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First evidence of globuli ossei in bird (Aves, Spheniciformes). Implications on paleohistology and bird behaviour

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ABSTRACT

Globuli ossei are subspherical structures of endochondral origin that are inserted in the hypertrophic cartilage of long bones, and are related to paedomorphic phenomena. These structures are here described for the first time in Aves, concretely in a postcranial bone of an extinct Spheniciformes from Antarctica (*Delphinornis arctowskii*). Although it is difficult to infer the significance of the presence of these structures, they appear not to be related to adaptations to aquatic life or depend on the small size or ontogenetic stage of the specimen. A pathological origin for these structures lacks evidence sustaining this hypothesis. The data provided in this contribution reveals that the presence of globuli ossei in vertebrate long bones is not always linked with adaptations to aquatic life and/or paedomorphic processes.

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Introduction

Cellular diversification of the skeletal tissues has played a preponderant role in the evolutionary history of the vertebrates so that their study is of singular importance. During the endochondral ossification, which is one of the essential embryological processes of bone formation, one of the potential results is the formation of globuli ossei. They are subspherical structures resulting from the partial conversion of tissue-skeletal cells (Cervantes-Diaz et al. 2016).

During the endochondral ossification the chondroclasts that resorpt calcified cartilage left trabeculae on which osteoblasts are deposited (Francillon Vieillot et al. 1990, Quilhac et al. 2014). The death of chondrocytes results in chondrocytic lacunae. Posteriorly the chondrocytic lacunae are invaded by new cells coming from the blood/marrow system. These new cells differentiate into osteoblasts, which produce new bone matrix inside the empty chondrocytic lacunae (Quilhac et al. 2014). These structures are known as globuli ossei. During the resorption, the globuli ossei are inserted in a hypertrophic cartilage zone (Francillon Vieillot et al. 1990, Quilhac et al. 2014).

Excluding birds, globuli ossei have a wide occurrence among tetrapods (e.g., Haines RW 1938; Gussen 1968; Buffrénil et al. 2014). Its occurrence is significant because they can provide information about bone growth and/or about some mechanisms regarding secondary adaptations to aquatic life. In the first case, the formation and amount of globuli ossei have been linked to slow longitudinal growth of long bones (Haines, 1942; De Ricqlès 1979). In cases in which globuli ossei (and calcified cartilage in general) occur in the shaft of the postcranial bones of tetrapods during relatively advanced ontogenetic stages, they have been associated with a delay or inhibition of endochondral osteogenesis (Buffrénil et al. 1990). Such reduction in the

condroclastic activity has been interpreted as one of the possible mechanism for the increasing of bone mass by reduction of medullary cavity called osteosclerosis (e.g. Buffrénil et al. 1990, 2010). Skeletal osteosclerosis in long bones has been reported in several groups of aquatic vertebrates, including penguins (De Ricqlès and De Buffrénil 2001; Ksepka 2008, Ksepka et al. 2015; Cerda et al. 2014) and has frequently been considered as evidence of secondary adaptation to aquatic life (De Ricqlès and De Buffrénil 2001). However, the osteosclerotic condition reported for penguins is due to a secondary infilling of the medullary cavity and is not related to a reduction or inhibition of the chondroclastic activity (Cerda et al. 2014, Ksepka 2008, Ksepka et al. 2015). Hence, remains of calcified cartilage including globuli ossei have never been reported in the shaft of postcranial bones of penguins nor any other bird.

The present study documents for the first time the presence of globuli ossei in a postcranial element of a fossil penguin, specifically in a tarsometatarsus of *Delphinornis arctowskii*, which also represents the first identification of this structure in birds. The specimen come from Eocene sediments of La Meseta Formation, Antarctica which contains the major taxonomic and body size diversity of stem Sphenisciformes, including 10–15 species (Myrcha et al. 2002; Jadwiszczak 2006; Tambussi et al. 2006; Jadwiszczak and Mörs 2011; Reguero et al. 2013; Acosta Hospitaleche et al. 2017). In this paper, a description of the structures is here performed, and its implications for ontogeny and behaviour are discussed.

Materials and methods

The tarsometatarsus of *Delphinornis arctowskii* (MLP 93-X-1-92) here studied is part of previously published sample (Cerda et al.

2014), which includes tarsometatarsi of eight specimens of different fossil penguins (*Palaeudyptes gunnari*, *P. klekowski*, *Anthropornis grandis*, *A. nordenskjoldi*, *Archaeospheniscus wimani*, *Marambiornis exilis*, *Delphinornis arctowskii* and *D. larseni*). The preparation of the histological sections was carried out at Departamento de Geología de la Universidad Nacional de San Luis (Argentina). The specimen was prepared for thin sectioning using the methods outlined in Chinsamy and Raath (1992). Because this is a destructive method, all specimens were photographed, and standard measurements were taken before sectioning. Two sections obtained at level of the midshaft of the tarsometatarsi were examined for *D. arctowskii* MLP 93-X-1-92. The samples are housed at Museo de La Plata (MLP).

The MLP 93-X-1-92 was recovered from the *Anthropornis nordenskjoldi* Biozone (Tambussi et al. 2006), Submeseta Allomember, Facies Association III, La Meseta Formation, Priabonian, ~ 34.2Ma, based on 87/86Sr dates (Marensi et al. 1998). Osteological nomenclature follows Baumel and Witmer (1993).

Results

The tarsometatarsus studied here is conformed by four distinct medullary cavities which are located in the internal areas of the *os metatarsale* II, III and IV and below the sulcus *longitudinalis dorsalis lateralis*. The *os metatarsale* III has the largest medullary cavity, which is partially divided by bony trabeculae of irregular shape.

Because a detailed histological study of the complete sample has previously been carried out (Cerdea et al. 2014), we only provide here a general characterization of the tissues and emphasis will be placed on the microstructure of the globuli ossei. At microanatomical level, the tarsometatarsus is composed of a compact cortex that surrounds four distinct medullary cavities (Figure 1A), which occupy 30.3% of the cross section of tarsometatarsus diaphysis. At histological level, the cortical bone is composed of both primary and secondary bone tissue. The primary bone consists of well-vascularised fibro-lamellar bone. The vascular channels in the bone are organized as primary osteons, which are mostly longitudinally and radially oriented. Secondary bone reconstruction is high, and several specimens exhibit dense Haversian bone tissue in their cortices (mostly at the mid and perimedullary regions of the compacta). The medullary cavities are entirely or partially coated by a thick layer of endosteally deposited lamellar bone tissue (inner circumferential layer).

Globuli ossei (Brandt 1852; see also Buffrénil et al. 1990), are embedded in primary bone and recorded in a reduced area located at the dorsal side of the *os metatarsale* III (Figure 1B). They are grouped in numerous clusters of vitreous appearance and are finely granulated. Some globuli ossei have been infilled with calcite. These globules are bone structures that are small and rounded in contour, conforming excrescences that protrude from the lamellar deposits to bone matrix (previously cartilage) and fill the empty chondrocyte lacunae. Globuli ossei are demarcated by a small cementing line. The clusters are connected to the main base

sometimes by a broad base, and sometimes only by a narrow stalk, and some of these bosses contain one or more cells.

The globuli ossei diameter ($\varnothing = 20$ to $30\mu\text{m}$) is correlated with known parameters for other taxonomic groups. These histological features are congruent with previous reports of globuli ossei in postcranial bones of other fossil and extant tetrapods, including non-amniotes (e.g. Sanchez et al. 2010; Buffrénil et al. 2014; Quilhac et al. 2014), mammals (e.g. Buffrénil et al. 1990, 2008; 2010), lizards (Bailleul et al. 2011) and non-avian archosaurs (Haines RW 1938).

Discussion

The presence of globuli ossei (and calcified cartilage in general) in the shaft of long bones have been commonly associated with osteosclerosis in vertebrates that are secondarily adapted to aquatic life (De Ricqlès and De Buffrénil 2001; Houssaye 2009). Although penguins show osteosclerotic long bones, the presence of globuli ossei reported here does not appear to be linked to such skeletal mass increasing. Osteosclerosis produces an increase in the internal compactness of the bone without modifying external dimensions. This condition may result from incomplete endochondral ossification, inhibition of secondary remodelling, or filling of internal cavities (Houssaye 2009; Quilhac et al. 2014). This increase of bone mass is due to the secondary infilling of the medullary cavity with lamellar bone of endosteal origin (Cerdea et al. 2014 and literature cited) as seen in different extant and extinct penguins (e.g., *Pygoscelis adeliae*, *Aptenodytes forsteri*, *Aptenodytes patagonicus*, *Spheniscus demersus*, *Spheniscus* sp., *Anthropornis wimani*, *Anthropornis gunnari*, *Palaeudyptes gunnari*, *Palaeospheniscus patagonicus*; Meister 1962; Chinsamy et al. 1998; Jadwiszczak and Acosta Hospitaleche 2013; Cerdea et al. 2014; Ksepka et al. 2015). Osteosclerosis has not been reported in taxa retaining a well-developed medullary cavity, as *Marambiornis exilis*, *Delphinornis larseni* and *D. arctowskii* (Cerdea et al., 2014). Because globuli ossei are present in *D. arctowskii*, a taxon with well-developed medulla, their presence do not appear to be correlated with this later anatomical feature.

The growth dynamics of the long bones could be other possible explanation for the presence of calcified cartilage with globuli ossei. Quilhac et al. (2014) indicated that the local occurrence of globuli ossei in the long bones of post-metamorphic urodeles is correlated with a low rate of longitudinal growth. Following this hypothesis, the presence of globuli ossei in the tarsometatarsus of *Delphinornis arctowskii* and the absence in other bones, could be explained by relatively low rate of growth in comparison with other bone elements. However, this hypothesis does not explain the absence of globuli ossei in the tarsometatarsi of taxa sampled by Cerdea et al (2014). Variation in body size that could be invoked to explain such absence. In this sense, the small body size of *Delphinornis arctowskii* (11.5 kg, Jadwiszczak, 2001) implies a relatively slow growth rate in comparison with larger penguins. Nevertheless, globuli ossei are also absent in the tarsometatarsus of the tiny *Marambiornis exilis* with a low body mass estimation (6.1 kg; Jadwiszczak, 2001).

Finally, globuli ossei have been occasionally associated to osteological pathologies. During imperfect osteogenesis of otic

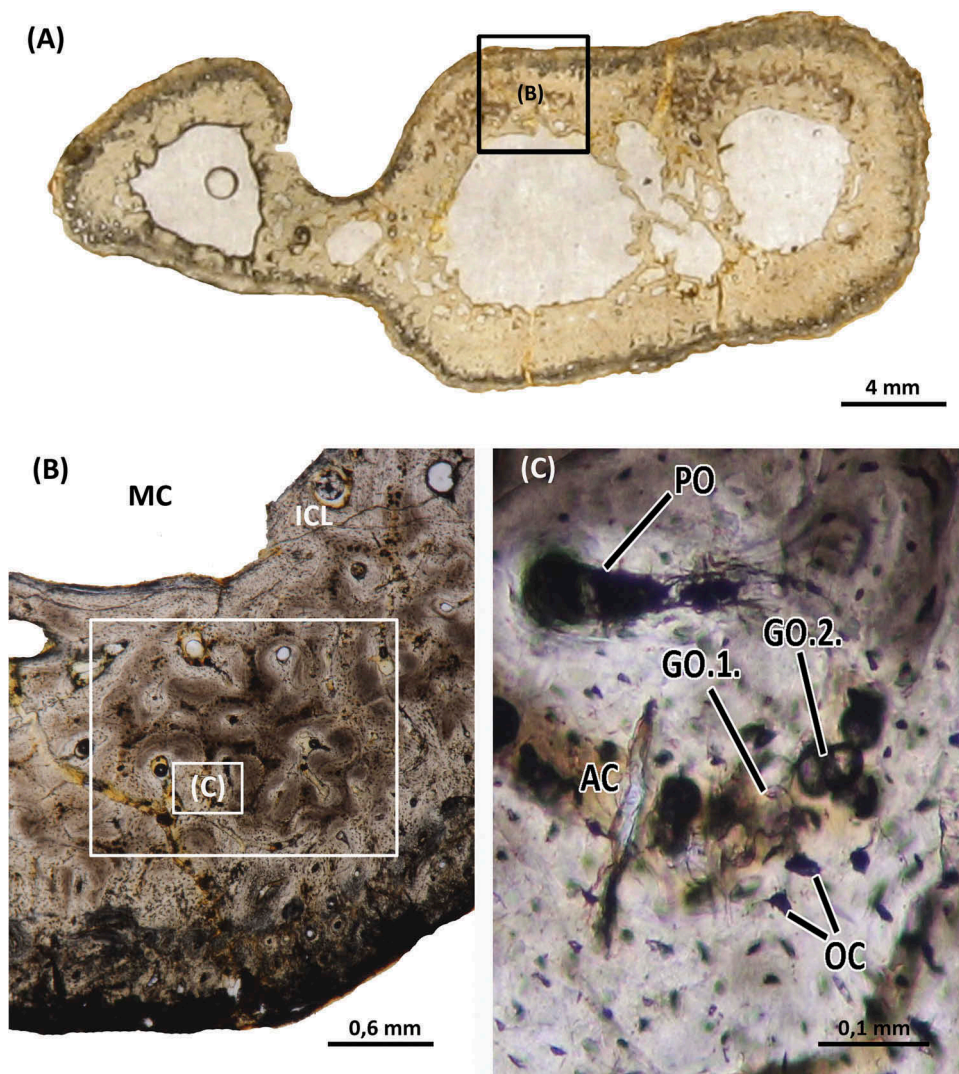


Figure 1. (A) View of the bone histology of tarsometatarsus cortex of *Delphinornis arctowskii* (MLP 93-X-1–92). (B) Detail of (A) remarking the inner circumferential layers (icl) surrounding the medullary cavity (mc), and with a big rectangle delimiting area where are present the *globuli ossei*, which are embedded in the fibro-lamellar matrix; and small rectangle delimiting the (C) detail image. (C) Detail of the *globuli ossei* well preserved (go.1) and *globuli ossei* altered by the calcite action (go.2), as well as other structures, the osteocyte lacunae (oc), the primary osteons (po) and ancient cartilage (ac) where are embedded the *globuli ossei* of *D. arctowskii* (MLP 93-X-1–92). Both images were taken under normal transmission light.

capsule, the poor formation of collagen leads to abnormally thin bony trabeculae (Milroy and Michaels, 1990). In the case of osteopetrosis, the otic capsule is greatly expanded by increasing of *globuli ossei*, as result of pathological osteoclast function (Milroy and Michaels, 1990). The relative scarcity and patchy distribution of *globuli ossei* in *Delphinornis arctowskii*, appears not to be associated with a pathological origin.

Conclusions

The presence of *globuli ossei* in tarsometatarsus of an Eocene penguin is documented for the first time. Although it is not possible to infer the significance for the presence of these structures in the tarsometatarsus of *D. arctowskii*, it appears to be clear that are not related with adaptation to aquatic life, small body size or ontogenetic stage. A pathological origin for these structures is also discarded. A detailed investigation on

the ossification process of the tarsometatarsus and other long bones in extant penguin taxa could provide insights on the nature of the *globuli ossei* in this group of diving birds.

The link between certain histological structures and the animal way of life must be taken with caution and discussed in a broader context.

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References

- Acosta Hospitaleche CA, Reguero M, Santillana S. 2017. *Aprosdokitos mikrotero* gen. et sp. nov., the tiniest Sphenisciformes that lived in Antarctica during the Paleogene. *Neu Jahr Geol Paläo- Abh.* 283:25–34.
- Bailleul A, Ségalen L, Buscalioni AD, Cambra-Moo O, Cubo J. 2011. Palaeohistology and preservation of tetrapods from Las Hoyas (lower cretaceous, Spain). *Comp Ren Palevol.* 10:367–380.
- Baumel JJ, Witmer LM. 1993. Osteologia. In: Baumel JJ, King AS, Breazile J, Evans HE, Vanden Berge JC, editors. *Handbook of Avian Anatomy: Nomina Anatomica Avium*. 2nd ed. Cambridge: Publications of Nuttall Ornithological Club; p. 779.
- Brandt A. 1852. *Disquisitiones des ossificationis processu*. Inaugural Dissertation, Tartu (Estonia); p. 353.
- Buffrénil V, Canoville A, D'Anastasio R, Domning DP. 2010. Evolution of Sirenian Pachyosteosclerosis, a model-case for the Study of Bone Structure in Aquatic Tetrapods. *J Mammal Evol.* 17:101–120.
- Buffrénil V, Canoville A, Evans SE, Laurin M. 2014. Histological study of karaurids, the oldest known (stem) urodeles. *Hist Biol.* 27:109–114.
- Buffrénil V, De Astibia H, Pereda Suberbiola X, Berreteaga A, Bardet N. 2008. Variation in bone histology of middle Eocene sirenians from western Europe. *Geodiversitas.* 30:425–432.
- Buffrénil V, De Ricqlès A, De Ray CE, Domning DP. 1990. Bone histology of the ribs of the archaeocetes (Mammalia: cetacea). *J Vert Paleo.* 10:455–466.
- Cerda IA, Tambussi CP, Degrange FJ. 2014. Unexpected microanatomical variation among Eocene Antarctic stem penguins (Aves: sphenisciformes). *Hist Biol.* 27:549–557.
- Cervantes-Diaz F, Contreras P, Marcellini S. 2016. Evolutionary origin of endochondral ossification: the transdifferentiation hypothesis. *Dev Genes Evol.* 227:121–127.
- Chinsamy A, Martin LD, Dodson P. 1998. Bone microstructure of the diving *Hesperornis* and the volant *Ichthyornis* from the Niobrara Chalk of western Kansas. *Cret Res.* 19:225–235.
- Chinsamy A, Raath MA. 1992. Preparation of fossil bone for histological examination. *Palaeo Afr.* 29:39–44.
- De Ricqlès A. 1979. Quelques remarques sur l'histoire évolutive des tissus squelettiques chez les vertèbres et plus particulièrement chez les tétrapodes. *Ann Biol.* 18:1–35.
- De Ricqlès A, De Buffrénil V. 2001. Bone histology, heterochronies and the return of the tetrapods to life in water. where are we? In: Mazin J, De Buffrénil V, editors. *Secondary adaptation of tetrapods to life in water*. Friedrich Pfeil. Munchen: Verlag Dr; p. 367.
- Francillon-Vieillot H, de Buffrénil V, Castanet J, Géraudie J, Meunier F, Sire JY, de Ricqlès A. 1990. Microstructure and mineralisation of vertebrate skeletal tissue. In : Carter JG editor. *Skeletal biomineralizations: patterns, processes and evolutionary trends*. New York: Van Nostrand Reinhold; p. 471–530.
- Gussen R. 1968. The labyrinthine capsule: normal structure and pathogenesis of otosclerosis. *Acta Oto.* 235:5–55.
- Haines RW. 1938. The primitive form of epiphysis in the long bone of tetrapods. *J Anat.* 72:323–343.
- Haines RW. 1942. The evolution of epiphyses and of endochondral bone. *Biol Rev.* 17:267–292.
- Houssaye A. 2009. “Pachyostosis” in aquatic amniotes: a review. *Integ Zool.* 4:325–340.
- Jadwiszczak P. 2001. Body size of Eocene Antarctic penguins. *Pol Polar Res.* 22:147–158.
- Jadwiszczak P. 2006. Eocene penguins of Seymour Island, Antarctica: taxonomy. *Pol Polar Res.* 27:3–62.
- Jadwiszczak P, Acosta Hospitaleche C. 2013. Distinguishing between two Antarctic species of Eocene *Palaeudyptes* penguins: a statistical approach using tarsometatarsi. *Pol Polar Res.* 34:237–252.
- Jadwiszczak P, Mörs T. 2011. Aspects of diversity in early Antarctic penguins. *Acta Pal Pol.* 56:269–277.
- Ksepka DT. 2008. *Phylogeny, histology and functional morphology of fossil penguins (Aves: sphenisciformes)* [dissertation]. USA: Columbia University.
- Ksepka DT, Werning S, Sclafani M, Boles ZM. 2015. Bone histology in extant and fossil penguins (Aves: sphenisciformes). *J Anat.* 227:611–630.
- Marenssi SA, Santillana SN, Rinaldi CA. 1998. Stratigraphy of the La Meseta Formation (Eocene), Marambio (Seymour) Island, Antarctica. In: Casadio S, editor. *Paleógeno de América del Sur y de la Península Antártica*. Publicación Especial 5. Argentina: Asociación Paleontológica; p. 137–146.
- Meister W. 1962. Histological structure of the long bone of penguins. *Anat Rec.* 143:377–386.
- Milroy CM, Michaels L. 1990. Pathology of the otic capsule. *J Laryngol Otol.* 104:83–90.
- Myrcha A, Jadwiszczak P, Tambussi CP, Noriega JI, Gazdzicki A, Tatur A, Del Valle R. 2002. Taxonomic revision of Eocene Antarctic penguins based on tarsometatarsal morphology. *Pol Polar Res.* 23:5–46.
- Quilhac A, De Ricqlès A, Lamrous H, Zylberberg L. 2014. Globuli ossei in the long bones of *Pleurodeles waltl* (Amphibia, Urodela, Salamandridae). *J Morph.* 275:1226–1237.
- Reguero MA, Goin FJ, Acosta Hospitaleche C, Marenssi S, Dutra T. 2013. Late cretaceous/paleogene west Antarctica terrestrial biota and its intercontinental affinities. *Dordrecht: Springer Brief in Earth System Sciences*; p. 120.
- Sanchez S, De Ricqlès A, Schoch RR, Steyer JS. 2010. Developmental plasticity of limb bone microstructural organization in *Apateon*: histological evidence of paedomorphic conditions in branchiosaurs. *Evol Devel.* 12:315–328.
- Tambussi CP, Acosta Hospitaleche CI, Reguero MA, Marenssi SA. 2006. Late Eocene penguins from West Antarctica: systematics and biostratigraphy. In: Francis JE, Pirrie D, Crame JA, editors. *Cretaceous–tertiary high-latitude palaeoenvironments, James Ross Basin, Antarctica, special publications 258*. London: Geological Society; p. 145–161.