

# Evolution of Sirenian Pachyosteosclerosis, a Model-case for the Study of Bone Structure in Aquatic Tetrapods

Vivian de Buffrénil · Aurore Canoville ·  
Ruggero D'Anastasio · Daryl P. Domning

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**Abstract** Osteosclerosis, or inner bone compaction, and pachyostosis, or outer hyperplasia of bone cortices (swollen bones), are typical features of tetrapods secondarily adapted to life in water. These peculiarities are spectacularly exemplified by the ribs of extant and extinct Sirenia. Sea cows are thus the best model for studying this kind of bone structural specializations. In order to document how these features differentiated during sirenian evolution, the ribs of 15 species, from the most basal form (*Pezosiren portelli*) up to extant taxa, were studied, and compared to those of other mammalian species from both morphometric and histological points of view. Pachyostosis was the first of these two specializations to occur, by the middle of the Eocene, and is a basal feature of the Sirenia. However, it subsequently regressed in some taxa that do not exhibit hyperplastic rib cortices. Osteosclerosis was only incipient in *P. portelli*. Its full development occurred later, by the end of the Eocene. These two structural specializations of bone are variably pronounced in extinct and extant sirenians, and relatively independent from each other, although frequently

associated. They are possibly due to similar heterochronic mechanisms bearing on the timing of osteoblast activity. These results are discussed with respect to the functional constraints of locomotion in water.

**Keywords** Sirenia · Bone · Histology · Osteosclerosis · Pachyostosis · Evolution

## Introduction

Since the pioneer works by Kiprijanoff (1881–1883), Nopcsa (1923), Sickenberg (1931), and Nopcsa and Heidsieck (1934), an abundant literature has confirmed that secondary adaptation of tetrapods to an aquatic life always induced modifications of the inner architecture and histological characteristics of bones. As compared to the basal condition of tetrapod skeletal elements, at least in the Amniota (tubular long bones, cancellous short bones, diploë-containing flat bones; see Francillon-Vieillot et al. 1990), this process led to variably pronounced conditions oriented either towards increase in bone compactness and volume, or towards lightening of bone structure (reviewed in Ricqlès and Buffrénil 2001). A general trend appears among marine tetrapods: in the beginning of their evolutionary radiation, when taxa are incipiently adapting to life in water and have not yet developed highly efficient propelling modes, their skeletons display a local or general increase in density and mass, resulting from higher compactness of bone inner architecture (osteosclerosis) and/or from hyperplasia of periosteal cortices (pachyostosis). All lineages went through this initial stage during their evolution (Ricqlès 1989). The evolution of the opposite condition, i.e., osteoporotic-like skeletal lightening, followed this first stage (Ricqlès and Buffrénil 2001; Gray et al. 2007; Madar 2007; see also Buffrénil et al. 1990a), and

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V. de Buffrénil (✉) · A. Canoville  
Département Histoire de la Terre, UMR 7207 (CR2P),  
Muséum National d'Histoire Naturelle,  
Bâtiment de Géologie CC 48, 57 rue Cuvier,  
Paris F-75005, France  
e-mail: vdebuff@mnhn.fr

R. D'Anastasio  
Faculty of Medicine (Section of Anthropology),  
State University of Chieti,  
Trento e Trieste,  
Chieti, Italy

D. P. Domning  
Department of Anatomy (Laboratory of Evolutionary Biology),  
Howard University,  
Washington, DC, USA

occurred only in the groups that acquired advanced capabilities for fast swimming in open seas (e.g., ichthyosaurs, cetaceans).

Although recent studies on bone histology in early Cetacea (Madar 2007; Gray et al. 2007) clearly illustrate the reversion processes that can affect skeletal inner structure during the history of a clade, studies on a large time scale are lacking. Information is indeed meager on whether the evolutionary process that transforms an initial pachyosteosclerotic condition into an osteoporotic-like one is a general trend, linked to fundamental neuroendocrine adaptations of the amniotes to the peculiar constraints of marine life (as suggested by the interpretations of some authors, e.g., Ricqlès 1989), or a facultative one mainly dependent on the ecological specializations finally acquired by the clades. Moreover, available data give only marginal information on the relationships that may exist between the outer, morphological modification of the bones, and that of their inner, histological organization. Significant advances on these questions require the study of relatively complete and well-dated phyletic series, including basal taxa that do not exhibit conspicuous anatomical adaptation to life in water, and much more specialized forms, highly designed for swimming and no longer able to move on land. Of course, such fossils are very rare. However, the series of fossils presently available for the order Sirenia is remarkably complete. It includes basal taxa in which there are few important morphological specializations unambiguously related to life in water (e.g., *Pezosiren portelli*, from the lower middle Eocene of Jamaica; Domning 2001a), taxa still partly dependent on land (e.g., the genus *Protosiren*, from the middle Eocene of Egypt and Pakistan; see Zalmout et al. 2003), and taxa highly transformed for marine life, including the five recent species of sea cows.

The present article gives morphometric data and describes bone histology in the ribs of extant and extinct sirenian species that span the diversity of this order. These species are compared to a sample of terrestrial, amphibious, or fully aquatic mammals. The aim of this study is to define quantitatively the specializations of bone structure in the Sirenia, assess the mechanisms from which they arise, and reconstruct the evolutionary history of such specializations.

## Material and methods

The biological sample (Tables 1 and 2) includes 15 extant or extinct sirenian species (Table 1, 38 specimens) representing 13 genera and the four families of this order, one desmostylian (*Paleoparadoxia* sp.), and 42 extant species (53 specimens) of other mammals (Table 2), representing ten orders of terrestrial, aquatic, or amphibious forms.

Within the sirenian sub-sample, the most basal form is the prorastomid *Pezosiren portelli*, from the base of the middle Eocene of Jamaica. In the description of the type specimen, Domning (2001a) estimates that this species had the same size as a pig (some 2.1 m long), a short neck, a relatively long, barrel-shaped body, and four well-developed legs (Fig. 1). Morphologically, neither its vertebrae nor its limbs are indicative of a special adaptation to life in water. Conversely, *Pezosiren* ribs are described as “swollen and dense”. A complete rib and a rib fragment (distal two thirds) of *P. portelli* were used in this study. Both are from the middle third of the rib series and, considering their sizes, they must have belonged to one (perhaps two) adult specimens. The next evolutionary grade is represented in the sample by *Protosiren fraasi*, from the early middle Eocene of Egypt. This genus (as shown by the more-specialized species *Protosiren smithae*) still had complete pelvic limbs, but the sacrum, pelvis, and other elements had begun to be reduced, and the sacroiliac articulation was less firm than that of *Pezosiren*. This is why *Protosiren* is supposed to represent a stage in aquatic adaptation between that of fully quadrupedal Prorastomidae, and that of totally aquatic Dugongidae and Trichechidae (Domning 2001b). Conversely, the slightly more recent European form, *Protherium monserrattense*, a primitive dugongid represented in the sample by one rib from an adult specimen from the Bartonian of Castellvell I Vilar, Catalonia (eastern Pyrenees, Spain; see Pilleri et al. 1989), is supposed to have reached a complete or nearly complete specialization for life in water. The Egyptian forms *Eotheroides aegyptiacum*, *Eosiren libyca*, and *Eosiren stromeri* (middle to late Eocene of Fayoum), are also considered to have been well adapted to life in water. The eight other sirenian species represented in the sample range in age from late Eocene (*Halitherium*) to present. They are characterized by variable specific sizes (up to 9 m for *Hydrodamalis gigas*), an exclusive swimming locomotion, and the lack of differentiated pelvic limbs. Figure 1 summarizes both the phylogenetic trends within the Sirenia, and the main evolutionary steps that conditioned their adaptation to life in water.

The comparative sample of extant placental mammals (Table 2) includes ribs from artiodactyls (11 species), proboscidians (1 species), perissodactyls (4 species), rodents (2 species), lagomorphs (1 species), insectivores (Eulipotyphla: 1 species), non-pinniped carnivores (10 species), pinnipeds (7 species), primates (1 species), and cetaceans (4 species). As far as possible, domestic species or zoo specimens were avoided (due to possible disuse osteoporosis). In all extant species, the ribs selected for this study were from the middle third of the rib series (e.g., ribs n° 8 to 12 for extant sirenian species).

Entire ribs were measured as indicated in Fig. 2. Gross morphometric data were aimed at quantifying the relative

**Table 1** List of sirenian and desmostylian specimens

Genus & species	Sample	Geol. age	Reference
<b>Sirenia</b>			
<b>Prorastomidae</b>			
<i>Pezosiren portelli</i> Domning, 2001a	Rib A (R10)	Early Mid. Eoc. JM	DPD - 911
<i>Pezosiren portelli</i> Domning, 2001a	Rib B (indet.)	Early Mid. Eoc. JM	DPD - 1067
<b>Protosirenidae</b>			
<i>Protosiren fraasi</i> Abel, 1907 (n=1)	Rib compl.	Middle Eoc. EG	MNHN-PAL/LBE 690
<b>Dugongidae</b>			
<b>Halitheriinae</b>			
<i>Eotheroides aegyptiacum</i> Owen, 1875 (n=2)	Ribs compl. + part	Middle Eoc. EG	SMNS 43989, 10576
<i>Eosiren stromeri</i> Sickenberg, 1934 (n=1)	Rib compl.	Upper Eoc. EG	Holotype SMNS no ref.
<i>Eosiren libyca</i> Andrews, 1902 (n=2)	Ribs compl. + part	Late Eoc. EG	SMNS 11246, SMNS 11242
<i>Prototherium montserratense</i> Pilleri, 1989 (n=1)	Rib part	Late Mid. Eoc. SP	MCSB 44892
<i>Halitherium schinzii</i> Kaup, 1838 (n=3)	Ribs compl. + part	Early Oligoc. Eur.	MNHN-PAL/BTA 8, BTA 13, Cat 6
<i>Metaxytherium krahuletzii</i> Depéret, 1895 (n=2)	Ribs compl. + part	Early Mioc. FR	MNHN-PAL/FS 2858, -AC 1921.10
<b>Hydrodamalinae</b>			
<i>Hydrodamalis gigas</i> Zimmerman, 1780 (n=1)	Ribs compl.	Recent (extinct)	MNHN-AC 1919.48
<b>Dugonginae</b>			
<i>Crenatosiren olseni</i> Reinhart, 1976 (n=1)	Rib 16 compl.	Late Oligoc. US	SC 90.104.1.114
<i>Nanosiren garciae</i> Domning, 2008 (n=1)	Rib compl.	Early Plioc. US	USNM 377502
<i>Dioplotherium</i> cf. <i>allisoni</i> (Kilmer, 1965) (n=1)	Rib part	Middle Mioc., US	USNM 521235
<i>Dugong dugon</i> Müller, 1776 (n=7)	Ribs 9-11 compl.	Recent, Red Sea	MNHN-AC <sup>a</sup> , and MNH
<b>Trichechidae</b>			
<i>Trichechus senegalensis</i> Link, 1795 (n=11)	Ribs 9-11 compl.	Recent, WA	MNHN-AC <sup>a</sup>
<i>Trichechus manatus</i> Linnaeus, 1758 (n=2)	Ribs compl.	Recent, Cent. Amer.	MNHN-AC 1893.384, 1939.123
<b>Desmostyilia</b>			
<i>Paleoparadoxia</i> sp. Tokunaga, 1939 (n=1)	Rib 9 left, cast	Mioc., JP	USNM 26375 (cast); NSM-PV 5601 (original)
<i>Paleoparadoxia</i> cf. <i>tabatai</i> Tokunaga, 1939 (n=1)	Rib part	Mioc. California	Univ. Calif. (Berkeley), no ref. number

*Geol. Age* geological age; *compl.* complete bone; *Mid.* Middle (followed by geological age); *Eoc.* Eocene; *Mioc.* Miocene; *Oligoc.* Oligocene; *Plioc.* Pliocene; *EG* Egypt; *Eur.* Europe; *FR* France; *JM* Jamaica; *US* United States of America; *WA* West Africa. For scientific institutions, the abbreviations are as follows: *DPD* personal collection of Daryl P. Domning (Washington, USA); *MCSB* Museo Catalan de Ciencias Biológicas (Barcelona, Spain); *MNH* Muséum d'Histoire Naturelle (Lyon, France); *MNHN-AC* Museum National d'Histoire Naturelle-Anatomie Comparée (Paris, France); *MNHN-PAL* Museum National d'Histoire Naturelle – Paléontologie (Paris, France); *NSM-PV* National Science Museum (Tokyo, Japan); *SC* South Carolina State Museum (Columbia, USA); *SMNS* Staatliches Museum für Naturkunde Stuttgart (Stuttgart, Germany); *Univ. Calif.* University of California in Berkeley (USA); *USNM* United States National Museum (Washington DC, USA).

<sup>a</sup> The catalog numbers of the 11 *Trichechus senegalensis* specimens are as follows: MNHN-AC (Paris, FR) 1994.148, 1895.393, 1898.163, 1908.93, 1909.357, 1912.488, 1913.103, 1924.354, 1928-250, 1945.231, 1974.69; and those of the 7 *Dugong dugon* are: MNHN-AC 1875.242, 1883.241, 1980.68, 1981.06, 1981.152, 1981.153; MNH (Lyon, FR) 50-002521.

development of rib periosteal cortices, and assessing, on a comparative basis, whether or not a species displays signs of pachyostosis *sensu stricto* (cortical hyperplasy). Two main measurements (in mm  $\pm$  0.1 or  $\pm$  1 mm, according to specific sizes) were taken in this purpose. A) Rib length, an index corresponding to the sum of rib chord + mean rib arrow. Mean rib arrow refers to the mean of the lengths of two vectors projected perpendicularly from the chord to, respectively, the inner and outer rib surfaces at maximum rib bend (see Fig. 2); this measurement was added to rib chord in order to take into account the curvature of rib shaft. B) Rib mean

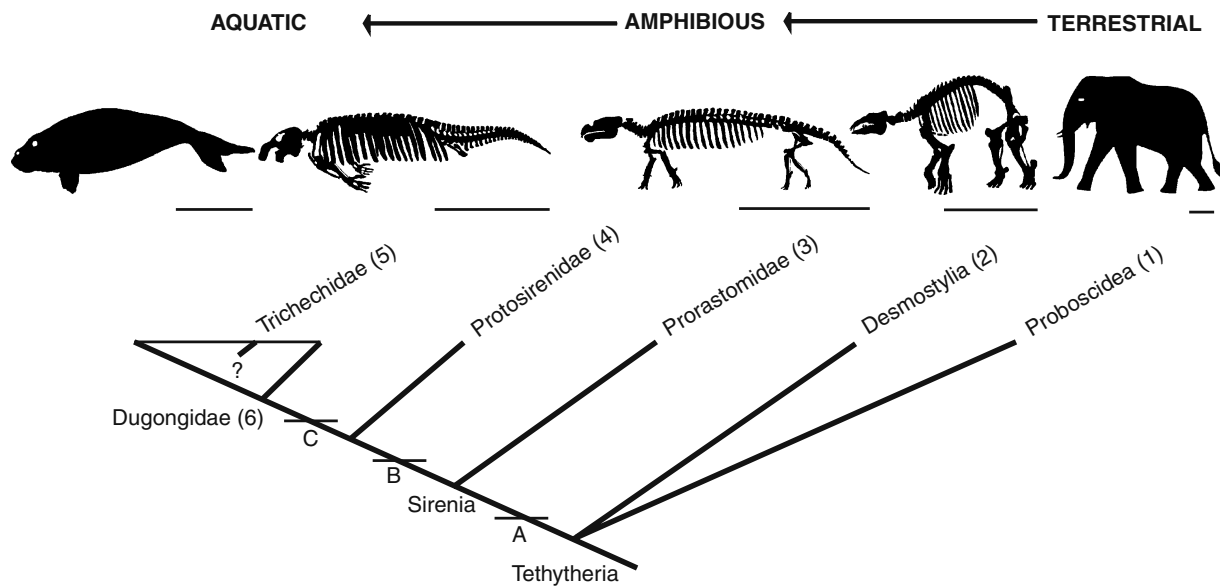
circumference, measured at the proximal, middle, and distal thirds of the bone. Cortical development, or *CD*, corresponds to the division of mean rib circumference by rib length.

In all sirenian and most non-sirenian species, the bone samples used for making ground sections were taken from the middle of the shaft of one rib (Fig. 2), a bone region represented in all our specimens, even fragmentary ones. Methods of processing these samples followed standard procedures, and thin sections 80–120  $\mu$ m thick were obtained. These were used for two purposes: basic histology and histomorphometry. For basic descriptive histology, the

**Table 2** Comparative sample of extant placental mammals used in our analysis and sources of biological material

Species	Reference
<b>Artiodactyla</b>	
<i>Cervus elaphus</i> Linnaeus, 1758 ( $n=1$ )	50002211 MNH (Lyon, FR)
<i>Rangifer tarandus</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Capreolus capreolus</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Aepyceros melampus</i> (Lichtenstein, 1812) ( $n=1$ )	p.c. VB
<i>Ovis aries</i> Linnaeus, 1758 ( $n=1$ )	p.c. VB
<i>Bison bison</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Hemitragus jayakari</i> Thomas, 1894 ( $n=1$ )	p.c. VB
<i>Sus scrofa</i> Linnaeus, 1758 ( $n=1$ )	p.c. VB
<i>Hippopotamus amphibius</i> Linnaeus, 1758 ( $n=1$ )	50002123 MNH (Lyon, FR)
<i>Giraffa camelopardalis</i> (Linnaeus, 1758) ( $n=1$ )	50002060 MNH (Lyon, FR)
<i>Camelus dromedarius</i> Linnaeus, 1758 ( $n=1$ )	50002063 MNH (Lyon, FR)
<b>Perissodactyla</b>	
<i>Equus caballus</i> Linnaeus, 1758 ( $n=1$ )	p.c. VB
<i>Equus asinus</i> Linnaeus, 1758 ( $n=1$ )	p.c. VB
<i>Rhinoceros sondaicus</i> Desmarest, 1822 ( $n=1$ )	50002041 MNH (Lyon, FR)
<i>Tapirus indicus</i> Desmarest, 1819 ( $n=1$ )	50002038 MNH (Lyon, FR)
<b>Proboscidea</b>	
<i>Elephas maximus</i> Linnaeus, 1758 ( $n=1$ )	50002671 MNH (Lyon, FR)
<b>Carnivora (Pinnipedia excluded)</b>	
<i>Ursus maritimus</i> Phipps, 1774 ( $n=1$ )	p.c. VB
<i>Canis lupus</i> Linnaeus, 1758 ( $n=1$ )	p.c. VB
<i>Vulpes vulpes</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Neovison vison</i> (Schreber, 1777) ( $n=1$ )	p.c. VB
<i>Enhydra lutris</i> (Linnaeus, 1758) ( $n=1$ )	50001023 MNH (Lyon, FR)
<i>Lutra lutra</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Martes martes</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Martes foina</i> (Erxleben, 1777) ( $n=1$ )	p.c. VB
<i>Felis s. silvestris</i> Schreber, 1777 ( $n=2$ )	p.c. VB
<i>Genetta genetta</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<b>Pinnipedia</b>	
<i>Arctocephalus forsteri</i> (Lesson, 1828) ( $n=2$ )	p.c. VB
<i>Otaria flavescens</i> (Shaw, 1800) ( $n=3$ )	p.c. VB
<i>Halichoerus grypus</i> (Fabricius, 1791) ( $n=1$ )	p.c. VB
<i>Mirounga leonina</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Monachus monachus</i> (Hermann, 1779) ( $n=2$ )	50001018 MNH (Lyon, FR), p.c. VB
<i>Phoca vitulina</i> Linnaeus, 1758 ( $n=2$ )	50001020 MNH (Lyon, FR), p.c. VB
<i>Odobenus rosmarus</i> (Linnaeus, 1758) ( $n=1$ )	50001014 MNH (Lyon, FR)
<b>Lagomorpha</b>	
<i>Lepus europaeus</i> Pallas, 1778 ( $n=1$ )	p.c. VB
<b>Rodentia</b>	
<i>Rattus norvegicus</i> (Berkenhout, 1769) ( $n=1$ )	p.c. VB
<i>Myocastor coypus</i> (Molina, 1782) ( $n=1$ )	p.c. VB
<b>Eulipotyphla</b>	
<i>Erinaceus europaeus</i> Linnaeus, 1758 ( $n=1$ )	p.c. VB
<b>Primates</b>	
<i>Gorilla gorilla</i> Savage & Wyman, 1847 ( $n=1$ )	50001762 MNH (Lyon, FR)
<b>Cetacea</b>	
<i>Delphinus delphis</i> Linnaeus, 1758 ( $n=6$ )	p.c. VB
<i>Orcinus orca</i> (Linnaeus, 1758) ( $n=1$ )	p.c. VB
<i>Monodon monoceros</i> Linnaeus, 1758 ( $n=1$ )	50001027 MNH (Lyon, FR)
<i>Phocoena phocoena</i> (Linnaeus, 1758) ( $n=1$ )	50001046 MNH (Lyon, FR)

MNH (Lyon, FR) Museum of Natural History of Lyon, France; p.c. VB private collection of V. de Buffr enil without specimen number.



**Fig. 1** Main morphofunctional stages in sirenian adaptation to life in water. *A* quadrupedal swimming (pelvic paddling). *B* reduced hind limbs (dorsoventral spinal undulations and bilateral thrusts of hind limbs). *C* vestigial pelvis (tail thrust only). 1: *Elephas maximus*; 2: *Paleoparadoxia* (strong hind limbs); 3: *Pezosiren portelli* (retracted nasal openings, well-developed hind limbs, strong sacroiliac articulation); 4: *Protosiren fraasi* (reduced hind limbs, weak sacroiliac

articulation); 5: *Trichechus manatus*, *Trichechus senegalensis* (vestigial pelvis); 6: *Eotheroides aegyptiacum*, *Eosiren stromeri*, *Eosiren libyca*, *Halitherium* cf. *schinzii*, *Metaxytherium krahuletzki*, *Dioplotherium* cf. *allisoni*, *Nanosiren garciae*, *Crenatosiren olseni* (vestigial or no hind limbs, reduced pelvis), *Dugong dugon*, *Hydrodamalis gigas* (lack of hind limbs, vestigial pelvis). Scale bar: 1 m.

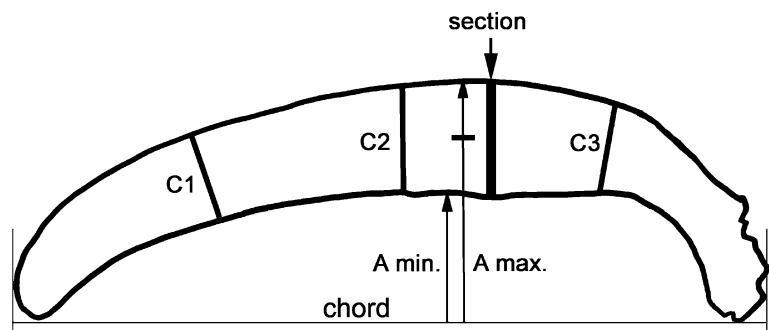
sections were observed at low and high magnifications using a petrographic microscope under normal and polarized light. Histomorphometric analyses were performed with the specialized image-processing software *Bone Profiler* (technical details in Girondot and Laurin 2003; Laurin et al. 2004; see also Figs. 3 and 4). In brief, *Bone Profiler* accurately quantifies (i) bone compactness and (ii) the tubular or non-tubular organization of the bones. For this purpose, this program measures the area of solid bone tissue in 60 radial sectors, each sector being subdivided into 51 unitary segments where measurements are made. Local measurements are then integrated for the whole sectional area, and the distribution of compactness in the section is expressed in the form of a sigmoid curve oriented in reference to section center and periphery (Fig. 3). Two main parameters given by *Bone Profiler* were considered. A)

Global bone compactness for the whole sectional area, or *BC*. This parameter is the ratio of solid bone tissue area to total sectional area. B) Compactness profile, or *CP*, that is the difference in compactness between the center (medullar region) and the periphery (peripheral cortex) of a bone section. The other parameters given by bone profiler (slope and inflexion point of the sigmoid) were of poor relevance for this study.

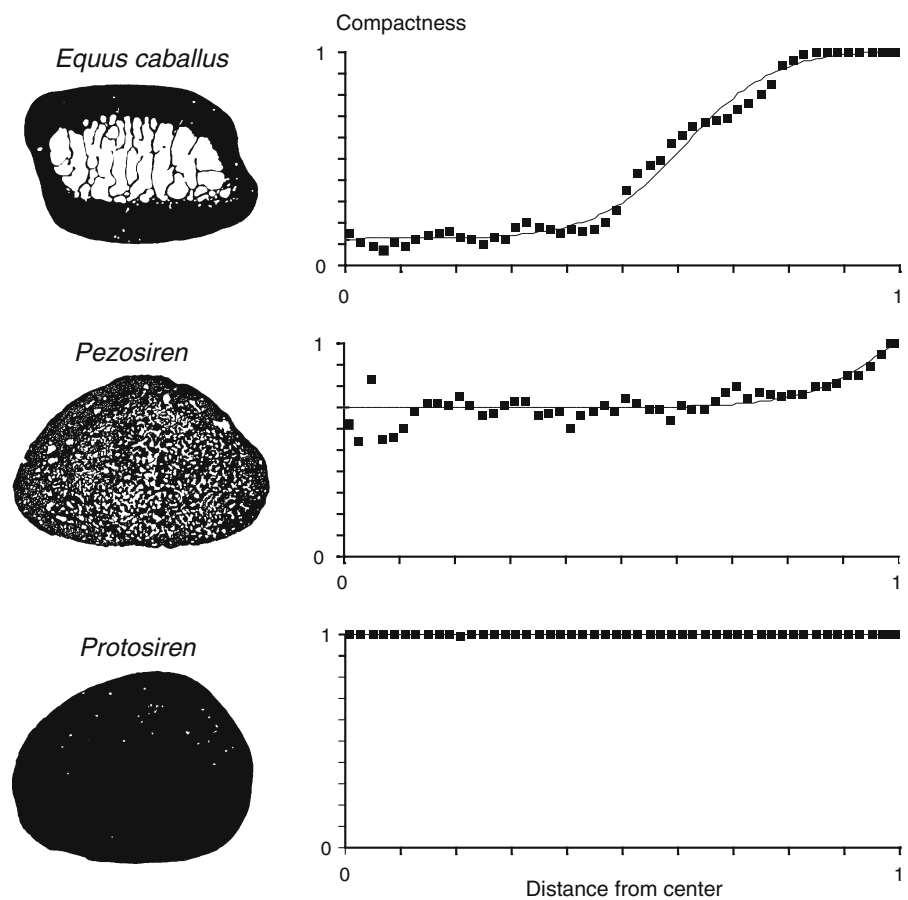
When remnants of calcified cartilage occurred in the medullar regions of the ribs, the relative area of this tissue was measured in four to six contiguous fields 2.5 x 2.5 mm, and averaged for each section, in order to have an index (*CC* index) quantifying chondroclastic activity.

Histological terminology and bone tissue typology used in this study refer to Francillon-Vieillot et al. (1990). All computations and statistical tests were performed with the

**Fig. 2** Basic measurements made on the ribs. *A* min.: small arrow at maximum bend; *A* max.: large arrow at maximum bend. The mean arrow (*black horizontal segment*) is:  $0.5(A \text{ min.} + A \text{ max.})$ . *C*1, *C*2, *C*3: circumferences at the proximal, middle, and distal thirds. Section: place where bone ground sections were made.



**Fig. 3** Bone Profiler analyses. The section sketches show the rough documents analyzed by Bone Profiler, and the resulting sigmoid curves of compactness profile. On top: *Equus caballus*; in central position: *Pezosiren portelli*; below: *Protosiren fraasi*. Bone sections are not to scale.



software PRISM (GraphPad TM). In statistical tests, null hypotheses were rejected for  $p < 0.05$ .

## Results

### Morphometric features of the ribs

Morphometric results are summarized in Table 3 and Fig. 5. In order to compare the Sirenia with other aquatic or non-aquatic tetrapods, the whole sample was subdivided when possible (based on size of data sets) into sub-samples corresponding to the gross qualitative degree of aquatic specialization of the taxa (taxonomic divisions are of lesser importance here).

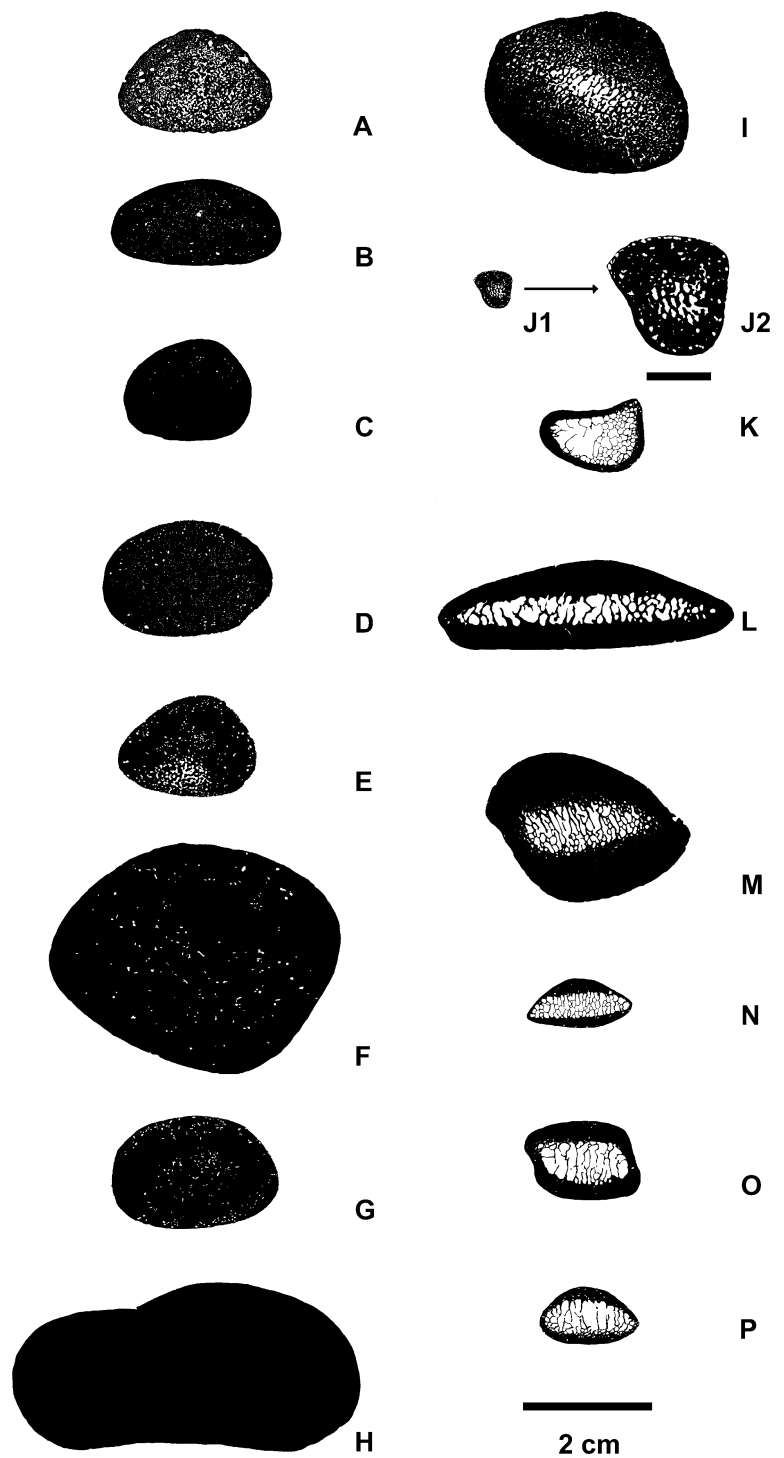
**Cortical Development (CD)** For this index, five sub-samples were distinguished: a) sirenian species; b) all non-sirenian species; c) terrestrial or occasionally amphibious species (i.e., whole sample less the Sirenia, pinnipeds, cetaceans, sea otter, and *Paleoparadoxia*); d) pinnipeds (i.e., Odobenidae, Otariidae, and Phocidae); e) cetaceans. Cortical development is significantly higher ( $p < 0.0001$ ) in the Sirenia than in all other groups (non-sirenian, terrestrial,

pinniped or cetacean species); however, the difference with the pinnipeds is somewhat less pronounced ( $p = 0.0051$ ) than with other groups.

The central tendencies for the terrestrial sub-sample are considered here as indicative of the unspecialized, or plesiomorphic, condition in the Eutheria. For a 95% confidence interval, *CD* in this group ranges from 0.0445 to 0.1769. Therefore, specialized pachyostotic conditions should correspond to  $CD > 0.1769$ . In this respect, *Protosiren fraasi* ( $CD = 0.167$ ) and *Dugong dugon* ( $CD = 0.1290$ ), as well as *Paleoparadoxia* ( $CD = 0.116$ ), cannot be considered as having hyperplastic, or pachyostotic, rib cortices. Moreover, bone pachyostosis in *Hydrodamalis gigas* ( $CD = 0.183$ ) is poorly pronounced. All other sirenian species display *CD* values between 0.200 and 0.300.

**Bone compactness (BC)** Three sub-samples (sirenians, non-sirenians, and terrestrial) were distinguished for this index, because the numbers of pinniped and cetacean species for which rib sections were available ( $n = 3$  for each) were too small for statistical analyses. Moreover, *BC* values for these two groups (mean = 0.550 for pinnipeds and 0.703 for cetaceans) are comparable to those of terrestrial forms. Conversely, mean bone compactness in sirenians (0.963) is

**Fig. 4** General view of bone compactness distribution in the sections. **A.** *Pezosiren portelli*; **B.** *Prototherium montserratense*; **C.** *Protosiren fraasi*; **D.** *Crenatosiren olseni*; **E.** *Nanosiren garciae*; **F.** *Halitherium schinzi*; **G.** *Dugong dugon*; **H.** *Trichechus senegalensis*; **I.** *Paleoparadoxia*; **J<sub>1</sub>** and **J<sub>2</sub>**. *Enhydra lutris* (bar=3.3 mm); **K.** *Ursus maritimus*; **L.** *Hippopotamus amphibius*; **M.** *Rhinoceros sondaicus*; **N.** *Sus scrofa*; **O.** *Equus caballus*; **P.** *Gorilla gorilla*.



very significantly higher than those of non-sirenian or terrestrial forms ( $p < 0.0001$ ).

In terrestrial taxa,  $BC$  ranges from 0.374 to 0.817 for a 95% confidence interval. This range is considered here to represent unspecialized, or plesiomorphic, values of bone compactness in mammals; therefore, only  $BC$  values above 0.817 should be indicative of a specialized osteosclerotic

condition of ribs. Nearly all sirenians display this condition with spectacular  $BC$  values ( $0.9600 < BC \leq 1$ ); however, *Pezosiren portelli* ( $BC = 0.755$ ) is an exception. The three pinniped species ( $BC = 0.449$  to  $0.655$ ) and the four cetaceans ( $BC = 0.658$  to  $0.748$ ) are well below this threshold, whereas *Paleoparadoxia* ( $BC = 0.850$ ), the Indonesian rhinoceros ( $BC = 0.857$ ), the sea otter ( $BC = 0.874$ ), and the Norway

**Table 3** Morphometric data

Taxon (specimen numbers)	L <sub>rib</sub> (mm) Min – Max	CD	BC	CP	CC (in %)
<b>Sirenia</b>					
<i>Pezosiren portelli</i>	325	0.226	0.755	0.749	15.40
<i>Protosiren fraasi</i>	339	0.167	0.998	0.026	0
<i>Prototherium montserratense</i>	–	–	0.981	0.113	19.93
<i>Eotheroides aegyptiacum</i>	277	0.284	0.959	0.286	7.09
<i>Eosiren stromeri</i>	430	0.283	–	–	–
<i>Eosiren libyca</i>	330	0.301	0.999	0.141	10.73
<i>Halitherium schinzii</i>	524	0.256	0.992	0.020	25.41
<i>Metaxytherium krahuletzii</i>	656	0.200	0.995	0.231	0
<i>Dioplotherium cf. allisoni</i>	–	–	0.963	0.207	0
<i>Nanosiren garciae</i>	323.5	0.229	0.949	0.496	–
<i>Crenatosiren olseni</i>	441.5	0.235	0.977	0.036	0
<i>Trichechus senegalensis</i>	209.5–546	0.224	0.991	0.000	0
<i>Trichechus manatus</i>	434–528	0.299	0.985	0.105	0
<i>Dugong dugon</i>	41.7–50.5	0.129	0.977	0.022	0 <sup>(1)</sup>
<i>Hydrodamalis gigas</i>	1184	0.183	–	–	0
<b>Desmostylia</b>					
<i>Paleoparadoxia</i> sp.	642.5	0.116	0.850	0.834	0
<b>Artiodactyla</b>					
<i>Cervus elaphus</i>	333–449	0.110	0.499	0.916	0
<i>Rangifer tarandus</i>	237	0.103	–	–	–
<i>Capreolus capreolus</i>	133	0.099	0.426	0.891	0
<i>Aepyceros melampus</i>	263	0.099	0.668	0.860	0
<i>Ovis aries</i>	276	0.105	0.553	0.817	0
<i>Bison bison</i>	687	0.097	–	–	–
<i>Hemitragus jayakari</i>	324	0.074	0.536	0.879	0
<i>Sus scrofa</i>	339	0.116	0.560	0.801	0
<i>Hippopotamus amphibius</i>	814	0.120	0.730	0.890	0
<i>Giraffa camelopardalis</i>	464.5	0.094	0.554	0.998	0
<i>Camelus dromedarius</i>	566	0.155	0.511	0.869	0
<b>Perissodactyla</b>					
<i>Equus caballus</i>	485–600	0.081	0.647	0.890	0
<i>Equus asinus</i>	526–549	0.085	–	–	–
<i>Rhinoceros sondaicus</i>	902	0.096	0.857	0.761	0
<i>Tapirus indicus</i>	524	0.095	0.563	0.803	0
<b>Proboscidea</b>					
<i>Elephas maximus</i>	963	0.101	0.461	0.761	0
<b>Carnivora (Pinnipedia excluded)</b>					
<i>Ursus maritimus</i>	426	0.104	0.525	0.893	0
<i>Canis lupus</i>	288.5	0.066	0.545	0.881	0
<i>Vulpes vulpes</i>	93.9–121	0.102	0.595	0.825	0
<i>Neovison vison</i>	43.6–46.2	0.149	0.667	0.790	0
<i>Enhydra lutris</i>	173	0.125	0.874	0.407	0
<i>Lutra vulgaris</i>	120	0.142	–	–	–
<i>Martes martes</i>	65.7	0.117	0.610	0.973	0
<i>Martes foina</i>	54.0	0.108	0.544	0.949	0
<i>Felis s. silvestris</i>	86.2	0.095	0.628	0.981	0
<i>Genetta genetta</i>	58.6	0.091	0.602	0.850	0



**Table 3** (continued)

Taxon (specimen numbers)	$L_{rib}$ (mm) Min – Max	CD	BC	CP	CC (in %)
<b>Pinnipedia</b>					
<i>Arctocephalus forsteri</i>	340–351	0.112	–	–	–
<i>Otaria flavescens</i>	319–376	0.136	–	–	–
<i>Halichoerus grypus</i>	248	0.173	–	–	–
<i>Mirounga leonina</i>	597	0.241	–	–	–
<i>Monachus monachus</i>	256	0.137	0.655	0.957	0
<i>Phoca vitulina</i>	248	0.145	0.545	0.900	0
<i>Odobenus rosmarus</i>	567	0.164	0.449	0.803	0
<b>Lagomorpha</b>					
<i>Lepus europaeus</i>	133.2	0.073	0.617	0.999	0
<b>Rodentia</b>					
<i>Rattus norvegicus</i>	33.5	0.089	0.913	0.997	0
<i>Myocastor coypus</i>	82.8–87.4	0.117	0.666	0.902	0
<b>Eulipotyphla</b>					
<i>Erinaceus europeus</i>	50.0	0.122	0.516	0.949	0
<b>Primates</b>					
<i>Gorilla gorilla</i>	323.5–368	0.101	0.530	0.869	0
<b>Cetacea</b>					
<i>Delphinus delphis</i>	307–326	0.075	0.748	0.902	0
<i>Orcinus orca</i>	965	0.092	–	–	–
<i>Monodon monoceros</i>	750	0.079	0.702	0.945	0
<i>Phocoena phocoena</i>	282	0.076	0.658	0.806	0

$L_{rib}$  length of the rib (chord + arrow, in mm); *CD* mean cortical development index; *BC* mean global bone compactness; *CP* mean compactness profile; *CC* mean relative area (in %) occupied by calcified cartilage.

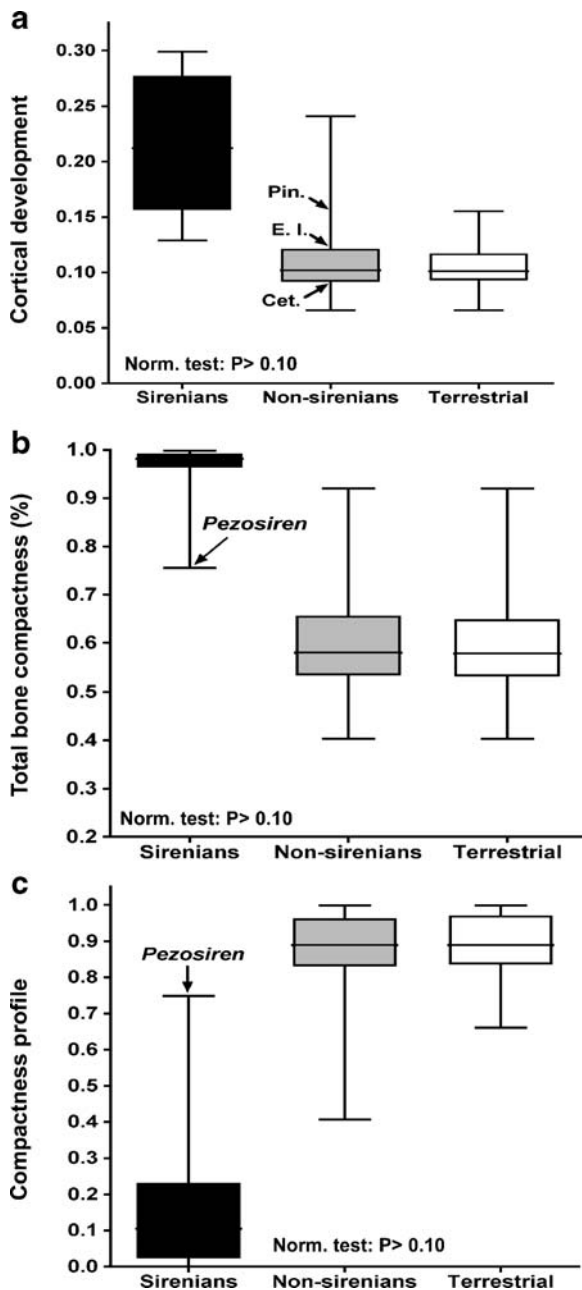
rat (0.913) are above it and should thus be considered as having osteosclerotic or incipiently osteosclerotic (but in no case pachyostotic) ribs.

**Compactness profile (CP)** There are spectacular and highly significant differences ( $p < 0.0001$ ) between the mean *CP* index of the Sirenia (mean  $CP = 0.187$ ) and those of non-sirenian (0.870) and terrestrial mammals (0.892). For a 95% confidence interval, *CP* for terrestrial mammals ranges from 0.731 to 1. Bone structure can thus be considered divergent from the generalized mammalian condition, and modified towards amedullar architecture, for *CP* values below 0.731. All sirenians have amedullar ribs ( $0 \leq CP < 0.496$ ), with the exception of *Pezosiren* ( $CP = 0.749$ ). However, *Nanosiren garciae* ( $CP = 0.496$ ) and *Eotheroides aegyptiacum* ( $CP = 0.286$ ) display some porosity in the medullar region of their ribs that could be the remnant of a former medullar resorption field. Pinnipeds ( $CP = 0.803$  to 0.957), cetaceans ( $CP = 0.806$  to 0.945), and *Paleoparadoxia* ( $CP = 0.834$ ) are within the normal mammalian range of compactness profile, whereas the sea otter ( $CP = 0.407$ ) is outside this range.

Histological features of the medullar and cortical regions of the ribs

*Pezosiren*, *Eotheroides*, *Eosiren*, and *Prototherium* Histological organization of ribs is comparable in these four taxa (beyond differences in bone compactness and compactness profile); therefore, a common description can be applied to them.

The medullar region is sharply distinct from the cortex (Fig. 6A), and separated from it by a thin (50 to 60  $\mu\text{m}$ ) layer of brightly birefringent lamellar bone tissue (Fig. 6B). The distinction between medulla and cortex is further enhanced by the distribution of bone remodeling: the latter is much more active in, and nearly restricted to, the medulla (Fig. 6B). The medulla is typically occupied by highly remodeled secondary lamellar tissue of endosteal origin, combined with more or less abundant remnants of calcified cartilage matrix. The latter appears as a vitreous, finely granulated substance containing numerous clusters of empty chondrocyte lacunae (Fig. 6C, D) and osseous globules (*globuli ossei* of Brandt 1852; see also Buffr nil et al. 1990a). These globules are small rounded bone



**Fig. 5** Results of the morphometric study. **a** Cortical development (*CD*): data for the sirenians, non-sirenian taxa and terrestrial species. **b** Bone compactness (*BC*): Data for the sirenians, non-sirenian taxa and terrestrial species. **c** Compactness profile (*CP*): Data for the sirenians, non-sirenian taxa and terrestrial species. Distributions of *CD*, *BC*, and *CP* values among terrestrial species are normal (normality test:  $P > 0.10$ ). Cet.: cetaceans; E. l. *Enhydra lutris*; Pin: pinnipeds.

formations ( $\varnothing=20$  to  $30\mu\text{m}$ ), protruding from the lamellar deposits into the cartilage matrix (Fig. 6D) and filling empty chondrocyte lacunae. The relative area of the cartilage remnants in *Pezosiren* ( $CC=15.40\%$ ) and *Prototherium* ( $CC=19.93\%$ ) is above that of *Eosiren* and *Eotheroides*, but exceeded by that of *Halitherium* (Table 3).

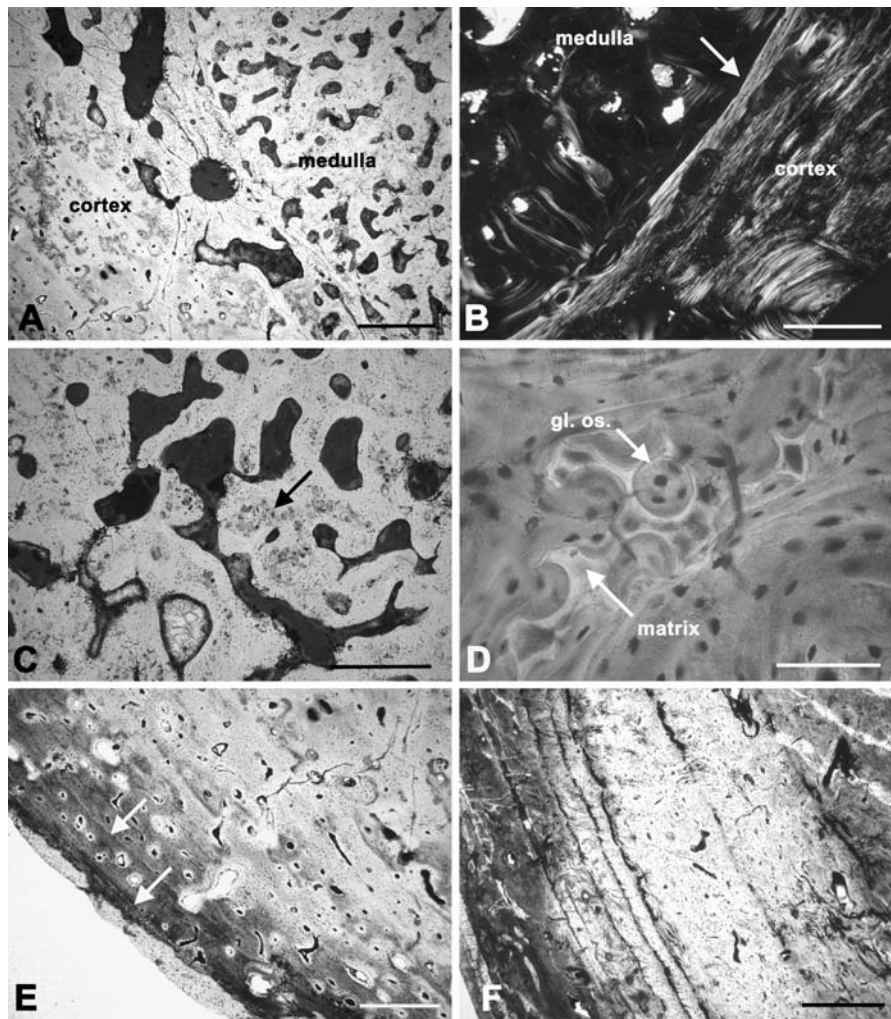
In deep cortical regions, primary periosteal deposits form a thick layer of “fibro–lamellar tissue”. This tissue is characterized by the association of a trabecular network made of woven–fibred bone, housing primary osteons, the walls of which are composed of endosteal lamellar tissue centripetally deposited around the lumen ( $\varnothing$ : 20 to  $60\mu\text{m}$ ) of vascular canals (Fig. 6E). Local vascular density is relatively high, and the canals are mainly oriented longitudinally (Fig. 6E). Cyclic growth marks (Fig. 6E, F) occur in the form of *annuli* (i.e., thin layers of parallel-fibred bone displaying birefringence in polarized light), and lines of arrested growth (peripheral cortex of *Eosiren*). In three of these four taxa, cyclic growth marks are blurred and poorly discernible, an aspect possibly due to the mineral impregnation of bone during fossilization. Conversely, in the fourth, *Eosiren*, annuli and lines of arrested growth are very sharp (Fig. 6F). Haversian remodeling can be relatively intense in periosteal cortices, but it is restricted to the deepest cortical regions. Towards the cortical periphery, the vascular network of bone becomes less dense, the intensity of Haversian remodeling is much reduced, and the histological characteristics of primary periosteal deposits change to a parallel–fibred tissue type (mass birefringence, spindle-like osteocyte lacunae parallel to each other) with *annuli* and lines of arrested growth. Our *Pezosiren* and *Eosiren* specimens display, respectively, 6 to 7 and 15 to 16 cyclic growth marks. In relation to the curvature of the ribs, there is an important asymmetry in cortical thickness and growth mark spacing (growth off-centering) between the medial (thin cortex) and lateral (thick cortex) sides of the ribs.

#### *Protosiren fraasi*

Though it displays a typical sirenian “style” due to the complete lack of a medullar cavity and extreme compactness (see Fig. 7A), the rib of *Protosiren fraasi* is histologically different from all other species considered in this study. There is no remnant of calcified cartilage, and most of the sectional area is occupied by a fibro–lamellar complex housing very thin vascular canals (lumen diameter:  $15\mu\text{m}$  on average). Three striking features characterize *P. fraasi* bone. A) Its vascular supply consists of a very convoluted, anastomoses-rich network, forming a reticular pattern (see Ricqlès 1975), that does not occur in other sirenian species (Fig. 7B). B) There is no Haversian remodeling at all. C) Cyclical growth marks do not occur in cortical tissue.

#### *Halitherium cf. schinzii*

There is no histological difference between our two *Halitherium* ribs, and a common description can be given



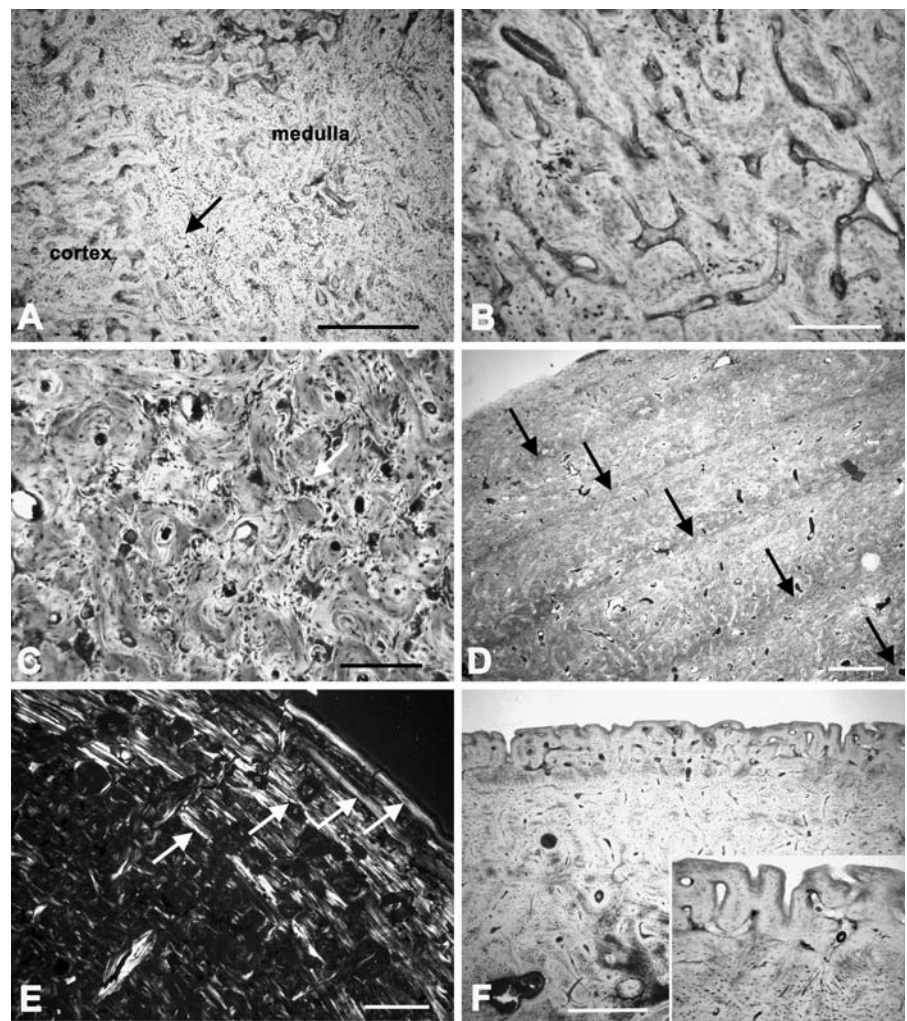
**Fig. 6** Histological features in *Pezosiren* and *Eosiren*. **A**. General view (natural transmitted light) of the medulla and cortex in *Pezosiren portelli*. Bar=950 $\mu$ m; **B**. Closer view (transmitted polarized light) of the birefringent bone layer marking the limit (indicated by arrows) between medulla and cortex in *Pezosiren portelli*. Polarized transmitted light. Bar=600 $\mu$ m; **C**. Bone structure in the medulla of *Pezosiren portelli*. The core of the primary trabeculae retains unresorbed

calcified cartilage (arrow). Bar= 600 $\mu$ m; **D**. Closer view of the remnants of calcified cartilage in the core of primary trabeculae in *Pezosiren portelli*. Numerous *globuli ossei* (gl. os.) protrude into the remnants of cartilage matrix. Bar=150 $\mu$ m; **E**. Cortical organization of *Pezosiren portelli*. Arrows point at lines of arrested growth. Bar=950 $\mu$ m; **F**. Conspicuous lines of arrested growth (LAGs) in the peripheral cortex of *Eosiren libyca*. Bar=950 $\mu$ m.

for them. Histologically, their medullar region resembles those of *Pezosiren* and *Prototherium*, but remnants of calcified cartilage matrix are more abundant ( $CC=25.41\%$ ), and bone remodeling is less pronounced (Fig. 7C). The medulla is also separated from the cortex by a brightly birefringent bone layer, and is thus clearly delimited. Relative medullar area is small in *Halitherium*; conversely, the periosteal cortex is thick (Fig. 7D), especially on the lateral side of the rib that grew faster (growth off-centering). Deep cortices are made of a typical fibro-lamellar complex with a plexiform network of primary osteons. Towards the cortical periphery, the orientation of the primary osteons turns to longitudinal, with numerous anastomoses. In the outermost cortical layers,

only longitudinal simple vascular canals occur, but the bone matrix still consists of woven-fibered tissue. Six sharp growth marks, mainly defined by a cyclic fluctuation in bone opacity (with no direct relationship to cell morphology or density), are distributed through the cortex of specimen MNHN BTA 8, and 11 growth marks in specimen MNHN BTA 13 (Fig. 7D). Growth mark spacing decreases progressively towards the bone periphery, but the presence of a relatively broad layer of bone between the last growth mark and the surface of the cortex in MNHN BTA 13 indicates that growth was still active by the time this specimen died (between 11 and 12 years, if growth marks had an annual periodicity, as in the *Dugong dugon* bones examined by Marsh 1980).

**Fig. 7** Bone histology in *Protosiren*, *Halitherium*, and *Crenatosiren*. **A.** General view of the medulla and cortex in *Protosiren fraasi* rib. Arrow: limit between cortex and medulla. Bar=950 $\mu$ m; **B.** Reticular pattern of the vascular network in the rib cortex of *P. fraasi*. Bar=500 $\mu$ m; **C.** Bone structure in the medulla of *Halitherium schinzii* rib. As in *Pezosiren* and *Prototherium*, the medulla comprises remnants of hypertrophic calcified cartilage (white arrow) and peri-vascular deposits of endosteal bone tissue. Bar=500 $\mu$ m; **D.** Cyclic growth marks (arrows) in the cortex of *Halitherium* rib. The last growth cycle remains relatively thick, suggesting that growth rate remained high at this growth stage. Bar=950 $\mu$ m; **E.** Remodeling of the deep cortex of a rib, and cyclic growth marks (arrows) in *Crenatosiren olseni*. Polarized transmitted light. Bar=950 $\mu$ m; **F.** Peripheral cortex in *Crenatosiren* (general view and detail). The rich vascular supply of the most peripheral bone layer suggests that growth was still active when the animal died. Bar=950 $\mu$ m for the general view, 450 $\mu$ m for the detail.



#### *Crenatosiren olseni* and *Dioplotherium cf. allisoni*

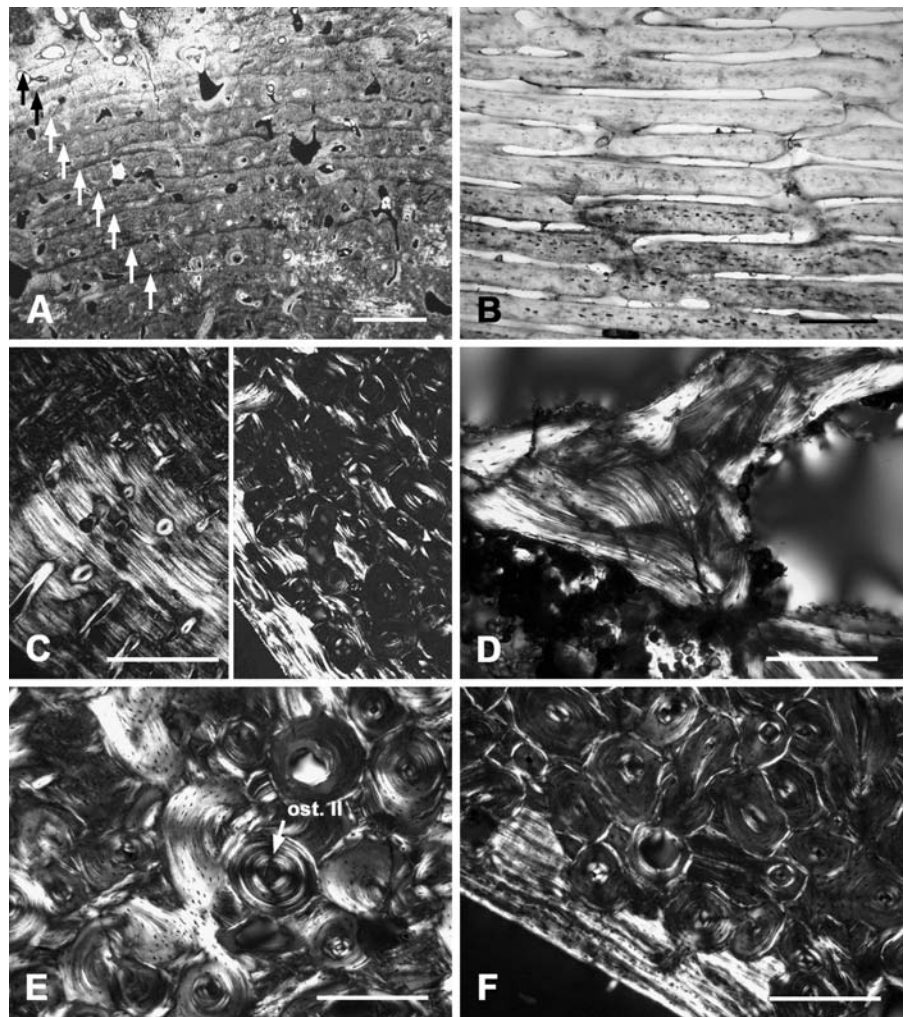
In addition to its great compactness, the rib of *Crenatosiren olseni* differs from the above-described species by two characteristics. **A)** Inner, Haversian remodeling is much more pronounced and involves not only the medullar region, but also the deep part of the periosteal cortex (Fig. 7E). As a consequence, the limit of the medullar territory is no longer visible. **B)** Because of the intensity of bone remodeling in the medulla, there are no remnants of calcified cartilage (Table 3).

Deep cortices are made of a dense Haversian tissue with only sparse remnants of the woven-fibered bone that composed the primary cortex. Haversian systems have very narrow lumina (some 10 $\mu$ m in diameter) that are often completely occluded by bone deposits. Peripheral, non-remodeled parts of bone cortex are made of a tissue showing characteristics intermediate between the woven-fibered and the parallel-fibered types (irregular mass birefringence combined with multipolar, unevenly oriented osteocytes). Paradoxically, local bone vascularization is more abundant than in deep cortices,

especially in the outermost cortical strata, where numerous, convoluted simple vascular canals form a plexiform network (Fig. 7F). Sharply defined *annuli* occur in the outer cortex (Fig. 7E). As suggested by the spacing pattern of the annuli and by the incorporation of vascular canals in the most superficial osseous layer, when this specimen died, sub-periosteal bone accretion on rib surfaces was still progressing at a rate comparable to that prevailing during the previous years.

Bone structure of *Dioplotherium cf. allisoni* is very close to that of *Crenatosiren olseni*: it is characterized by intense remodeling of the medullar region and a complete resorption of calcified cartilage. Both species also share the same tissue types in deep (dense Haversian bone) and peripheral (parallel-fibered tissue) primary cortices and an intense Haversian remodeling of deep cortices. In *D. cf. allisoni*, the vascular network of bone includes both primary and secondary osteons, and displays a typical plexiform geometry. Open resorption bays and secondary osteons in which reconstructive, perivascular bone deposits are not complete, are numerous in both the cortex and the

**Fig. 8** Bone histology in *Metaxytherium*, *Trichechus manatus*, *Dugong*, *Paleoparadoxia*, and *Gorilla*. **A**. Sharp cyclic growth marks (arrows) in the rib periosteal cortex of *Metaxytherium krahuletzki*. Bar=950  $\mu$ m; **B**. Laminar cortex in *Trichechus manatus*. Bar=950  $\mu$ m; **C**. Histology of the rib cortex in *Dugong dugon*. Left side: young individual with limited Haversian remodeling; right: adult with more intense cortical remodeling. Bar=400  $\mu$ m; **D**. *Paleoparadoxia*. Example of the intensely remodeled trabeculae forming the medullar spongiosa. Bar=300  $\mu$ m; **E**. *Paleoparadoxia*. Intensely remodeled cortex. Ost. II: secondary osteon. Bar=300  $\mu$ m; **F**. Cyclic growth marks in the periosteal cortex of a *Gorilla* rib. The deep cortical regions are intensely remodeled. Bar=350  $\mu$ m.



medulla. This confers on this rib a relatively high porosity as compared to most other sirenian species (Table 3). Relative cortical development (*CD* index) of *D. cf. allisoni* is unknown, but the apparent proportions of the bone fragment suggest an advanced degree of pachyostosis. At least 17 growth marks can be numbered.

#### *Metaxytherium krahuletzki*

Because of the intense remodeling activity that occurred in its medullar region, the rib of *Metaxytherium krahuletzki*, like those of the two previous taxa, displays no remnant of calcified cartilage. Periosteal cortices are of the woven-fibered type, with longitudinal simple vascular canals and primary osteons. Sharpey's fibers are particularly abundant. The most striking feature of our *M. krahuletzki* bone is the occurrence of 21 to 22 cyclical growth marks, sharply defined by differences in bone opacity (Fig. 8A). Again, when this specimen died, the accretion of new bone on the surface of periosteal cortices was still active, as evidenced by the relatively broad spacing of the most peripheral growth marks.

#### Remarks on extant sirenian species

In adult dugongs, and in the specimens of *Trichechus senegalensis* and *Trichechus manatus*, the medullar region of all ribs is occupied by a compact formation of intensely remodeled lamellar bone. Compactness and remodeling are somewhat lesser in the posterior ribs (n° 15 to 18) of juvenile dugongs. The ribs of extant sirenians display pronounced off-centered growth that results in peripheral relocation of the medullar region. The latter may even come to crop out on the medial side of the rib and be itself submitted to superficial resorption. This outcropping and resorption of the core region of ribs was also observed by Domning (1978) in *Hydrodamalis gigas* and *Hydrodamalis cuetae*.

Histological organization of periosteal cortices corresponds to the general fibro-lamellar type, associating periosteal woven-fibered osseous tissue with primary osteons. The latter are either deposited between bone laminae (Fig. 8B), thus forming laminar bone tissue, or oriented in radial directions and composing a radiating pattern, according

to the specimen considered or the various regions of a single section. Towards the cortical periphery, bone structure turns to the parallel-fibered type, especially in *Dugong dugon*, and primary osteons become longitudinal (Fig. 8C). Bone vascular supply, as well as the intensity of Haversian remodeling, decrease towards cortical periphery (Fig. 8C), whereas cyclic growth marks occur in the non-remodeled parts of the peripheral cortex.

#### Paleoparadoxia and non-sirenian mammals

Histologically, the structure of the *Paleoparadoxia* rib is strictly comparable to the generalized pattern encountered in terrestrial and amphibious mammals. The medullar region is occupied by an open cavity, containing a loose network of thin, remodeled trabeculae (Fig. 8D; see also Fig. 4I–P); while the compact cortex is exclusively composed of a dense Haversian tissue (Fig. 8E). No remnant of the primary, periosteal cortex persists in *Paleoparadoxia*; however, in several extant taxa (*Cervus*, *Sus*, *Tapirus*, *Enhydra*, *Rattus*, *Gorilla*, etc.), Haversian remodeling is more limited in space, and broad portions of untouched primary cortices persist. Primary periosteal tissue in mammalian ribs varies from the parallel-fibered type (*Rattus*, *Erinaceus*, etc.) to the woven-fibered type, according to specific sizes. Our *Paleoparadoxia* specimen displays no cyclic growth marks (the whole cortex is indeed remodeled), but about half of the non-sirenian taxa (*Sus*, *Cervus*, *Enhydra*, *Myocastor*, *Lagopus*, *Gorilla*, etc.) have conspicuous growth marks (Fig. 8F) that are generally less sharp, regular and complete in sequence than those of the Sirenia. In our sample, no remnants of calcified cartilage occur in non-sirenian species.

#### Discussion

Gross morphological, as well as histological observations show that sirenian ribs share some common basic features that were already present in early forms of this order, but they also display structural differences.

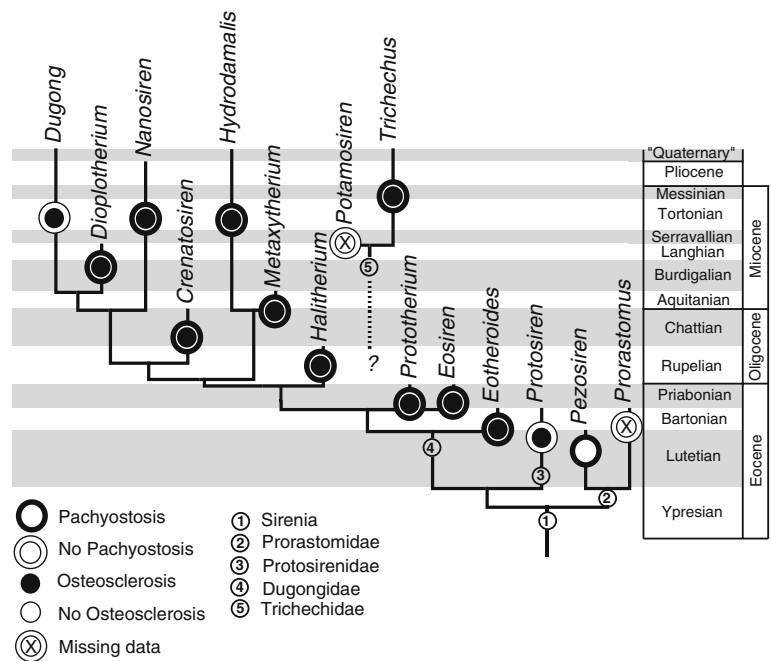
With a *CD* index of 0.226, *Pezosiren* had pachyostotic ribs (i.e., hyperplastic periosteal cortices) like most, but not all, sirenians described hitherto. Conversely, it differs from all of them if global inner bone compactness and compactness profile, two indices quantifying the degree of osteosclerosis, are considered. Although this taxon displays some incipient trend towards osteosclerosis, especially in the lack of a well-circumscribed medullar cavity, it nevertheless remains inside the 95% confidence interval of terrestrial mammals for these indices, and thus looks closer to the generalized (plesiomorphic) mammalian pattern than to the highly derived condition of more recent

sirenians. As mentioned above (see Material and Methods), there is no reason to consider that the *Pezosiren* rib in which the sections were made belonged to a young individual, or that its relatively low compactness is related to age. Morphometric data also reveal that pachyostosis *sensu stricto* does not occur in *Protosiren* and *Dugong dugon* (but both are strongly osteosclerotic). These taxa share a relatively small body size but otherwise represent quite distinct grades in sirenian history (Domning 2002): *Dugong dugon* displays advanced adaptation to swimming, whereas *Protosiren fraasi* had well-developed pelvic limbs and could have retained some dependence on land (see above: Material and Methods). According to Zalmout et al. (2003), the genus *Protosiren* can be “easily recognized” by several postcranial peculiarities, including a general lack of rib pachyostosis. Finally, the degree of cortical development of sirenian ribs displays an important variability (*CD* indices range from 0.129 to 0.299) that could possibly reflect subtle functional differences in the static or dynamic requirements of locomotion in each taxon, or be related to species-specific morphological peculiarities, such as lung volume or blubber thickness, that bear on buoyancy or trim control in water.

*Paleoparadoxia*, the sea otter, *Enhydra lutris*, and the Norway rat, *Rattus norvegicus*, are interesting comparative elements. The ribs of these species are not pachyostotic, but they show an obvious tendency towards osteosclerosis and, according to *BC* values, they are more advanced in this process than *Pezosiren* ribs. These four taxa represent a clear illustration of the basic dissociation of pachyostosis and osteosclerosis. Although these specializations of bone often occur conjointly, as in most sirenians and various other marine tetrapods (e.g., basilosaurid cetaceans; see Buffrénil et al. 1990a), they are neither necessarily linked to each other nor synchronous during the evolutionary history of a given lineage. In this respect, the “adaptive” trajectory followed by the basalmost sirenians slightly differed from that of pakicetids (Madar 2007) in which rib osteosclerosis seems to have preceded pachyostosis (the latter was additionally acquired by the Basilosauridae with further adaptation to marine life). The phyletic tree presented in Fig. 9 summarizes the distribution of pachyostosis and osteoclerosis among the sirenian taxa considered in this study. Finally, the relatively high value of *BC* index in a terrestrial species such as *Rhinoceros sondaicus* suggests that the occurrence of compact bones is not, per se, exclusive to aquatic forms, or due to determinisms that arise specifically from a direct influence of water. This question is further considered below.

The results of this study give some insight into the timing of appearance of the two main features, pachyostosis and osteosclerosis, constituting the heavy pachyosteosclerotic condition of most post-Eocene Sirenia. The following

**Fig. 9** Distribution of pachyostosis and osteosclerosis among the sirenian genera considered in this study (with exception for *Prorastomus* and *Potamosiren*). This simplified phyletic tree is based on Domning (1994) and Domning and Aguilera (2008).



reconstruction can be proposed. Incipient acquisition of aquatic habits by the earliest forms induced the need for ballast, allowing a passive control of buoyancy and trim. It thus favored, through positive selection, a relative increase in rib cortical development, resulting in pachyostosis. This evolutionary process was rapid and already completed in animals that basically remained terrestrial from an anatomic point of view. Inner bone compaction, or osteosclerosis, also occurred early in sirenian evolution, but as a progressive and somewhat slower process that was not closely synchronous with pachyostosis. *Pezosiren* typifies an initial adaptive stage combining advanced pachyostosis, incomplete osteosclerosis, and nearly non-existent gross morphological specializations for life in water, at least for the bones involved in locomotion (the nasal region suggests some aquatic adaptation; see Domning 2001a).

With further adaptation of the Sirenia to life in water, inner bone compaction increased, and finally resulted in completely solid, amedullar ribs as displayed by, e.g., *Protosiren* and *Prototherium*. In the meantime, the functional effects of this process were enhanced by additional cortical hyperplasia in some taxa (e.g., *Halitherium*). Since rib pachyostosis occurred in the basalmost sea cow, *Pezosiren*, and most subsequent sirenians, but in no other Tethytheria or Afrotheria, this feature should be considered as a synapomorphy of the Sirenia and a primitive trait within this order. This conclusion agrees with that of Savage (1977). If so, this feature would have undergone reversion (in addition to substantial variability) in some taxa, especially *Protosiren* ( $CD=0.167$ ) and *Dugong* ( $CD=0.129$ ). This remark is further developed and discussed

below. Osteosclerosis is less liable to represent a significant synapomorphic trait of the Sirenia, because it was not fully developed in *Pezosiren*, and was probably still less developed in the immediate ancestors of this genus.

At a tissue level, rib structure is roughly consistent among sirenian taxa, with the exception of *Prototherium*. From the earliest sirenian taxon up to extant species, *Protosiren* being left apart, the main trends of bone histogenesis in sirenian ribs can be reconstructed as follows, with reference to the present study and previously published observations (especially Fawcett 1942). The ribs of newborn individuals comprise a central medulla housing very thick trabeculae produced by an incomplete resorption of the hypertrophic calcified cartilage. At this stage, periosteal cortices are cancellous, and composed of woven-fibered bone most often organized in laminae (laminar bone).

Postnatal osteogenesis in all extinct and extant sirenian species is characterized by the following traits:

- A) In early growth stages, medullar trabeculae are superficially eroded but resorption stops before calcified cartilage is entirely destroyed. Periosteal accretion is fast, especially on the lateral (convex) surface of the ribs, and creates a thick layer of woven-fibered tissue organized either in laminae housing circular vascular canals, or as a less structured deposit with longitudinal or radial vascular canals.
- B) Eroded surfaces of medullar trabeculae are reconstructed by endosteal accretion of lamellar or pseudo-lamellar tissues. The trabeculae thus acquire the typical

structure of primary trabeculae, with a core of calcified cartilage covered with endosteal platings. In most tetrapods, such trabeculae are produced just under the growth plate of long bones as the first step of bone substitution for cartilage (endochondral ossification), but they disappear in metaphyseal regions to be replaced by secondary, highly-remodeled trabeculae. In sirenians, endosteo-endochondral trabeculae also undergo active remodeling, but there is an excess in the accretion of secondary (reconstructive) bone, as compared to the amount of tissue previously eroded. This process results in a progressive reduction of intertrabecular spaces that are finally reduced to the diameter of very thin blood vessels, if not entirely occluded. As evidenced by our results, remodeling of the medullar territory was less active in *Pezosiren*, *Prototherium*, and *Halitherium*, and remnants of calcified cartilage, in addition to some bone cavities in *Pezosiren* and *Prototherium*, were thus preserved.

In parallel with inner and outer growth, bone cortex is extensively eroded on the medial surface of the rib shaft, especially at mid-shaft, where bone curvature is maximal. Resorption can even reach the medullar region and remove it in part or in totality. The combination of fast subperiosteal accretion on the lateral rib surface, with slower growth or resorption on the medial surface, is partly responsible for maintaining rib curvature during growth.

In the cortex, centripetal perivascular deposits of lamellar tissue on the walls of bone laminae create primary osteons and contribute to further occluding bone cavities.

- C) With decrease of growth rate, the structure of new cortical layers gradually turns to the parallel-fibered type with longitudinal vascular canals and sharp cyclic growth marks.

The occurrence of cyclic growth marks in the ribs of mammals is poorly documented, mainly because intense Haversian remodeling entirely replaces primary periosteal tissues by secondary osteons. The present study shows that such marks are actually frequent, if not general, a situation that should tend to temper previous statements (see Buffrénil and Schoevaert 1989) relating the cyclic pattern of growth observed in various bones of the sirenian skeleton (see Domning and Myrick 1980 for ribs; Marsh 1980 for tympanic bones, ribs, and phalanges; Ricqlès and Buffrénil 1995 for skull; Marmontel et al. 1996 for periotic bones; etc.) with some physiological traits of these animals, especially their relatively low metabolic rate and limited thermogenetic capabilities (Cave and Aumonier 1967; Marsh et al. 1978; Gallivan et al. 1983; Irvine 1983). Actually, cyclic growth, at least in ribs, is a common condition

in mammals, even in primates (Klevezal 1996; Castanet 2006) and has no unequivocal physiological meaning (see also Sander and Andrassy 2006). In the Sirenia, growth marks are particularly conspicuous simply because cortical remodeling is mild, at least in peripheral regions.

In parallel with growth, bone remodeling develops from the medullar to the cortical territories, provoking further rarefaction of calcified cartilage remnants, if any.

- D) The growth of rib cortices is apparently long lasting, at least in the taxa displaying pachyostosis, and still proceeds at sustained speed after 11 (*Halitherium* MNHN BTA 13), 17 (*Dioplotherium*), or 22 (*Metaxytherium*) annual growth cycles. This feature suggests that cortical hyperplasy in sirenian ribs relies (at least in part) on a protraction of sub-periosteal accretion during ontogeny. This topic is further discussed below.

Histologically, the main differences between sirenian taxa are related to the intensity of bone remodeling (and consequently the completion of calcified cartilage resorption) and to the degree of cortical hyperplasy. Four species exhibit no cartilage remnants and intense Haversian remodeling of deep cortices (*Crenatosiren olseni*, *Dioplotherium* cf. *allisoni*, *Metaxytherium krahuletzki*, and *Trichechus senegalensis*) whereas five species display cartilage remnants and no, or mild, cortical remodeling (*Pezosiren portelli*, *Eosiren libyca*, *Eotheroides aegyptiacum*, *Prototherium montserratense*, and *Halitherium schinzii*). Observations made in *Dugong dugon* reveal that the degree of cartilage resorption can be influenced by the position of a rib in the rib series (Buffrénil and Schoevaert 1989). Moreover, bone remodeling develops over time and is a cumulative process; therefore, for a given rib, cartilage resorption depends on individual age. These two factors could possibly explain a part of the differences in the persistence of calcified cartilage observed among specimens, especially in the case of *Dioplotherium* (22 years at least) and *Metaxytherium* (17 years at least). However, they would not be decisive because the species in which calcified cartilage is most abundant, *Halitherium* cf. *schinzii* (CC index=25.41), is represented neither by a posterior rib nor by a particularly small or young individual (minimal age 11–12 years). In addition, our sections from *Prototherium montserratense* (CC=19.93) are from the middle of the shaft of an adult. Actual interspecific differences in the degree of bone remodeling and the persistence of calcified cartilage thus occur among sirenian taxa, geologically oldest forms being those with the feeblest remodeling activity and the most abundant cartilage remnants. Paradoxically, this statement means that *Pezosiren*, *Eosiren*, *Eotheroides*, *Prototherium*, and *Halitherium* were more derived relative to the plesiomorphic mammalian



condition (fast and complete resorption of calcified cartilage) than geologically more recent forms.

Osteogenic trends could have been fairly different in *Protosiren fraasi*. In this species, resorption of calcified cartilage was complete during the growth in length of the ribs, and thick endosteal trabeculae, poorly remodeled during subsequent growth, were formed. Periosteal accretion must have been fast, because the reticular tissue that was deposited is classically considered to result from fast growth (Francillon-Vieillot et al. 1990). This tissue was originally cancellous. It was then compacted by endosteal deposits, and no subsequent remodeling occurred. The specimen used in this study must have died when it was still actively growing, or conversely cortical growth must have stopped suddenly, because the same type of bone tissue occurs from the depth to the periphery of rib cortices. The genus *Protosiren* has several cranial and postcranial features unknown in other sirenians (Gingerich et al. 1994; Zalmout et al. 2003; see also Domning 2001b). Histological peculiarities displayed by the ribs of *P. fraasi* could represent an additional apomorphic feature supporting that the Family Protosirenidae be considered well apart from both the basal Prorastomidae and the more modern, fully aquatic, sirenians including Dugongidae and Trichechidae.

The possible cause of sirenian osteosclerosis and pachyosteosclerosis is an intriguing, but still poorly documented question. To go further with this question, two preliminary remarks must be considered.

- A) Though not otherwise observed among the Afrotheria or Paenungulata, these features are by no means unique to the Sirenia. They are also encountered with the same histological tableau in the long bones of most, if not all, other aquatic tetrapods at incipient stages of their adaptation to life in seas: e.g., nothosaurs (Zangerl 1935), champsosaurs (Buffr n il et al. 1990b), juvenile plesiosaurs (Wiffen et al. 1995), various snake-like squamates (Buffr n il and Rage 1993; Houssaye et al. 2008), early cetaceans (Buffr n il et al. 1990a; Gray et al. 2007; Madar 2007), and even some spheniscid birds (Meister 1962); data are lacking for early ichthyosaurs and marine turtles. The osteosclerotic or pachyosteosclerotic stages thus seem to be mandatory passages in the history of marine tetrapods. In semi-aquatic forms or fresh-water dwellers there is just a limited increase in bone density (Wall 1983; Stein 1989).
- B) The cells producing bone tissue, the osteoblasts, are of mesenchymal origin, whereas the cells resorbing cartilage and bone (chondroclasts and osteoclasts) are of hematopoietic origin (V n nen and Laitala-Leinonen 2008). They thus represent distinct and cytogenetically independent cell lineages.

Several hypotheses have been proposed to explain the pachyosteosclerotic condition of aquatic tetrapods. According to Nopcsa (1923) and Nopcsa and Heidsieck (1934), this feature could result from endocrine disturbance due to the peculiar abiotic factors (low temperature, high pressure, low lighting) prevailing in sea water. Species incipiently adapting to this milieu would lack the physiological ability to meet these constraints, which would finally interfere with normal osteogenic controls during growth (synthesis in Ricql s 1989). This hypothesis is, of course, irrelevant to the Sirenia because this order ranks among the most completely and most anciently transformed for life in water.

Another, more sophisticated endocrine interpretation was developed by Ricql s (1989; see also Cave and Aumonier 1967). In brief, this hypothesis, mainly relative to amphibians, considers that the abiotic conditions of marine life interfere with thyroid and parathyroid functions through a cascade of endocrine determinisms, starting at the level of the diencephalon, and mediated by alteration in hypophyseal secretions: decrease in TSH and increase in prolactin productions. Prolactin increase would favor bone apposition via its action on thyrocalcitonin, while thyroxine decrease would inhibit bone and cartilage remodeling (see Ricql s 1989: fig. 1). The combined results of these endocrine influences would be, respectively, inner bone compaction plus cortical hyperplasia, and incomplete cartilage resorption. This peculiar hormonal context would initially occur at an individual level; it would then be positively selected during subsequent evolution for the functional advantages resulting from it (buoyancy and trim control). Of course, in this form, the relevance of this hypothesis to marine mammals is difficult to assess because the hormonal context prevailing in these animals, and their sensibility to environmental factors, are quite different from those of amphibians. Otherwise, this hypothesis would agree with histological peculiarities that suggest hypofunctioning of the thyro-parathyroid gland complex in the manatee and dugong (Cave and Aumonier 1967; Husar 1975; Marsh et al. 1978).

The main objection to all hypotheses relating pachyosteosclerosis to a hormonal cause, or any other systemic factor, is that this feature is limited in Sirenia to the thorax (in addition to ribs, the scapula and the humerus are osteosclerotic; see Buffr n il and Schoevaert 1989) and the rear part of the skull, while the rest of the skeleton (vertebral centra, zeugo- and autopodials, etc.) has a more or less normal structure (Buffr n il and Schoevaert 1989). Though additional comparative data on this question are needed (most available data are from *Dugong dugon*), pachyosteosclerosis in the Sirenia appears as a local feature that would therefore depend on local factors. Moreover, the supposed decrease in bone remodeling in these animals is not entirely substantiated by the results of the present study, especially for the medullar region of post-Eocene

forms. Hypotheses referring to endocrine causes basically rely on the supposed impact of abiotic oceanic factors, especially low lighting and unfavorable temperature. As such, they fail to explain why the highly pelagic forms, which usually display advanced capabilities for deep diving (cetaceans, seals, possibly also ichthyosaurs, etc.) and are thus all the more submitted to the physical constraints of the marine environment, do not display the pachyostotic or osteosclerotic conditions that they exhibited when they were shallow water dwellers, at the beginning of their adaptive radiation.

Another kind of explanation would refer to a mutational cause comparable to those involved in the many types of osteoscleroses or hyperostoses described hitherto in man and other tetrapods (syntheses in, e.g., Vernejoul and Bénichou 2001; Key and Ries 2002; Karsenty 2007). The symptomatic tableau of such pathologies is relatively different from the condition of the sirenian skeleton because most of them are diffuse through the whole skeleton or have a topographic distribution quite different from the situation of sirenians (Bénichou et al. 2000; Key and Ries 2002). All forms of pathologic osteosclerosis are caused by a steep reduction in bone resorption (and consequently remodeling) due to deficiency in osteoclast recruitment or physiology (e.g., Popoff and Marks 1995; Key and Ries 2002; Asagiri and Takayanagi 2007). According to the mutational hypothesis, bone pachyosteosclerosis should be considered as originally pathologic, and this hypothesis would postulate that one mutation occurred very early in sirenian history (or in that of any zoological group displaying this feature), and was maintained in subsequent evolution by positive selection. A typical example of this interpretation was recently given by D'Anastasio's (2004; see also Kaiser 1970) study of "idiopathic hyperostosis" in various vertebrates including the Sirenia.

In the case of sirenians and other marine tetrapods, the "pathologic (or mutational) model" has to face at least four serious objections:

- A) An obvious objection arising from this study is that osteosclerosis was progressively acquired by the Sirenia, along a morphocline that started before *Pezosiren* (likely by the end of the early Eocene), and reached full achievement in the forms posterior to this taxon. Paleohistological data show that by no means did this feature appear abruptly with full completion, as should be the case if a pathological mutation was involved.
- B) At least ten orders and some 22 tetrapod families displayed osteosclerosis or pachyostosis during their history (Kaiser 1960; Ricqlès and Buffrénil 2001). The probability that the same mutation occurred and was positively selected in each of them seems to be, at best, extremely small.

- C) An important, severe genetic mutation occurring early in a clade (and not negatively selected) is very unlikely to be reversible, because this would require another subsequent mutation (or series of mutations) precisely correcting the effects of the first one, which is beyond reasonable expectancy. This mutation would thus be maintained in the whole subsequent history of this lineage. However, in many clades, the pachyosteosclerotic condition of early forms turned to the reverse condition in later evolutionary stages (Ricqlès and Buffrénil 2001; see also Wiffen et al. 1995). This situation is clearly exemplified by the Cetacea in which the heavy pachyosteosclerosis of the archaeocetes turned to the osteoporotic-like condition of later forms (Gray et al. 2007). Among the Sirenia, the lack of pachyostosis in *Protosiren* and *Dugong* suggests that this feature regressed during evolution, because basal dugongine forms, like *Crenatosiren* ( $CD=0.235$ ), were pachyostotic (Domning and Aguilera 2008).
- D) If not lethal, a massive genetic mutation with extensive impact on bone structure has many functional consequences, especially on the static and dynamic performances of an organism in water and on land. It can be beneficial only if this organism is behaviorally competent to exploit this transformation. It is, at best, very poorly conceivable that a series of concomitant mutations bearing on bone structure, behavior, etc. occur simultaneously.

In summary, the hypothesis of a severe mutation resulting in osteosclerosis is not very convincing and should be discarded.

An alternative interpretation is to attribute the whole set of bone peculiarities of the Sirenia to one single cause: an increase in bone accretion during remodeling of the medullar region (this would involve endosteal osteoblasts) and cortical growth (periosteal osteoblasts). In most mammals, bone is submitted to permanent resorption and reconstruction (remodeling). In terrestrial forms, the situation considered normal is a balance between these two processes and stability in bone volume (reviewed in Karsenty 2007). Negative imbalance is frequent and leads to osteoporotic states (e.g., Parfitt 1982; see also Buffrénil and Schoevaert 1988). Positive imbalance, resulting in excess reconstructive bone, is also known to occur under various influences (see, e.g., Karsenty 2007). In the ribs of the sirenians, the latter process would lead to a progressive compaction of originally cancellous tissue, with the complete closure of inter-trabecular spaces and even the lumina of vascular canals. It would be linked to remodeling but would also contribute to progressively constrain remodeling, because the cells responsible for bone resorption (the initial stage of remodeling) are of haematopoietic origin, and are brought in situ by the blood flow. This interpretation would explain why bone remodeling can be relatively intense in most taxa, but apparently limited in

space (medullar region) and time (as evidenced by the scarcity of open resorption bays).

Moreover, a protraction of periosteal accretion during ontogeny seems to be an obvious situation in sirenian ribs, as evidenced by several specimens in which annual growth marks were still deposited at advanced ages (more than 17 or 22 years).

According to this general interpretation, the prominent cause of sirenian pachyosteosclerosis would concern the timing and/or intensity of osteoblast activity at both endosteal and periosteal levels. To a large extent, this would represent a developmental heterochrony, bearing independently on endosteal and periosteal cell populations, and finally causing hypermorphosis (hyperplasia in periosteal and endosteal bone deposits) through post-displacement (delayed apposition cessation; see Cubo 2000; see also Ricqlès and Buffrénil 2001). The mechanism underlying this process is beyond reach for the present study; however, it would primarily involve local osteoblast populations (they respond to a highly complex set of influences), not a systemic cause.

The present study shows that such a process developed progressively and is therefore compatible with a classical Darwinian evolutionary scheme based on variability under strong selective pressure. All authors consider that osteosclerosis and/or pachyostosis have a hydrostatic role, and contribute, as ballast, to regulating buoyancy and trim in tetrapods incipiently adapting to life in water, or specialized in feeding on fixed or slow-moving prey in shallow waters. In the Sirenia, hydrostatic constraints would have controlled, through positive selection, the gradual differentiation of rib pachyosteosclerosis (Domning and Buffrénil 1991; Domning 2000). Opposite to predatory forms that shifted their locomotion towards fast swimming and high maneuverability, and modified accordingly the structure of their bones during evolution, the Sirenia retained or increased their skeletal ballast because their basic diet and foraging mode did not significantly change during geological times (Domning 2001c).

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