aptenodytes forsteri*. This means that females develop an ICL, but this structure is lost due to the osteoclastic action involved in the production of medullary bone.

Regarding medullary bone, its presence was identified in most of the bone elements analysed in *P. papua* (except the carpometacarpus), and in all the elements of *P. antarctica* and *A. forsteri*. However, *Spheniscus magellanicus* showed a partial distribution of medullary bone in the sections analysed, with this tissue being observed only in the carpometacarpus and tarsometatarsus. This result contrasts with what was expected, given that it has been proposed that medullary bone should be present in the entire appendicular and axial skeleton of the female (for more details, see Bailleul et al. 2019; Canoville et al. 2019, 2020). On the other hand, a previous study on this species suggests that the females obtain the calcium necessary for egg formation from the selective ingestion of mollusc shells, rather than from the mobilisation of calcium from their bones (Boersma et al. 2004). Although the prevalence of medullary bone is variable in different skeletal regions (Canoville et al. 2019), a calcium-rich diet (Boersma et al. 2004) may influence the formation and distribution of medullary bone, reducing its development at the skeletal level. This observation underlines the need to evaluate a larger number of individuals under controlled dietary conditions to test the hypothesis that diet affects the presence and distribution of medullary bone in *Spheniscus magellanicus*.

Is there histovariability within the genus pygoscelis?

Genus *Pygoscelis* comprises three extant species (*Pygoscelis adeliae*, *Pygoscelis antarctica* and *Pygoscelis papua*), with Antarctic and sub-Antarctic biogeographic ranges (García Borboroglu and Boersma 2015). Adélie's penguin (*P. adeliae*) has a circumpolar distribution, with major breeding aggregations in ice-free areas arround the Ross Sea, along the Antarctic coast, on the west coast of the Antarctic Peninsula and the islands of the Scotia Arc. On the other hand, the chinstrap penguin (*P. antarctica*) is distributed mainly on the Antarctic Peninsula, south of 64° S, and the South Shetland, South Orkney and South Sandwich Islands. Finally, the gentoo penguin (*P. papua*) has a circumpolar breeding distribution spanning in latitude from Cape Tuxen on the Antarctic Peninsula (65°16' S) to the Crozet Islands (46°00' S).

Unlike the other *Pygoscelis* species, *P. papua* does not exhibit migratory habits. These variations in migratory or non-migratory habits, biogeographic range and even diet and feeding stress, raise questions about the presence of histological variability within the genus. Wilson and Chin (2014) were the first to assess histovariability in pygoscelids by analysing the bone microstructure of migratory and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◂**Fig. 5** Histological features of *Spheniscus magellanicus* (MLP-O 937). (**a**, **b**) humerus, (**c**, **d**) radius, (**e**, **f**) ulna, (**g**, **h**) carpometacarpus, under normal transmitted light (left) and under polarized light (right). (**a**, **c**, **e**, **g**) cortical and perimedullary region, (**b**, **d**, **f**, **h**) medullary region. (do?) drifting osteon-like structure, (is) intertrabecular space, (lt) lamellar tissue, (mb) medullary bone, (po) primary osteon, (so) secondary osteon, (vc) vascular canal and (yellow triangle) LAGs. Scale 200 µm

non-migratory species. Given that migrations reduce the amount of seasonal environmental stress experienced by individuals (Tütken et al. 2004), the absence or reduction of cyclic growth marks in migratory species could be expected. In line with this, Wilson and Chin (2014) sought to identify zonal tissue (with the presence of LAGs) in non-migratory species and azonal tissue in migratory species. The authors found an outer circumferential layer (OCL) without LAGs in all pygoscelids. These data suggest that the absence of LAGs is not necessarily related to migratory habits, at least in pygoscelids.

Conversely, in the present and previous works (Garat et al. 2023), the presence of LAGs in an avascular lamellar matrix within the cortical margin, forming an OCL, was identified in all three *Pygoscelis* species. These data revealed that LAGs may be more related to the somatic maturity of individuals (Padian and Woodward 2021) than to migratory habits. Furthermore, the arrangement of LAGs varied in number and presence both within the skeleton of the same individual and among species of the genus. One LAG was observed on the radius, tibiotarsus and ribs (vertebral and sternal), and two LAGs on the femur and tarsometatarsus of *P. adeliae*. For the humerus, ulna and carpometacarpus, two LAGs were identified in the male specimen and one LAG in the female specimen of *P. adeliae* (Garat et al. 2023). On the other hand, two LAGs were observed for the femur and tarsometatarsus of *P. papua* and one LAG for the humerus, femur, tibiotarsus, vertebral and sternal ribs of *P. antarctica*.

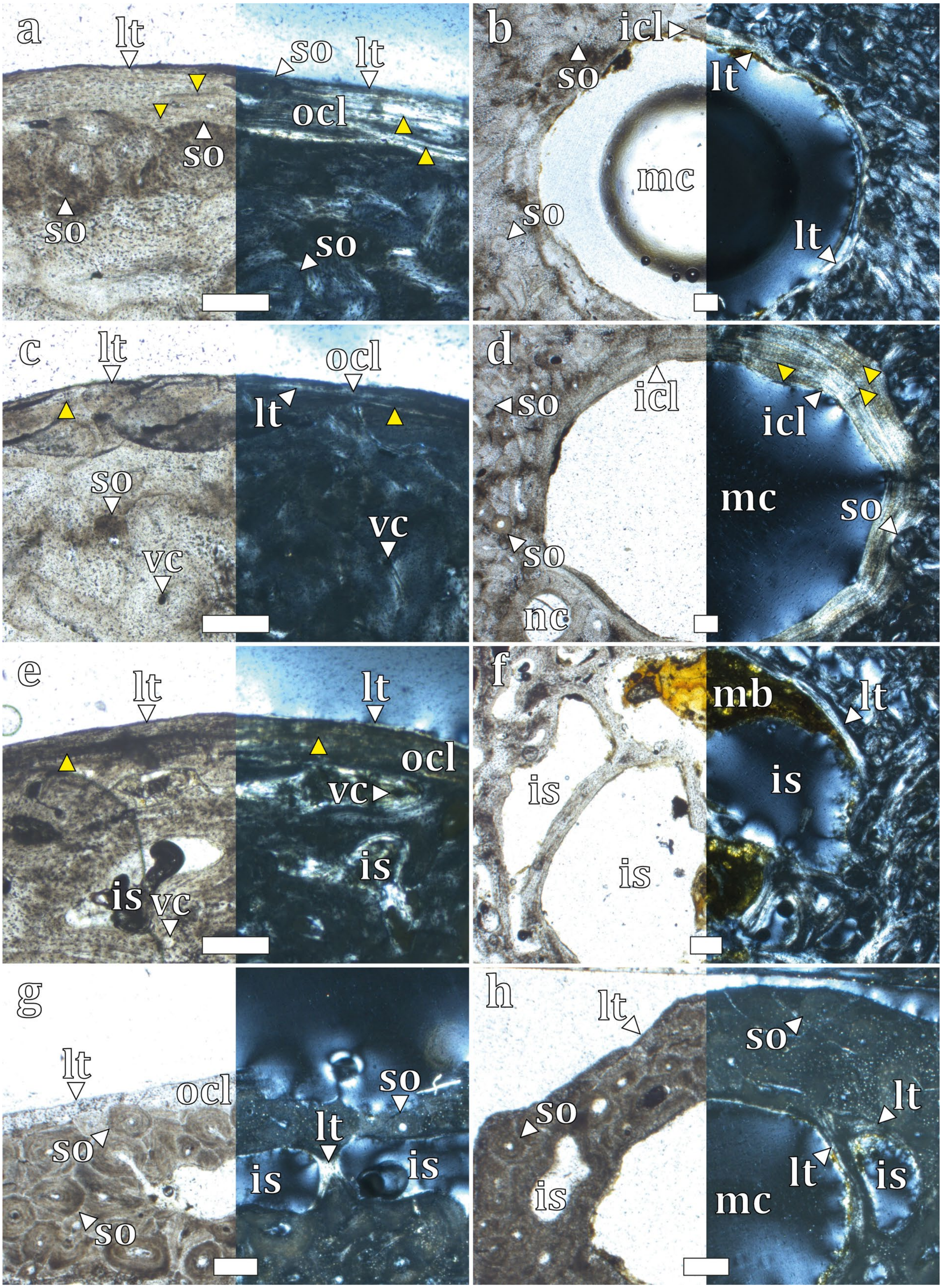
However, these differences could be related to the degree of bone remodelling present in the specimens. In an analysis of a series of different ontogenetic stages of *Pygoscelis antarctica*, the vascular arrangement varies according to the degree of remodelling (pers. obs. Garat). When the remodelling front does not reach the cortical margin, the vascular pattern is laminar, with longitudinal and circular channels; however, the pattern becomes predominantly longitudinal when remodelling invades the cortex. Future studies on the ontogeny of *P. antarctica* may provide further details on the dynamics of growth and its relationship with vascularisation and the somatic maturity of the individual.

Finally, in both *P. antarctica* (MLP 930) and *P. papua* (MLP 38), the same patterns reported by Garat et al. (2023) were identified: three tissue regions (a cortical, perimedullary and medullary region) defined by their bone matrix, organisation of vascular channels and degree of compaction. In the present study, all pygoscelids show compact bone tissue as a result of secondary compaction of trabecular bone and extension of the ICL. Consequently, major osteohistological variations within the genus have not been identified.

Conclusions

According to the analysis, the ICL appears to develop in both females and males. However, in females, it remodels (and probably does not re-form) due to osteoclastic action linked to the medullary bone, whereas in males it remains intact. Although the presence of a well-developed ICL is not a reliable indication of sex, its absence in adult stages or its presence with signs of remodelling would suggest the specimen is a female. Based on these results, further sampling to identify taxonomic variations due to sex in future studies of fossil species may be planned.

Regarding the distribution of medullary bone in *Spheniscus magellanicus*, its variation may be explained by the availability of calcium in its diet. Finally, no significant histological differences were observed within the genus *Pygoscelis*.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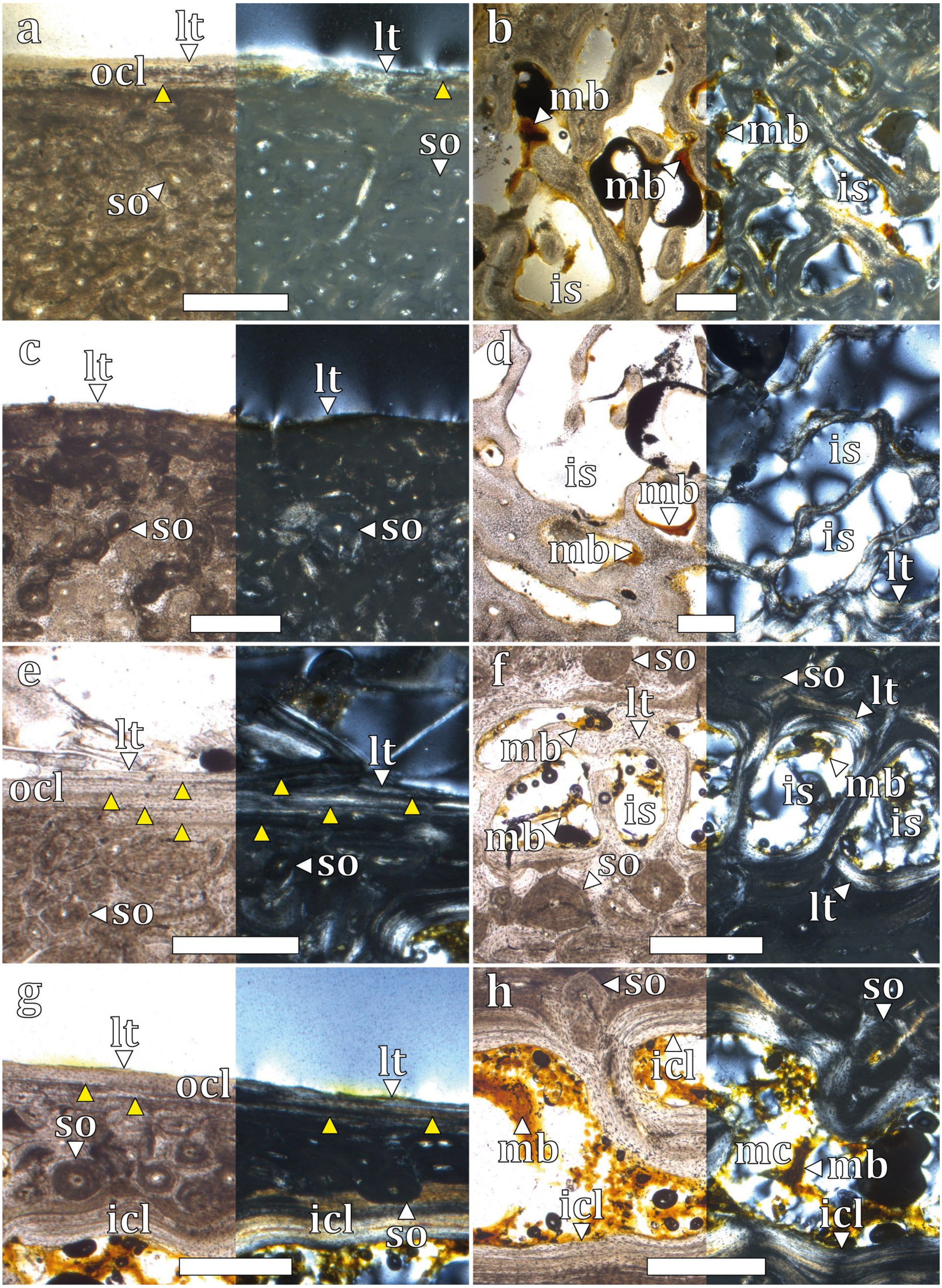
◂**Fig. 6** Histological features of *Spheniscus magellanicus* (MLP-O 937). (**a**, **b**) femur, (**c**, **d**) tibiotarsus, (**e**, **f**) tarsometatarsus, (**g**) vertebral rib, (**h**) sternal rib, under normal transmitted light (left) and under polarized light (right). (**a**, **c**, **e**, **g**, **h**) cortical and perimedullary region, (**b**, **d**, **f**, **g**, **h**) medullary region. (icl) inner circumferential layer, (is) intertrabecular space, (lt) lamellar tissue, (mb) medullary bone, (mc) medullary cavity, (nc) nutrient canal, (so) secondary osteon, (vc) vascular canal and (yellow triangle) LAGs. Scale 200 µm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◂**Fig. 7** Histological features of *Aptenodytes forsteri* (MLP-O 15192). (**a**, **b**) humerus, (**c**, **d**) femur, (**e**, **f**) vertebral rib, (**g**, **h**) sternal rib, under normal transmitted light (left) and under polarized light (right). (**a**, **c**, **e**, **g**) cortical and perimedullary region, (**b**, **d**, **f**, **h**) medullary region. (icl) inner circumferential layer, (is) intertrabecular space, (lt) lamellar tissue, (mb) medullary bone, (mc) medullary cavity, (so) secondary osteon and (yellow triangle) LAGs. Scale 500 µm

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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