Variation in bone histology of middle Eocene sirenians from western Europe

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ABSTRACT

The histological characteristics of the ribs of several sirenians from the middle Eocene of the Pyrenees (the dugongid *Prototherium montserratense* from Catalonia and two undetermined dugongid species from Navarre) reveal that the histogenetical mechanisms causing bone pachyosteosclerosis in the extant Sirenia, i.e. hyperplasy of periosteal and endosteal deposits, and inhibition of the chondroclastic and osteoclastic activities involved in bone remodelling, were already present by the middle of the Eocene, and have not greatly changed since then. However, in one of the two undetermined specimens examined, bone remodelling in the cortical and medullar regions remained fairly active, a characteristic that is reminiscent of the condition commonly encountered in mammals. These observations suggest that the mechanisms involved in the peculiar form of pachyosteosclerosis displayed by the sirenians did not act with the same intensity, and with identical results, in all Eocene forms.

KEY WORDS
Mammalia,
Sirenia,
Prototherium,
palaeohistology,
pachyosteosclerosis,
middle Eocene,
Pyrenees.

RÉSUMÉ

Variation de l'histologie osseuse chez les siréniens de l'Éocène moyen d'Europe occidentale.

Les caractéristiques histologiques des côtes de plusieurs siréniens de l'Éocène moyen des Pyrénées (le dugongidé *Prototherium montserratense* de Catalogne et deux espèces indéterminées de dugongidés de Navarre) montrent que les mécanismes histogéniques qui provoquent la pachyostéosclérose des siréniens actuels : l'hyperplasie des dépôts périostiques et endostéaux, ainsi que l'inhibition des activités chondroclasiques et ostéoclasiques impliquées dans le remaniement osseux, étaient déjà présents à l'Éocène moyen et n'ont pas notablement varié depuis. Cependant, chez l'un des deux spécimens indéterminés, le remaniement des territoires corticaux et médullaires demeurait relativement actif, caractéristique qui rappelle l'état communément rencontré chez les mammifères. Ces observations suggèrent que les mécanismes intervenant dans la forme particulière de pachyostéosclérose que présentent les siréniens ne s'exprimaient pas avec la même intensité, et ne produisaient pas des résultats identiques, chez toutes les formes de l'Éocène.

INTRODUCTION

One of the most common features of the tetrapods secondarily adapted to life in water is a more or less spectacular modification of the histological characteristics of their skeletons, with a concordant influence on bone volume and density (synthesis in Ricqlès & Buffrénil 2001). Depending on the general adaptations of the animals, this process leads during evolution either to an osteoporotic-like state, or to pachyostotic and/or osteoclerotic conditions (these terms are used here without pathologic connotation). 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 actual evolutionary time.

The cephalic and thoracic regions of the Sirenia display a heavy pachyosteosclerotic condition, in which two distinct osteogenic mechanisms are involved: a sub-periostic hyperostosis (cortical hyperplasy, or pachyostosis *sensu stricto*), associated with a hyperplasy of endosteal deposits and an inhibition of chondroclastic and osteoclastic activities (osteosclerosis; cf. Fawcett 1942; Buffrénil & Schoevaert 1989; Ricqlès & Buffrénil 2001; D'Anastasio 2004). This highly derived feature has been known for

a long time in extant and extinct forms (Nopcsa 1923) and represents a typical autapomorphy of the Sirenia order (Savage 1976; see also Domning 2001). However, the main stages of the evolution of this complex characteristic during sirenian evolution remain poorly documented. From comparative, evolutionary, and ontogenetic points of view, the question can be complex, as exemplified, on one hand, by the Cetacea, which first displayed pachyostosis and osteosclerosis, at least in their ribs, before acquiring the reverse adaptation that is an extremely light, osteoporotic-like skeleton (Buffrénil *et al.* 1990; Robineau & Buffrénil 1993), or, on the other hand, by plesiosaurs, which seem to have possessed a pachyostotic skeleton when juvenile, and osteoporotic-like bones when adult (Wiffen *et al.* 1995). In extant dugongids and trichechids, the morphological and histological appearance of pachyosteosclerosis, as well as the basic mechanisms from which this peculiarity arises, seem to be uniform among taxa (Buffrénil & Schoevaert 1989). However, among earlier forms, and especially in the rich sirenian radiation of the Eocene, differences in the achievement of this osseous specialisation could have existed between taxa, and there is no evidence that pachyosteosclerosis, a feature extremely prone to homoplasy, evolved in strict parallelism

and synchrony in all clades. The present note gives comparative elements relevant to these questions in some sirenian specimens from the middle Eocene of western Europe.

MATERIAL AND METHODS

The palaeontological sample consists of fossil sirenian ribs from the middle Eocene of the South Pyrenean Realm: the dugongid *Prototherium montserratense* Pilleri, 1989 and two unidentified species provisionally referred to Dugongidae indet. (Astibia *et al.* 2006).

The fragmentary rib of *P. montserratense* (reference: MGSB 44892 in the collection of the Museu Geològic del Seminari, Barcelona) was collected in the Bartonian beds of Castellvelli El Vilar, Catalonia (eastern Pyrenees) (Pilleri *et al.* 1989). The fragments of this specimen used for histology come from the middle of the shaft, and from the distal extremity (the last five centimetres) of the rib.

The unidentified fossil ribs were collected in two middle Eocene outcrops, called Uztarrotz (UZ) and Ardanatz (AR), located near the city of Pamplona/Iruñea, Navarre (western Pyrenees). This material was recently described by Astibia *et al.* (2006). The outcrops correspond to different lower Bartonian lithostratigraphic units of the Pamplona Basin: the lower part of the Pamplona Marl Formation (Uztarrotz site) and the upper part of the Ardanatz Sandstone (Ardanatz site). The former represents a deep and low-energy sea floor far away from a deltaic slope; the Ardanatz environment probably corresponds to a semi-closed deltaic bay periodically affected by catastrophic floods (see Astibia *et al.* 2005).

In addition to the rib fragments (UZ1.29-31), the Uztarrotz outcrop has mostly yielded neural arches of thoracic vertebrae. The fossils were found in anatomical relation, so they presumably come from the same individual. At least some neural arches from Uztarrotz exhibit sutural surfaces; so this material could be from an immature individual. The Ardanatz fossils consist of ribs (AR1.32-34), as well as fragmentary dorsal, sacral and caudal vertebrae. Most of the remains were collected together and probably belong to a single individual. Based
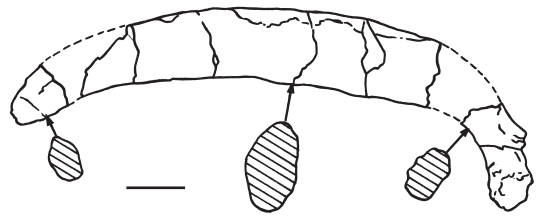

FIG. 1. — Reconstruction of a sirenian rib from the middle Eocene of Uztarrotz (Navarre, western Pyrenees), based on specimens UZ1-21, 22 and 31, showing the outlines in cross section where the histological sections were made. Scale bar: 25 mm.

on the fusion of the centra to the neural arches, the Ardanatz vertebrae belong to a mature individual. All the Navarrese sirenian remains are provisionally kept in the Departamento de Estratigrafía y Paleontología of the Universidad del País Vasco (UPV/EHU) at Bilbao.

The superficial erosion and the evidence of epibiontic activity suggest that in both specimens the bones were exposed on the sea floor for a while prior to burial (biostratinomic processes). The palaeohistological structures are well preserved except in the peripheral region, where there is an altered layer filled by pyrite and oxides that may correspond to microbial bioturbation.

Sirenian fossil remains found in the middle and upper Eocene of western Europe have been referred to species of the dugongid genera *Protosiren* Abel, 1907, *Prototherium* De Zigno, 1887, *Eotheroides*, Palmer, 1899 and *Halitherium*, Kaup, 1838 (Bizzotto 1983; Pilleri *et al.* 1989; Sagne 2001). Nevertheless, some of these genera are currently regarded as non-monophyletic (Domning 1994; Sagne 2001). Morphologically, the ribs and vertebrae from Navarre are comparable to those described from the localities of the eastern Pyrenees of Catalonia (Pilleri *et al.* 1989). Pending the discovery of diagnostic skull remains and teeth, these remains are provisionally referred to as Dugongidae indet. (see Astibia *et al.* 2006 for more details).

Methods of thin-sectioning have followed standard procedures. The ribs of specimens AR1 and UZ1 were sectioned transversely at the mid-length, and at the proximal and distal parts of the bones (Fig. 1). For the specimen of *P. montserratense*, samples were

from the mid-diaphyseal region only. The thin sections were ground to a thickness of 100 to 150 µm. They were studied at low and high magnifications using a petrographic microscope under normal and polarized light. The histological terminology and the typology of bone tissues used in this study refer to Francillon-Vieillot *et al.* (1990).

DESCRIPTION AND COMPARISONS

In cross section at mid-diaphysis, all the ribs display the same general structure: they are oval and massive, with a particularly thick periosteal cortex and a compact medullar region nearly devoid of any cavity. Conversely, the bone texture in the proximal and distal regions of the ribs differs among the three specimens: these regions are as compact as the rest of the rib in *P. montserratense* and in specimen AR1, whereas they are much more cancellous (though relatively dense in texture) in specimen UZ1.

Histologically, the medullar region in all specimens has a composite structure (Fig. 2A). It includes, on one hand, thick perivascular layers of endosteal lamellar bone showing evidence of moderate remodelling activity and, on the other hand, small amounts of calcified cartilage matrix, appearing as an acellular, vitreous material interspersed between the osseous deposits (Fig. 2B). The endosteal deposits display numerous *globuli ossei*, in the form of rounded burgeoning excrescences protruding into the cartilage matrix (Fig. 2B). Such structures (described in various aquatic tetrapods) correspond to the infilling of the chondrocyte lacunae by bone deposits. Cartilage remnants are very discrete in specimen UZ1.

The deep regions of the periosteal cortices are occupied by a thick layer of fibro-lamellar tissue (association of primary osteons with the so-called woven-fibered, or fibrous, periosteal bone tissue), housing a vascular network that displays a reticular to plexiform spatial organisation (Fig. 2C). The presence of well-defined cyclic growth marks (annuli) in this tissue is remarkable (Fig. 2C; see also Fig. 2D). Haversian remodelling can be intense in deep cortical regions, especially in the specimen from Uztarrotz. Towards the cortical periphery, the vascular

network of bone becomes less dense, the intensity of Haversian remodelling is reduced, and the histological characteristics of the primary periosteal tissue forming the cortex turn to a parallel-fibered type (mass birefringence, spindle-like osteocyte lacunae parallel to each other) with sharply defined annuli and lines of arrested growth (Fig. 2D). Specimen AR1 (Ardanatz) shows at least 10 to 12 main (annual?) growth cycles, which would confirm that this specimen was not a juvenile.

In relation to the curvature of the ribs, there is an important dissymmetry in cortical thickness and growth mark spacing between the mesial and lateral sides of these bones (growth off-centring). In specimen AR1, both sides display a periosteal cortex, but the lateral one is thicker and has more widely spaced growth marks, two features that indicate a faster lateral accretion. *Prototherium* rib also displays periosteal cortices on both sides however, in addition to being thinner; the mesial cortex shows evidence of superficial resorption that eroded away the outermost (non-remodelled) part of the cortex. Resorption was still more pronounced in specimen UZ1, and reached the medullary territory that finally outcrops on the shaft surface.

The main difference between our specimens is relative to the intensity of bone remodelling in the cortical and medullar regions. Remodelling was clearly more intense in the Uztarrotz specimen than in the other two fossils, a process that resulted in a more extensive erosion of the remnants of calcified cartilage matrix (they were replaced by secondary osteons; Fig. 2E). As a consequence, the characteristics of the primary, periosteal bone tissue in this specimen, especially the cyclic growth marks, are hardly discernible and a large part of sectional area is occupied by dense Haversian tissue. In specimen AR1, the bone histological features in the proximal and distal extremities of the rib (close to growth plates) do not differ markedly from those observed in the central part of the shaft. Conversely, in specimen UZ1, the medullar region in the bone extremities is not occupied by compact tissue, but rather by a dense spongiosa, with intensively remodelled trabeculae and a nearly complete absence of residual calcified cartilage matrix (Fig. 2F).
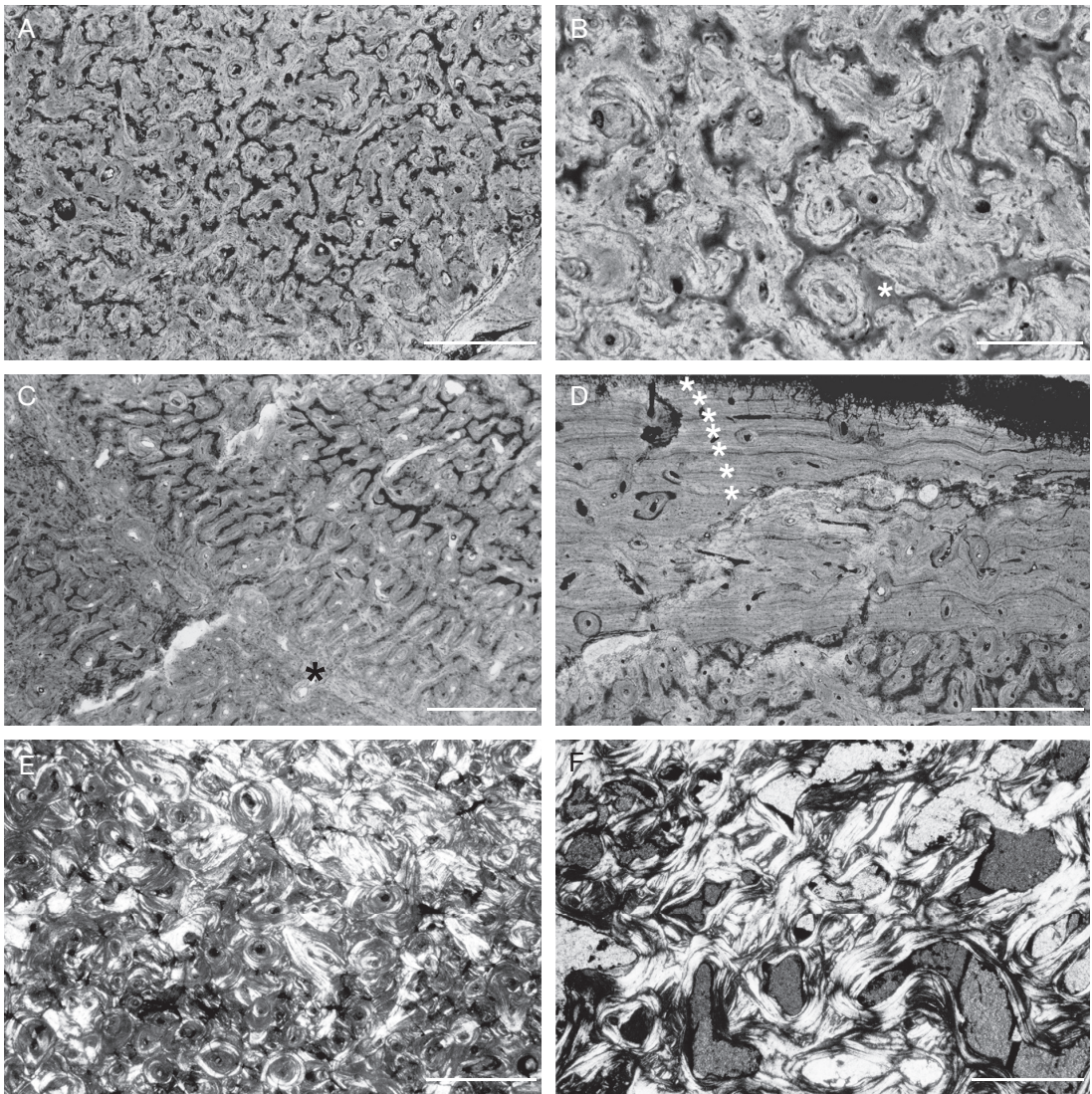
Fɪɢ. 2. — Histological characteristics of the ribs: **A-D**, specimen AR1-33 from Ardanatz (Navarre); **A**, general view of the medullar region at mid-diaphysis. The medulla is entirely compact and includes deposits of endosteal bone tissue (light areas), with remnants of calcified cartilage matrix interspersed between them (dark areas); **B**, endosteal deposits, *globuli ossei* and remnants of calcified cartilage matrix (*) in the diaphyseal medulla; **C**, deep region of the periosteal cortex at mid-diaphysis. The cortex is composed of variably oriented primary osteons (light areas), surrounded by "woven-fibered" periosteal tissue (dark areas). The asterisk indicates one annulus; **D**, peripheral region of the cortex. The asterisks point to several conspicuous cyclic growth marks, represented here by lines of arrested growth. Haversian remodelling is feeble; **E**, medullar remodelling at mid-diaphysis in the rib of specimen UZ1-30 from Uztarrotz (Navarre). The spatial density of the secondary osteons produced by Haversian remodelling is clearly more elevated than in specimen AR1. Polarized light; **F**, Remodelled medullar spongiosa in the proximal extremity of the rib of specimen UZ1-29 from Uztarrotz. The osseous trabeculae are extensively remodelled, and the residues of calcified cartilage are sparse, as compared to specimen AR1. Polarized light. Scale bars: A, C-F, 950 µm; B, 400 µm.

DISCUSSION AND CONCLUSION

Primary periosteal cortices in the three fossil ribs are histologically similar to those of *Halitherium* (Spillmann 1959), *Dugong* Lacépède, 1799 (Buffrénil & Schoevaert 1989), *Trichechus* Linnaeus, 1758 (Fawcett 1942), *Hydrodamalis* Retzius, 1794 (Ricqlès & Buffrénil 1995) and *Pezosiren* Domning, 2001 (D'Anastasio 2004). As suggested by the local succession of bone tissue types in the rib cortex, and by the number and spacing of the cyclical growth marks displayed by specimen AR1, the pachyostotic, or hyperplastic, development of the cortices ("banana-like" bones; cf. Fawcett 1942; Kaiser 1966), was due to the combination of the fast initial deposition of fibro-lamellar tissue followed by a slower, but protracted, accretion of pseudo-lamellar bone (see e.g., Margerie *et al.* 2002 for the dynamics of bone accretion). This mechanism does not differ from that observed in the skeleton of *Dugong dugon* Müller, 1776 (Buffrénil & Schoevaert 1989). Although cyclic growth marks are infrequent in the bones of mammals (and other homeotherms), they are nevertheless encountered in various, phylogenetically unrelated, species (synthesis in Klevezal 1996; Castanet 2006; see also Sander & Andrassy 2006). In extant Sirenia, conspicuous growth marks exist in all taxa and most skeletal parts, a feature due to pronounced growth cycles (Marsh 1979, 1980) strongly influenced by environmental conditions (Best 1983). The results of the present study point out the persistence of this eco-physiological peculiarity through the evolutionary history of the Sirenia: it was already settled by the middle Eocene, at least, and remained unchanged in subsequent stages of sirenian evolution.

Prototherium montserratense and specimen AR1 share another feature with extant sirenians: the transformation of the medullary spongiosa formed by endochondral ossification during growth in length of the bones into a very compact tissue. This process, described in detail in *D. dugon* (Buffrénil & Schoevaert 1989), results from a nearly total filling of intertrabecular spaces by endosteal, perivascular deposits of lamellar bone. This is a typical osteosclerotic mechanism observed in numerous aquatic tetrapods belonging to phylogenetically distant taxa (Nopcsa 1923; Ricqlès & Buffrénil 2001).

However, in specimen UZ1, this process was less active, and the rib extremities regions retained a relatively cancellous (though dense) texture closer to the generalized condition of long bones.

One of the most obvious peculiarities of the endochondral bones of extant sirenians, that is the persistence of calcified cartilage remnants up to late ontogenetic stages (Fawcett 1942), was fully achieved in *P. montserratense* and specimen AR1. The generalised condition in tetrapods is a complete and rapid erosion of the calcified cartilage by the so-called conjunctivovascular invasion front. Cartilage disappears in the metaphyses, where the medullary trabeculae become entirely composed of secondary, heavily remodelled endosteal lamellar deposits. In the Sirenia, the destruction of the cartilage is not complete. Residues of cartilage matrix persist in the core of the medullary trabeculae, and are progressively relocated towards the diaphyseal regions during growth in length of the bones. The ontogenetic trajectory of endochondral ossification is thus shortened, resulting in a neotenous condition of bone structure (Ricqlès & Buffrénil 2001). In this feature, specimen UZ1 differs from *P. montserratense* and specimen AR1: the remnants of calcified cartilage were much less abundant in this taxon, which suggests that the ontogenetic trajectory of endochondral ossification was less modified.

The low intensity of Haversian remodelling in sirenians is believed to reflect a local inhibition of osteoclastic activities (Buffrénil & Schoevaert 1989). In this respect, specimen UZ1, the bones of which show evidence of relatively intense remodelling, again appears closer to the generalized mammalian condition than *P. montserratense* and specimen AR1. Since bone remodelling is a long-lasting process with cumulative effects on bone structure, a possible explanation of this difference could be that the specimen exhibiting the most intense remodelling was ontogenetically the oldest one. However, this explanation must be rejected because it is inconsistent with the estimations of individual ages based on the morphological features of each specimen: UZ1 must have been an immature individual and AR1 an adult (see above).

The results of this study finally suggest that *P. montserratense* and specimen AR1 are "histologically

compatible" with each other; whereas specimen UZ1 has proved to be fairly different. The latter could possibly represent a distinct lineage differing from the other two by the intensity of chondroclastic and osteoclastic activities, and closer to the plesiomorphic condition of mammals in this respect. On a broader scale, the structure of bone in sirenians seems to be consistent with some physiological peculiarities of these animals: a low metabolic rate and a depression of thyroid and parathyroid functions due to atrophy and morphological abnormalities of these glands (Sickenberg 1931; Scholander & Irving 1941; Cave & Aumonier 1967; Marsh *et al.* 1978; Irvine 1983; see also Gallivan *et al.* 1983). The results of this study suggest that this ecophysiological specialisation could have been achieved to different degrees in Eocene taxa.

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