



The phylogenetic origin and evolution of acellular bone in teleost fishes: insights into osteocyte function in bone metabolism

Donald Davesne, François Meunier, Armin Schmitt, Matt Friedman, Olga Otero, Roger Benson

► To cite this version:

Donald Davesne, François Meunier, Armin Schmitt, Matt Friedman, Olga Otero, et al.. The phylogenetic origin and evolution of acellular bone in teleost fishes: insights into osteocyte function in bone metabolism. *Biological Reviews*, 2019, 94 (4), pp.1338-1363. 10.1111/brv.12505 . hal-02167990

HAL Id: hal-02167990

<https://hal.science/hal-02167990>

Submitted on 4 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The phylogenetic origin and evolution of acellular bone in teleost fishes: insights into osteocyte function in bone metabolism

Donald Davesne^{1,*}, François J. Meunier², Armin D. Schmitt¹, Matt Friedman³, Olga Otero⁴ and Roger B. J. Benson¹

¹ Department of Earth Sciences, University of Oxford, OX1 3AN Oxford, U.K.

² BOREA (UMR 7208 CNRS, IRD, MNHN, Sorbonne Université), Muséum national d'Histoire naturelle, 75005 Paris, France

³ Museum of Paleontology and Department of Earth and Environmental Sciences, University of Michigan, Ann Arbor, MI 48109-1079, U.S.A.

⁴ PalEvoPrim (UMR 7262 CNRS), Université de Poitiers, 86000 Poitiers, France

* Corresponding author. ORCID: 0000-0002-4775-2360
donald.davesne@earth.ox.ac.uk

ABSTRACT

Vertebrate bone is composed of three main cell types: osteoblasts, osteoclasts and osteocytes, the latter being by far the most numerous. Osteocytes are thought to play a fundamental role in bone physiology and homeostasis, however they are entirely absent in most extant species of teleosts, a group that comprises the vast majority of bony 'fishes', and approximately half of vertebrates. Understanding how this acellular (anosteocytic) bone appeared and was maintained in such an important vertebrate group has important implications for our understanding of the function and evolution of osteocytes. Nevertheless, although it is clear that cellular bone is ancestral for teleosts, it has not been clear in which specific subgroup the osteocytes were lost. This review aims at clarifying the phylogenetic distribution of cellular and acellular bone in teleosts, to identify its precise origin, reversals to cellularity, and their implications. We surveyed the bone type for more than 600 fossil and extant ray-finned fish species and optimised the results on recent large-scale molecular phylogenetic trees, estimating ancestral states. We find that acellular bone is a probable synapomorphy of Euteleostei, a group uniting approximately two-thirds of teleost species. We also confirm homoplasy in these traits: acellular bone occurs in some non-euteleosts (although rarely), and cellular bone was reacquired several times independently within euteleosts, in salmonids and relatives, tunas and the opah (*Lampris* sp.). The occurrence of peculiar ecological (e.g. anadromous migration) and physiological (e.g. red-muscle endothermy) strategies in these lineages might explain the reacquisition of osteocytes. Our review supports that the main contribution of osteocytes in teleost bone is to mineral homeostasis (*via* osteocytic osteolysis) and not to strain detection or bone remodelling, helping to clarify their role in bone physiology.

Keywords: osteocyte, acellular bone, anosteocytic bone, Actinopterygii, Teleostei, Salmoniformes, Scombridae, ancestral state reconstruction, bone remodelling, endothermy

I. INTRODUCTION

(1) General introduction

Vertebrate bone is a living tissue that, besides its mineralised extracellular component, comprises cells of three different types. Surface-based osteoblasts and osteoclasts synthesise and resorb bone, respectively, and osteocytes are more versatile cells that fulfil various functions (Francillon-Vieillot *et al.*, 1990; Ricqlès *et al.*, 1991; Bonewald, 2011; Shahar & Dean, 2013; Hall, 2015). Osteocytes are by far the dominant cellular component, constituting up to 95% of bone cells in mammals. They derive from osteoblasts of the bone surfaces that become embedded into the bone matrix in cavities called osteocyte lacunae (Franz-Odenaal, Hall & Witten, 2006) and communicate with each other through a network of canaliculi (Cao *et al.*, 2011).

Osteocytes play a key role in bone physiology: (1) they act as mechanical sensors detecting changes in bone strain; (2) they guide bone remodelling by activating or deactivating the osteoclasts they communicate with; (3) and they are involved in calcium and phosphorus metabolic regulation through direct resorption of the bone around their lacunae (Witten & Huysseune, 2009; Rochefort, Pallu & Benhamou, 2010; Bonewald, 2011; Wysolmerski, 2012; Shahar & Dean, 2013). This double role in mineral and mechanical homeostasis would suggest that osteocytes are indispensable for bone to function normally (Moss, 1961*b*; Shahar & Dean, 2013). However, bone is entirely devoid of osteocytes in most teleosts, (Kölliker, 1859; Stéphan, 1900; Enlow & Brown, 1956; Moss, 1963; Meunier, 1987, 1989; Meunier & Huysseune, 1992; Huysseune, 2000; Witten *et al.*, 2004; Shahar & Dean, 2013) a group of ray-finned fishes that comprises more than half of modern vertebrate species.

Nineteenth century histologists noted the absence of 'bone corpuscles' (i.e. osteocyte lacunae) in the bone of some teleosts (Williamson, 1851; Gegenbaur, Kölliker, & Müller, 1853; Mettenheimer, 1854; Quekett, 1855). This inspired Kölliker (1859) to undertake a remarkable survey of more than 250 ray-finned fish species, distinguishing those with acellular bone (improperly named 'osteoid' at the time) from those with cellular bone. Moss and colleagues later described the structure, mineral composition and development of teleost acellular bone, confirming its nature as true bone (Moss & Posner, 1960; Moss, 1961*a,b*, 1962, 1963, 1965; Moss & Freilich, 1963). Later, Weiss & Watabe (1979) proposed the term 'anosteocytic bone', which is more precise because this tissue still bears other cell types (osteoblasts and osteoclasts) on its surface. Nevertheless, the term 'acellular bone' remains widely used in modern literature, and we apply that term here.

That bone is acellular in such a large and ecologically important group as teleosts raises numerous questions pertaining to: (1) the distribution of bone type within teleosts (does it follow ecological, physiological or phylogenetic patterns?), (2) the origin of acellular bone (does it have a unique origin, or multiple convergent appearances?), and (3) the function of such a bone type (does the absence of osteocytes impact bone structure, function and homeostasis?). Addressing these questions has critical implications to understanding the evolution of bone within vertebrates as a whole, and the role of osteocytes in bone physiology (Huysseune, 2000; Witten *et al.*, 2004; Shahar & Dean, 2013; Currey, Dean & Shahar, 2017).

(2) The evolution of acellular bone: state of the art

Following the surveys of K  lliker (1859) and Moss (1961*b*), researchers attempted to explain the distributions of cellular and acellular bone among teleost species. For example, an early hypothesis proposed that acellular bone occurs because marine environments are richer in dissolved calcium, decreasing the need to use bone as an additional source of metabolic minerals (Moss, 1961*b*, 1963). However, acellular bone is also present in freshwater teleost taxa such as esocids (pikes), centrarchids (sunfishes), percids ('true' perches), and cichlids (Moss, 1965). In virtually all species, the entire skeleton seems to be composed exclusively of either cellular or acellular bone, and closely related species mostly seem to share the same bone type (K  lliker, 1859). Following these observations, cellularity was quickly recognised as a potentially significant phylogenetic character (e.g. K  lliker, 1859; Berg, 1947). Indeed, at least two studies have used the presence or absence of osteocytes to discuss the systematic position of enigmatic fossil taxa (Gaudant & Meunier, 2004; Mayrinck *et al.*, 2017).

Deep divergences in teleost phylogeny have been poorly resolved until recently, meaning that the phylogenetic distribution of cellularity has not been clear. Nevertheless, there is broad consensus on two statements: (1) that cellular bone is the plesiomorphic condition for teleosts, actinopterygians and osteichthyans in general (  rvig, 1951, 1967; Moss, 1961*b*, 1963); and (2) that acellular bone is found in 'advanced' or 'higher' teleost groups (Moss, 1961*b*, 1963; Meunier, 1987, 1989; Ricql  s *et al.*, 1991; Meunier & Huysseune, 1992; Witten *et al.*, 2004). As noted by the past authors themselves, these propositions are imprecise and potentially misleading. Indeed, the pattern appears to be much more complex: for example, acellular bone is found in certain 'lower' teleosts such as pikes and cellular bone is found in some 'higher' taxa such as tunas (Amprino & Godina, 1956; Moss, 1963; Meunier, 1989; Meunier & Huysseune, 1992). Moreover, the systematic distributions of both bone types have been described using subjective and poorly defined systematic categories (e.g. 'advanced teleosts'), not on an explicit phylogenetic framework based on character analysis.

Several authors used cellularity as a phylogenetic character: acellular bone is proposed as a synapomorphy uniting (1) Osmeriformes (true smelts) and Neoteleostei (the clade including spiny-rayed fishes, amongst others) by Rosen (1985); (2) Esociformes (pikes and mudminnows), Osmeriformes and Neoteleostei by Parenti (1986); (3) Esociformes and Neoteleostei by Johnson & Patterson (1996), the latter being the only phylogeny based on the analysis of a character matrix. However, the usefulness of this previous work is limited because the underlying phylogenetic frameworks have been superseded by more recent classifications based on molecular phylogenies that extensively sample both taxa and loci (e.g. Near *et al.*, 2012; Betancur-R. *et al.*, 2013, 2017). The most relevant changes relative to anatomical hypotheses include: (1) Esociformes do not form an exclusive clade with Neoteleostei, but instead consistently appear to be sister to Salmoniformes (Ramsden *et al.*, 2003; Wilson & Williams, 2010; Near *et al.*, 2012; Betancur-R. *et al.*, 2013; Campbell *et al.*, 2013); (2) Neoteleostei *sensu* Rosen (1973, 1985) is not a monophyletic group, with Stomiiformes (viperfishes and relatives) now considered sister to Osmeriformes (Li *et al.*, 2010; Near *et al.*, 2012; Betancur-R. *et al.*, 2013, 2017).

(3) Aim of this review

While most research on acellular teleost bone has been focused on its structure, development and function (Moss, 1961a; Meunier, 1989; Meunier & Huisseune, 1992; Cohen *et al.*, 2012; Dean & Shahar, 2012; Shahar & Dean, 2013), the evolutionary origin and phylogenetic distribution of this bone type has not been studied in detail.

Explaining the evolutionary origins of acellular bone requires an explicitly phylogenetic approach that can distinguish the role of adaptation from that of phylogenetic history in the distribution of bone types among species. This review aims to clarify the distribution of cellular and acellular bone in teleosts within a phylogenetic context that is now available thanks to an array of recently published large-scale molecular analyses (e.g. Near *et al.*, 2012; Betancur-R. *et al.*, 2013; Hughes *et al.*, 2018). We also review the structure of acellular bone, emphasising its functional similarity to cellular bone (Witten & Huisseune, 2009; Cohen *et al.*, 2012; Shahar & Dean, 2013; Currey *et al.*, 2017). Our review of the existing literature, complemented by our own observations, brings together most of the data published to date on actinopterygian bone to constitute a data set covering the whole diversity of the group. Including this data into an explicit phylogenetic framework for the first time, finally allows us to draw a possible historical scenario for the loss of osteocytes in teleosts.

II. TELEOST ACELLULAR BONE: STRUCTURE AND FUNCTION

(1) Structure and development

In teleosts, bone is found in the endoskeleton (the skeleton proper, i.e., cranial, axial and appendicular skeleton; Fig. 1A, C-F) and in the dermal exoskeleton, including scales. In teleosts, bone is found in the cranial, axial and appendicular skeleton (Fig. 1A, C-F) and in scales (Fig. 1B), lepidotrichia (fin rays) and the tissues that derive from them (Patterson, 1977; Schaeffer, 1977; Francillon-Vieillot *et al.*, 1990). Despite its structural peculiarities that led historical authors to improperly designate it under other names (e.g. 'osteoid', Kölliker, 1859), teleost acellular bone is considered true bone because it shares its developmental origin and main characteristics with every other vertebrate bone tissue (Moss, 1961b; Witten & Huisseune, 2009; Dean & Shahar, 2012): (1) it is composed of hydroxyapatite crystals in a mesh of type I collagen fibres; (2) it has the same functional properties as other bone tissues (muscle insertion and organ support); (3) its extracellular matrix is secreted by osteoblasts and resorbed by osteoclasts; (4) it can be submitted to active remodelling.

Typical cellular bone contains numerous mature osteocytes that, despite being completely surrounded by mineralised tissue, communicate with each other and with the bone surface *via* a network of canaliculi containing cytoplasmic processes (Fig. 1C, D). This lacunocanicular system permeates bone and gives osteocytes their characteristic star-shaped appearance (Meunier, 1987; Cao *et al.*, 2011). It is however not clear whether osteocytes form a proper lacunocanicular network in all teleosts with cellular bone (Fiaz, van Leeuwen & Kranenbarg, 2010; Totland *et al.*, 2011). In acellular bone, on the other hand, there are no osteocytes or lacunae within the bone mineral matrix (Fig. 1E, F), but it is sometimes penetrated by osteoblastic canaliculi from the bone surface (Francillon-Vieillot *et al.*, 1990; Sire & Meunier, 1994, 2017). In the 'tubular acellular bone' of a few taxa, tubules containing a bundle of collagen fibres and numerous osteoblastic cytoplasmic processes permeate acellular primary bone (Hughes, Bassett, & Moffat, 1994; Sire & Meunier, 2017; Meunier & Béarez, 2019).

These tubules are superficially similar, but structurally distinct from the canals of Williamson (Fig. 1D) that are known only from the cellular bone of holosteans and fossil stem teleosts (Williamson, 1849; Ørvig, 1951; Sire & Meunier, 1994; Meunier & Brito, 2004). Acellular bone can be vascular or avascular, osteoblastic canaliculi being more numerous in avascular acellular bone than in vascular acellular bone (Francillon-Vieillot *et al.*, 1990).

In cellular bone, osteocytes originate from osteoblasts that become surrounded by the mineral matrix they secreted (Franz-Odenaal *et al.*, 2006). Conversely, in acellular bone osteoblasts remain on the outer surface and secrete extracellular matrix exclusively towards the interior of bone, never ending up surrounded by bone to turn into osteocytes (Weiss & Watabe, 1979; Ekanayake & Hall, 1987, 1988). The hypothesis that acellular bone could form through intracellular mineralisation of osteocytes that are already entrapped in bone (Moss, 1961a) has been rejected since a study on the medaka *Oryzias latipes* (Ekanayake & Hall, 1987).

(2) Functional properties of acellular bone

(a) Mechanical properties

The mineral fraction in acellular bone is proportionally slightly higher than in cellular bone (Meunier, 1984a; Cohen *et al.*, 2012). This higher mineral content, along with the reduction in porosity associated with the absence of osteocytes have been hypothesised to increase the stiffness of acellular bone (Horton & Summers, 2009). However, comparative studies of structural stress have suggested that acellular and cellular bone have equivalent stiffness (Horton & Summers, 2009; Cohen *et al.*, 2012; Dean & Shahar, 2012; Currey & Shahar, 2013). On the other hand, the collagen fibre ultrastructure in acellular teleost bone gives it an increased toughness compared to tetrapod (e.g. human) cellular bone (Atkins *et al.*, 2015b).

(b) Resorption

Osteoclasts, the cells primarily responsible for bone resorption, were long thought to be absent from acellular teleost bone, although resorption was still observed (Blanc, 1953; Moss, 1963; Weiss & Watabe, 1979; Glowacki *et al.*, 1986). Indeed, osteoclasts in acellular bone are structurally different from the 'typical' osteoclasts found in cellular bone, explaining why they long went undetected: they are generally mononucleated instead of multinucleated as in cellular bone (Sire, Huysseune, & Meunier, 1990; Witten, 1997; Witten & Villwock, 1997; Witten & Huysseune, 2009). This structural difference may be explained by the absence of osteocytes, which promote the growth of multinucleated osteoclasts (Witten & Huysseune, 2009, 2010).

(c) Mineral metabolism

Bone plays a crucial role in calcium metabolism in vertebrates, both as a consumer and as a source of calcium. However, this role seems less critical in teleosts than in terrestrial vertebrates since, as aquatic animals, teleosts can mobilise calcium and other elements directly from the ambient water *via* their gills and/or digestive system (Takagi & Yamada, 1992; Witten & Huysseune, 2009; Shahar & Dean, 2013). Phosphorus availability appears to be more critical than that of calcium for healthy growth in both marine and freshwater teleosts (Witten & Huysseune, 2009; Shahar & Dean, 2013), and bone does not seem to mineralise when phosphorus is absent from the diet

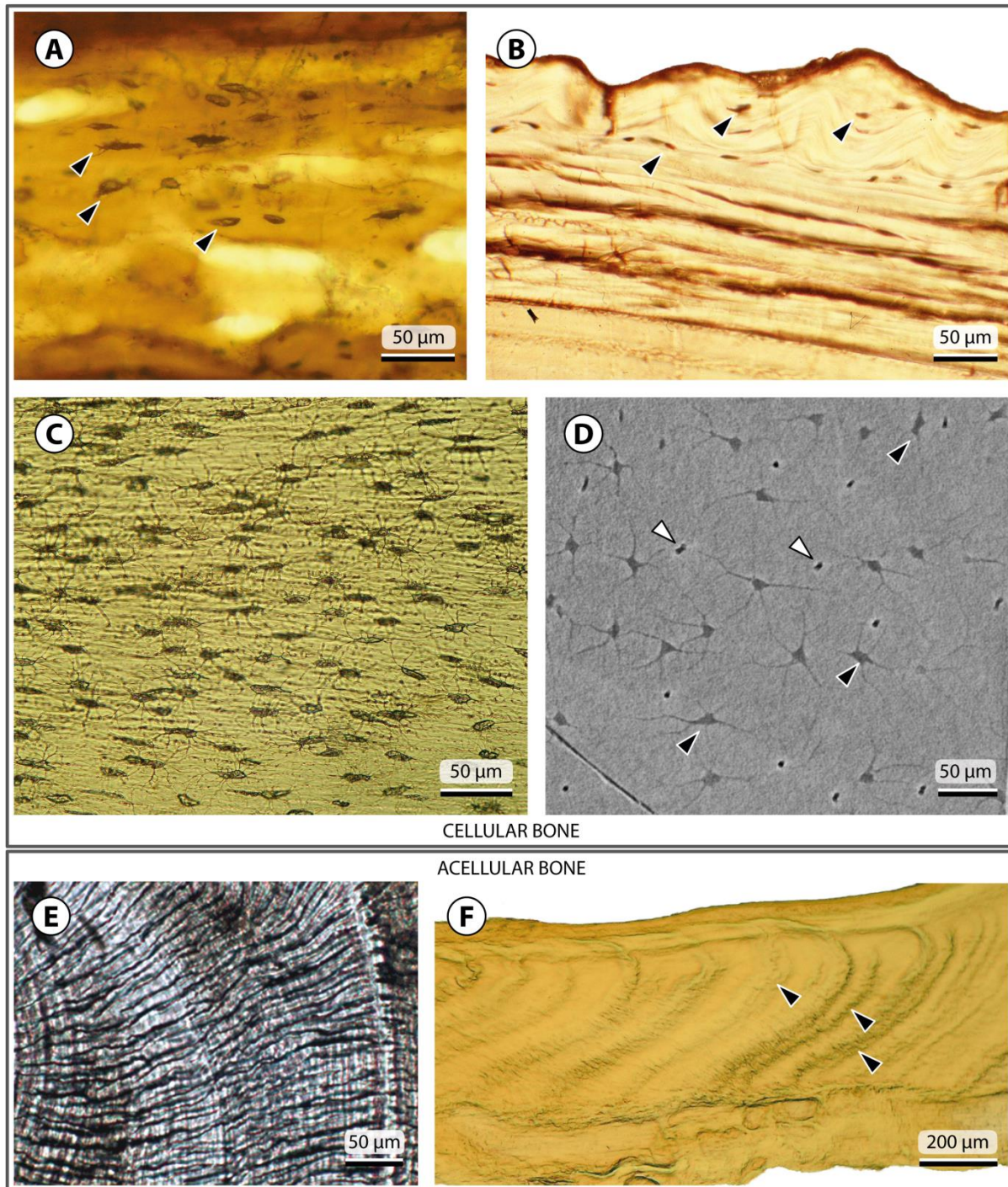


Fig. 1. Examples of cellular (A–D) and acellular (E, F) bone in teleosts and close relatives. (A) Ground section through cellular bone in the jaw of the Devonian actinopterygian †*Cheirolepis canadensis* (MHN 05-340), observed in transmitted natural light. Osteocyte lacunae are marked with black arrowheads. Modified from Meunier *et al.* (2018c). (B) Thin section through a scale of an osteoglossomorph, the arowana *Osteoglossum bicirrhosum*, observed in transmitted natural light. Osteocyte lacunae are visible in the superficial bony layer, and marked with black arrowheads. Photograph by F. J. Meunier. (C) Thin section through cellular bone in the rib of an ostariophysan, the barbel *Barbus barbus*, observed in transmitted natural light. Osteocyte lacunae and their associated lacunocanalicular network are clearly visible. Modified from Meunier & Herbin (2014). (D) ‘Virtual thin section’ obtained by stacking synchrotron

tomographic slices of the dentary of the Jurassic stem teleost †*Dorsetichthys bechei* (OUMNH J.3369). Star-shaped osteocyte lacunae and their canaliculi are visible (black arrowheads), as well as canals of Williamson in cross-section (white arrowheads). Image produced by D. Davesne and A. D. Schmitt. (E) Thin section through acellular bone in the rib of an acanthomorph, the sea bass *Dicentrarchus labrax*, showing numerous radially arranged osteoblastic canaliculi. Photograph by D. Davesne. (F) Thin section through acellular bone in the vertebra of an acanthomorph, the anglerfish *Lophius* sp. Bone is relatively featureless, apart from visible successive growth marks (black arrowheads). Photograph by F. J. Meunier.

(Witten *et al.*, 2016, 2018). Nevertheless, a specific type of bone resorption (osteocytic osteolysis) is undertaken by the osteocytes themselves and may be linked to periods of increased metabolic calcium and/or phosphorus requirement, as it occurs conspicuously in certain diadromous teleost species [e.g. European eel (*Anguilla anguilla*), salmoniforms] before and during migration (Kacem & Meunier, 2000, 2003; Sbahi *et al.*, 2007). In teleosts with acellular bone, osteocytic osteolysis is impossible, potentially making calcium and phosphorus more difficult to mobilise from and into the skeleton than in those with cellular bone (Moss, 1962; Simmons, Simmons & Marshall, 1970; Witten, 1997; Witten & Huysseune, 2009).

(d) Remodelling

Teleost bony tissues consist mainly of primary bone in most species (Meunier, 1987) and bone remodelling appears to be less abundant in teleosts than in tetrapods – it was even long thought to be absent (Moss, 1961a). Nevertheless, bone remodelling occurs in teleosts, in taxa with both cellular (Witten, Hansen, & Hall, 2001; Witten & Hall, 2003; Nemoto *et al.*, 2007; Witten & Huysseune, 2009) and acellular bone (Castanet & Ricqlès, 1986; Witten & Huysseune, 2009; Dean & Shahar, 2012; Shahar & Dean, 2013; Atkins *et al.*, 2014, 2015a; Currey *et al.*, 2017). For instance, hyperostoses are widespread in teleosts with acellular bone, and their formation requires an important remodelling activity (Meunier & Desse, 1986; Smith-Vaniz, Kaufman & Glowacki, 1995). In billfishes (Istiophoriformes), that lack osteocytes, bone in the rostrum is riddled with secondary osteons overlapping primary osteons, akin to what is found in the haversian bone of tetrapods and suggesting very intense remodelling activity as a response to fracture and load (Amprino & Godina, 1956; Poplin, Poplin & Ricqlès, 1976; Castanet & Ricqlès, 1986; Atkins *et al.*, 2014). These examples suggest that, in the absence of osteocytes as sensors, acellular bone is nevertheless capable of detecting strain and damage by some mechanism that is yet not fully understood (Kranenbarg *et al.*, 2005; Witten & Huysseune, 2009; Fiaz *et al.*, 2010; Dean & Shahar, 2012; Shahar & Dean, 2013; Atkins *et al.*, 2014, 2015a).

In its general structure, biomechanics, and mechanisms of bone resorption and remodelling, acellular teleost bone then appears to be functionally very similar to cellular teleost bone. This suggests that the presence of osteocytes is not strictly necessary to achieve these functions. This leaves osteocytic osteolysis, a potentially important mechanism involved in calcium and/or phosphorus metabolism (Witten & Huysseune, 2009; Cohen *et al.*, 2012; Shahar & Dean, 2013; Doherty, Ghalambor & Donahue, 2015), as the main function entirely lacking in acellular bone.

III. PHYLOGENETIC DISTRIBUTION OF ACELLULAR BONE

(1) Acellular bone outside of actinopterygians

(a) Palaeozoic jawless vertebrates

A peculiar bone-like tissue devoid of osteocytes, called aspidin, has long been known in the dermal skeleton of †heterostracans, a group of Palaeozoic jawless vertebrates (Gross, 1930; Halstead, 1969). Similar tissues were later described in other early jawless stem gnathostome lineages, such as †anaspids, †thelodonts and †galeaspids (Stensiö, 1958; Sire, Donoghue & Vickaryous, 2009; Keating & Donoghue, 2016). Aspidin appears to be structurally very similar to teleost acellular bone, with probable collagen bundles (akin to the ‘tubules’ of teleosts) penetrating the mineralised tissue (Keating *et al.*, 2018). The occurrence of either cellular or acellular bone in various early vertebrate lineages (Fig. 2) led to a debate over which one was phylogenetically older (Ørvig, 1951; Denison, 1963; Halstead, 1963; Smith & Hall, 1990). The earliest vertebrates with cellular bone are the jawless †osteostracans that appear in the Silurian (Stensiö, 1958; Smith & Hall, 1990; Donoghue & Sansom, 2002), although osteocytes have also been described in the dermal bone of a late Ordovician †arandaspid (Sansom *et al.*, 2013). Abundant evidence supports the placement of †osteostracans as the sister group to gnathostomes (jawed vertebrates): it seems likely that cellular bone would then be a synapomorphy of the clade uniting †osteostracans and gnathostomes (Donoghue & Sansom, 2002; Brazeau & Friedman, 2014), with a potential convergent appearance in †arandaspids (Fig. 2). This would imply that bone in †anaspids, †thelodonts, †heterostracans and †galeaspids is primitively devoid of osteocytes, making acellular bone the plesiomorphic state for skeletonising vertebrates (Denison, 1963; Halstead, 1963, 1969; Donoghue & Sansom, 2002; Keating *et al.*, 2018).

(b) Jawed vertebrates

As a plesiomorphic character for gnathostomes (Fig. 2), cellular bone is found in Palaeozoic jawed stem gnathostomes such as †‘placoderms’ (Ørvig, 1951; Downs & Donoghue, 2009; Sire *et al.*, 2009; Giles, Rücklin & Donoghue, 2013) and in fossils interpreted as stem osteichthyans, such as †*Andreolepis*, †*Lophosteus* and †*Psarolepis* (Jerve *et al.*, 2016; Qu *et al.*, 2017). Bone is cellular in sarcopterygians, the sister group to actinopterygians, including modern coelacanths, modern lungfishes, modern tetrapods (lissamphibians, mammals, diapsids) and fossil taxa falling on their respective stem groups (Sire *et al.*, 2009; Zylberberg, Meunier & Laurin, 2010; Schultze, 2016; Meunier, Cupello & Clément, 2019).

On the other hand, acellular bone also occurs in different gnathostome lineages. A prominent example is the basal bone layer in the odontodes of various chondrichthyans (cartilaginous fishes) and their close relatives, including Palaeozoic †‘acanthodians’ (Sire *et al.*, 2009; Chevrinais, Sire & Cloutier, 2017). Acellular perichondral bone is also found in the modified dorsal fin of the Palaeozoic stem holocephalan †*Akmonistion* (Coates *et al.*, 1998), while the fin rays of the African lungfish *Protopterus* are composed of acellular dermal bone (Géraudie & Meunier, 1984). Finally, acellular bone is found in very localised zones of specialised tissues in a few tetrapods, for example in cranial bones and sutures of †pachycephalosaurid and †ceratopsian dinosaurs (Goodwin & Horner, 2004; Bailleul & Horner, 2016). In all these taxa, acellular bone is found exclusively in dermal bone, leaving teleosts as the only known vertebrates with occurrence of acellular endochondral bone.

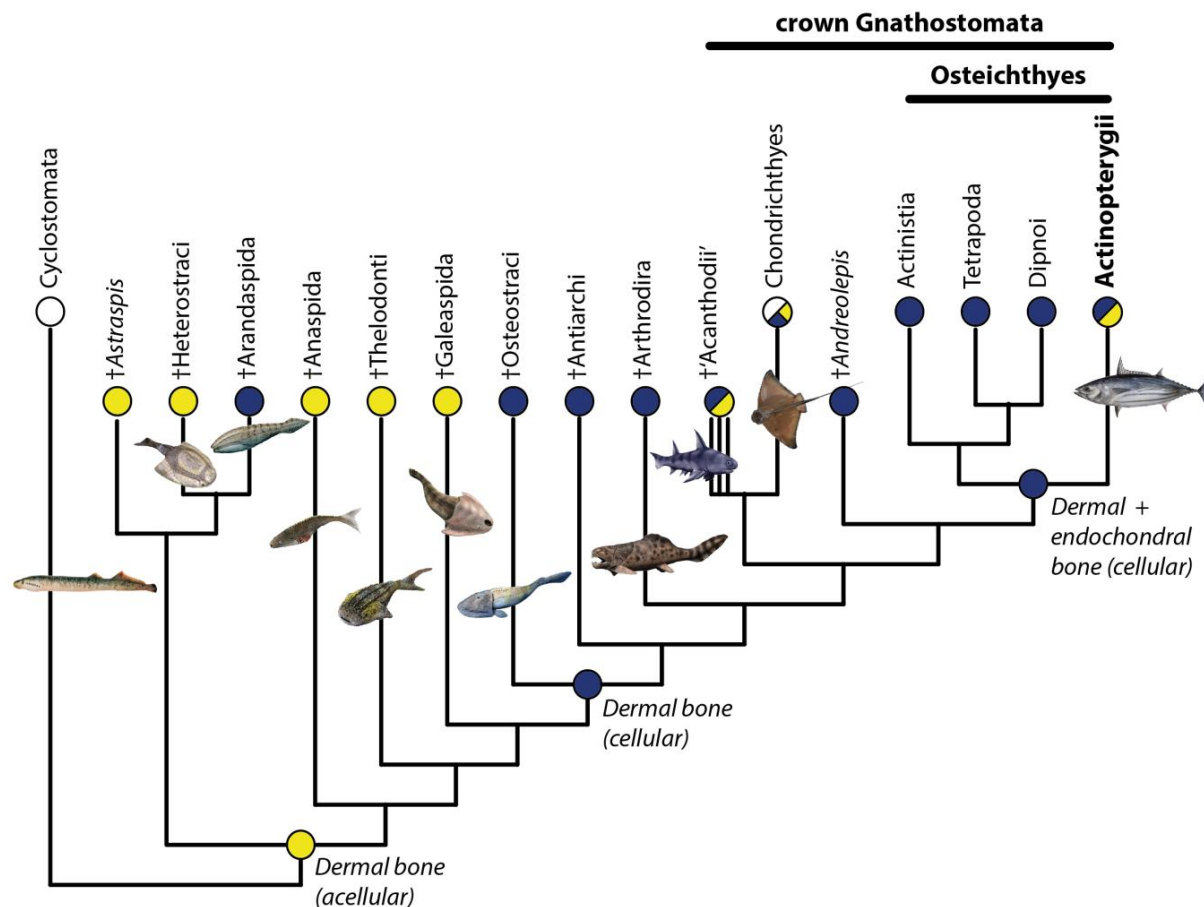


Figure 2. Distribution of cellular and acellular bone in the phylogeny of vertebrates (modified from Keating *et al.*, 2018). The coloured circles at the tip of branches reflect bone type in the clade: acellular (yellow), cellular (dark blue), or bone absent (white). Taxon pictures from N. Tamura, and Iglésias (2014a, 2014b).

(2) Phylogenetic distribution of acellular bone in teleosts and other actinopterygians

(a) Material of study

To evaluate the phylogenetic distribution of cellular and acellular bone in actinopterygians, we reviewed more than 150 years of literature on ray-finned fish bone. The most comprehensive sources of information were the extensive surveys by K  lliker (1859) and Moss (1961*b*, 1965), to which we added data from various fossil and extant species where required to better resolve the phylogenetic and temporal distribution (see online Supporting information, Table S1, for details on these sources). In total, our database includes 677 fossil and extant taxa. In addition, we obtained propagation phase contrast synchrotron microtomography (PPC-SR  CT) data from museum specimens of 108 extant and fossil species (Table 1, Table S1), bringing new information or corroborating our knowledge on the presence or absence of osteocytes in their bones. The SR  CT scans were carried out at the ID-19 (microtomography) beamline of the European Synchrotron Radiation Facility (ESRF), using a white beam with energy levels between 35 and 105 keV, obtaining a voxel size of 0.72   m.

For all extant and fossil taxa, we used the dentary as a bone of study (and in some cases, a rib). This bone appears to be cellular, even when both bone types coexist in the skeleton (Weigle & Franz-Odenaal, 2016). We then consider that the lack of osteocytes in the dentary is likely to reflect genuine acellularity in a given taxon.

(b) *Non-teleost actinopterygians*

Cellular bone is present in the earliest actinopterygians from the Devonian (Table S1): for example, in the bones and scales of †*Cheirolepis* (Zylberberg, Meunier & Laurin, 2016) and †*Moythomasia* (Sire *et al.*, 2009; Schultze, 2016) and in the scales of †*Mimipiscis* (Richter & Smith, 1995). Bone and scales are always cellular in modern non-teleost actinopterygians, for example in bichirs (Polypteriformes), bowfins and gars (Holostei) (Kölliker, 1859; Moss, 1961*b*; Sire & Meunier, 1994; Daget *et al.*, 2001; Sire *et al.*, 2009) and in their Mesozoic fossil relatives (Goodrich, 1907; Ørvig, 1978; Gayet & Meunier, 1992; Meunier & Brito, 2004; Meunier *et al.*, 2016). Sturgeons and paddlefishes (Acipenseriformes) have a poorly mineralised skeleton, but it is nonetheless composed of cellular bone (e.g. Kölliker, 1859; Stéphan, 1900; Buffrénil *et al.*, 2016; Leprévost *et al.*, 2017). Finally, many clades of extinct Mesozoic actinopterygians have been surveyed histologically and show cellular bone, for example: †saurichthyids (Scheyer *et al.*, 2014), †aspidorhynchids (Bruto & Meunier, 2000), †pachycormids (Meunier & Brito, 2004; Liston *et al.*, 2013), †pholidophorids (Meunier & Brito, 2004). Our SRμCT data provide additional information on a series of fossil non-teleost actinopterygians, revealing the presence of cellular bone in the Jurassic stem chondrosteian †*Chondrosteus acipenseroides*, the Jurassic †pseudodontiform †*Proscinetes elegans*, the Jurassic †dapediid †*Dapedium* sp., the Triassic holosteans †*Heterolepidodus dorsalis* and †*Eoeugnathus megalepis* and the Jurassic stem bowfin †*Caturus furcatus*. These data also confirm the presence of cellular bone in 17 Jurassic and Cretaceous taxa (Tables 1, S1) interpreted as stem-group teleosts (e.g. Arratia, 2015).

(c) *Elopomorpha*

Within Elopomorpha, cellular bone is found in tarpons and their relatives (Elopiformes), including in scales (Kölliker, 1859; Meunier & Brito, 2004). Several eels (Anguilliformes) are described as having acellular bone by Kölliker (1859). However, they all seem to pertain to an outdated taxonomy that treated leptocephalus larvae as separate taxa (Table S1). For example, Kölliker (1859) reports cellular bone in the sorcerer eel *Nettastoma melanurum* and acellular bone in '*Hyoprurus messanensis*', corresponding to the larva of *N. melanurum* (Eschmeyer, Fricke & van der Laan, 2018). Although Moss (1961*b*) reports acellular bone in the moray eel *Gymnothorax moringa*, we confirm the presence of osteocytes in this species, as well as in the adults of every other anguilliform surveyed, including the freshwater eels *Anguilla anguilla* and *A. rostrata* (Stéphan, 1900; Moss, 1965; Lopez, 1970), the conger eel *Conger conger* and the pike conger *Muraenesox cinereus* (Table 1). The bonefish *Albula vulpes* was described as having a mix of cellular and acellular bone (Moss, 1961*b*), but this is contradicted by our observations (see Section III.3a). Finally, our SRμCT data reveal cellular bone in several fossil albuliforms (e.g. †*Istieus*, †*Lebonichthys*), elopiforms (e.g. †*Ichthyemidion*, †*Anaethalion*, †*Flindersichthys*) and anguilliforms (†*Urenchelys*). In conclusion, we find that cellular bone is present in post-larval individuals of all elopomorphs surveyed so far.

(d) *Osteoglossomorpha*

Fossil and extant bony-tongue fishes (Osteoglossomorpha) have cellular bone in their skeleton, including scales (Kölliker, 1859; Meunier & Brito, 2004; Meunier, Brito & Leal, 2013a; Meunier, Dutheil & Brito, 2013b). Moss (1965) reported acellular bone in the two modern mooneye (Hiodontidae) species, *Hiodon alosoides* and *H. tergisus*. However, Kölliker (1859) described cellular bone in '*Hyodon claudulus*' that could be synonymised with *H. alosoides* (Eschmeyer *et al.*, 2018). We resolved this uncertainty using unambiguous observations of osteocyte lacunae in SRμCT images of dentaries and/or ribs from *H. alosoides*, *H. tergisus* and their Eocene close relative †*Eohiodon falcatus*, confirming the presence of cellular bone in hiodontids. We also find cellular bone in *Arapaima gigas*, in the arowana *Osteoglossum bicirrhosum* and its extinct Eocene relatives †*Brychaetus muelleri* and †*Phareodus encaustus*, as well as in the featherback *Chitala chitala* (Table 1). In conclusion, it is likely that cellular bone is present in all osteoglossomorphs (Table S1).

(e) *Clupeomorpha*

Herrings and their relatives (Clupeomorpha) appear to have cellular bone (Kölliker, 1859; Moss, 1961b, 1965). Although Moss (1961b) reported acellular bone in the anchovy *Anchoviella* sp. and the American shad *Alosa sapidissima*, he later updated this observation by reporting cellular bone in *A. sapidissima* and three other *Alosa* species (Moss, 1965). Our SRμCT data reveal cellular bone in all clupeomorphs surveyed (Table 1), including the Cretaceous †*Armigatus namourensis* and †*Ellimmichthys longicostatus* and the Eocene †*Knightia* sp., as well as the extant wolf-herring *Chirocentrus dorab*, the Pacific sardine *Sardinops sagax* and the alewife *Alosa pseudoharengus*. In conclusion, it is likely that cellular bone is present in all clupeomorphs (Table S1), with the possible exception of *Anchoviella* that needs further appraisal.

(f) *Ostariophysii*

Kölliker (1859) and Moss (1961b, 1965), extensively sampled the considerable diversity of the mostly freshwater ostariophysans, including milkfishes (Gonorhynchiformes), carps and relatives (Cypriniformes), characins and relatives (Characiformes), catfishes (Siluriformes) and electric 'eels' (Gymnotiformes). Their surveys totalled 115 species, virtually all of which appear to have cellular bone (Table S1). We also observed cellular bone in our SRμCT images of the carp *Cyprinus carpio*, the tench *Tinca tinca*, the bream *Abramis brama* (Cypriniformes), the trahira *Hoplias malabaricus*, the payara *Hydrolycus scomberoides*, the piranha *Serrasalmus spilopleura* (Characiformes), the catfishes *Ariopsis felis*, *Galeichthys feliceps* and *Pimelodella gracilis* (Siluriformes), and the banded knifefish *Gymnotus carapo* (Gymnotiformes), as well as in the Early Cretaceous gonorhynchiform †*Tharrias araripes* (Table 1). Acellular bone is only described in two ostariophysan species (Table S1): in the diminutive pencil catfish *Trichomycterus punctulatus* (Kölliker, 1859), which is confirmed by our SRμCT data from the dentary of another *Trichomycterus* species, and in some cranial dermal bones of the zebrafish *Danio rerio* (Weigle & Franz-Odenaal, 2016). In conclusion, cellular bone is present in all ostariophysans surveyed so far, with the notable exceptions of *Trichomycterus*. In addition, slickheads (Alepocephaliformes) are consistently recovered as sister to ostariophysans in molecular phylogenies (Lavoué *et al.*, 2008; Near *et al.*, 2012;

Betancur-R. *et al.*, 2013; Straube *et al.*, 2018; Hughes *et al.*, 2018). The only species surveyed from the group, *Alepocephalus rostratus*, has cellular bone (Kölliker, 1859).

(g) *Non-neoteleost Euteleostei*

Bone type is variable amongst Euteleostei, but generally homogeneous within a given lineage (Table S1). Acellular bone is found in galaxiids (but only two species of *Galaxias* have been surveyed), pikes and mudminnows (Esociformes; Kölliker, 1859; Moss, 1961*b*, 1965), smelts (Osmeridae; Moss, 1961*b*, 1965) and viperfishes and their relatives (Stomiiformes; Kölliker, 1859; Germain, Schnell & Meunier, in press). Conversely, cellular bone is found in *Argentina silus* (the only member of Argentiniformes that was sampled) and we observe it in the Late Cretaceous †*Spaniodon elongatus*, a taxon whose phylogenetic position within euteleosts is uncertain (e.g. Taverne & Filleul, 2003). Salmons, trouts and their relatives (Salmoniformes) are generally described as having cellular bone (Kölliker, 1859; Moss, 1961*b*, 1965; Hughes *et al.*, 1994; Witten & Hall, 2002; Totland *et al.*, 2011), but our extensive SRμCT sampling within the group complicates this pattern (Table 1). Bone appears always to be cellular in the ‘typical’ trouts and salmon (Salmoninae). We confirm this for extant and fossil representatives of *Oncorhynchus*, *Salmo*, *Salvelinus* and *Parahucho*. The whitefishes *Coregonus reighardi*, *Prosopium williamsoni* and *Stenodus leucichthys* (Coregoninae) also seem to have osteocytes, but they are much scarcer than in salmonines, and irregularly distributed inside of bone. This is consistent with the observation of Moss (1965), who described variation in osteocyte abundance within the skeleton in some salmoniforms. Finally, in the grayling *Thymallus thymallus* (Thymallinae), bone seems to be acellular.

(h) *Neoteleostei, including Acanthomorpha*

Within the euteleost subclade Neoteleostei (*sensu* Betancur-R. *et al.*, 2017), acellular bone is found in various lizardfishes (Aulopiformes), including the Late Cretaceous †*Eurypholis* sp., in the lanternfish (Myctophiformes) *Notoscopelus elongatus* and in the Cretaceous genus of uncertain placement †*Ctenothrissa vexillifer* (Kölliker, 1859; Moss, 1961*b*; Davesne *et al.*, 2018). Spiny-rayed fishes (Acanthomorpha) contribute the greatest fraction of neoteleost species diversity. Amongst the approximately 17,000 acanthomorph species (more than 300 being surveyed in the present study), acellular bone is virtually universal (Kölliker, 1859; Moss, 1961*b*, 1965) and is found throughout taxa displaying a broad range of morphologies and ecologies (Table S1), from marine benthic taxa such as toadfishes and sculpins (Simmons *et al.*, 1970; Horton & Summers, 2009), to pelagic fast-swimming taxa like jacks and billfishes (Smith-Vaniz *et al.*, 1995; Atkins *et al.*, 2014), deep-sea eelpouts (Meunier & Arnulf, 2018), or freshwater ricefishes and tilapias (Ekanayake & Hall, 1987; Cohen *et al.*, 2012). Within acanthomorphs, cellular bone is only known conclusively in two relatively species-poor lineages: the ‘true’ tunas *Auxis*, *Euthynnus*, *Katsuwonus* and *Thunnus* (Kölliker, 1859; Stéphan, 1900; Amprino & Godina, 1956; Moss, 1961*b*; Meunier & Huysseune, 1992; Santamaria *et al.*, 2018) and the opah *Lampris* (Davesne *et al.*, 2018). At least in tunas, osteocytes are present not only in bones, but also in scales, fin rays and spines (Meunier *et al.*, 2008*a*; Wainwright, Ingersoll & Lauder, 2018; Santamaria *et al.*, 2018).

(3) Intra-specific and intra-individual variation

(a) Occurrence of mixed bone types

Comparative literature generally states that when cellular or acellular bone is found, it occurs throughout the whole skeleton, including dermal and endochondral bone, fin rays and spines (Kölliker, 1859; Moss, 1961*b*, 1963; Meunier & Huysseune, 1992). The incompletely mineralised elasmoid scales of most modern teleosts are an exception: they are often acellular when the rest of the skeleton is cellular (see Section III.4). Moss (1961*b*) reported that the bonefish *Albula vulpes* displayed a mix of cellular and acellular bone, with the latter being found in the operculum and gill arches. However, our SRμCT data including the operculum and gill arches show osteocytes in all of these elements. These observations suggest that the whole skeleton of *A. vulpes* is cellular, contradicting Moss' (1961*b*) statement.

Weigle & Franz-Odenaal (2016) showed that in the zebrafish *Danio rerio*, bones with and without osteocytes coexist within the cranial skeleton of a given individual. Both dermal and endochondral bones can be cellular or acellular, but dermal intramembranous bones of the neurocranium seem more likely to be acellular, while endochondral bones of the splanchnocranium (i.e. palatoquadrate, hyoid and branchial arches) are all cellular. These results imply that using only the dermal neurocranium to describe bone type in a teleost species can potentially be misleading. Conversely, jaw bones (such as the dentary) and the postcranium (vertebrae excepted) are all cellular in *D. rerio* (Weigle & Franz-Odenaal, 2016). This suggests that our SRμCT data (Table 1), which rely on dentaries and/or ribs, accurately reflect cellularity: if acellular bone is found in these elements it is most likely to reflect the rest of the skeleton.

A possibility is that this pattern of mixed bone types stems from the very small adult body size of *D. rerio*. In very thin bones, there might not be enough bone matrix for osteoblasts to become entrapped and turn into osteocytes. For instance, some of the acellular bones observed in *D. rerio* are approximately 10 μm thick (Weigle & Franz-Odenaal, 2016), in an animal which rarely exceeds 40 mm in total adult length (Spence *et al.*, 2008). While the frontal bone is described as acellular in *D. rerio* (Weigle & Franz-Odenaal, 2016), we observe with SRμCT osteocytes in the frontal bone of the carp *Cyprinus carpio*, a closely related cypriniform. Since this observation comes from a carp of 452 mm in total length, it would corroborate our hypothesis of a size-related acellularity in *D. rerio*, and potentially other teleosts. Surveying various cranial bones in other teleost taxa and on specimens of various sizes would help clarify whether this pattern of mixed bone types is widespread in teleosts, or specific to *D. rerio*. Observations based on ontogenetic series of other taxa also corroborate that the absence of osteocytes might be explained by the size of the bone. For example, Huysseune (2000) reports that very young individuals of teleosts with cellular bone often lack osteocytes, which appear once bone becomes thicker. This would also explain Kölliker's (1859) observations of acellular bone in larval anguilliforms (see Section III.2*c*).

(b) Alleged osteocytes in tubular and hyperostotic bone

The presence of few osteocytes in very localised zones of otherwise acellular bone has been suggested for some species, relying upon two specific cases. In the first case, osteocytes were detected in tubules containing collagen bundles and osteoblastic canaliculi in three species of sparids (sea breams), an acanthomorph family otherwise

characterised by acellular bone (Hughes *et al.*, 1994). However these results are seemingly contradicted by more recent data (Sire & Meunier, 2017): at least in the case of *Sparus aurata* these tubules do not appear to contain osteocyte nuclei. In the second case, osteocytes were described within areas of hyperostosis in the cleithrum of the jack *Caranx latus* (Smith-Vaniz *et al.*, 1995) and in dorsal pterygiophores of the oarfish *Regalecus russellii* (Paig-Tran, Barrios & Ferry, 2016), two acanthomorphs that otherwise have acellular bone. However, such osteocytes do not appear to be present systematically in acanthomorph hyperostotic bone: they are absent from the hyperostoses of the scabbardfish *Trichiurus lepturus*, the jack mackerel *Trachurus trachurus*, the sicklefish *Drepane africana*, the grunt *Pomadasys kaakan* and the searobin *Prionotus stephanophrys* (Desse *et al.*, 1981; Meunier & Desse, 1994; Meunier, Béarez & Francillon-Vieillot, 1999; Meunier, Gaudant & Bonelli, 2010). The black skipjack tuna *Euthynnus lineatus* has cellular bone in its hyperostotic vertebrae (Béarez, Meunier & Kacem, 2005), however this is consistent with the presence of cellular bone throughout the rest of its skeleton. The occurrence of osteocytes in hyperostotic regions of an otherwise acellular skeleton then appears to be the exception rather than the rule; it nevertheless requires explanation. A possibility is that these localised osteocytes could form *via* an accidental incorporation of osteoblasts during the exceptionally rapid growth of hyperostotic bone. This arrangement may be temporary and accidental, and would differ from ‘true’ cellular bone. Determining whether these osteocytes are present in all hyperostotic individuals of a given species, for example, would help to assess the nature of this phenomenon.

(4) Phylogenetic distribution of acellular bone in actinopterygian scales

The phylogenetic distribution of osteocytes in actinopterygian scales (Table S1) has been less studied than in the rest of the skeleton (Parenti, 1986). Scales in actinopterygians primitively consist of a bony basal plate covered by dentine and ganoine (an enamel-like tissue). The bony component remains as a thin external layer in the elasmoid scales of most teleosts (Francillon-Vieillot *et al.*, 1990; Meunier & Huysseune, 1992; Sire *et al.*, 2009). In ganoid scales, bone is always cellular, as shown in early actinopterygians (Richter & Smith, 1995; Sire *et al.*, 2009; Zylberberg *et al.*, 2016), bichirs (Daget *et al.*, 2001; Sire *et al.*, 2009), holosteans (Meunier, François & Castanet, 1978; Meunier *et al.*, 2016; Brito, Meunier & Gayet, 2000) and stem teleosts (Bruto & Meunier, 2000; Meunier & Brito, 2004). In elasmoid scales, found in all teleosts but also in amiids and the extant coelacanth *Latimeria* (Smith, Hobdell & Miller, 1972; Meunier, 1984b; Meunier *et al.*, 2008b; Sire *et al.*, 2009), the situation is more complex. In this type of scales, the basal layer develops into an incompletely mineralised plywood-like structure called elasmodine (previously described as isopedine). The basal layer in the scales in amiids and some teleosts (e.g. *Megalops*, *Hiodon*, *Arapaima*, *Chanos*) incorporates cells superficially similar to osteocytes, called elasmocytes (Meunier, 1984b, 1987; Meunier & Brito, 2004). The bony layer is cellular in the elasmoid scales of amiids (Meunier & Poplin, 1995), elopomorphs (e.g. *Megalops*, *Elops*, *Albula*) and at least some osteoglossomorphs (Meunier, 1984b; Meunier & Brito, 2004). It is, however, acellular in other taxa with cellular bone including clupeomorphs, ostariophysans and salmoniforms (Meunier, 1987; Meunier & Brito, 2004; Meunier, Sorba & Béarez, 2004; Sire *et al.*, 2009). Taxa with acellular bone always seem to have acellular scales as well (Kölliker, 1859). In the tunas *Thunnus alalunga* and *T. obesus* scales are composed of

cellular bone (Meunier & Sire, 1981; Wainwright *et al.*, 2018), in agreement with the rest of the skeleton. Since many teleosts with cellular bone lack osteocytes in their scales, it then seems that acellularisation in scales phylogenetically precedes that of the rest of the skeleton (Kölliker, 1859; Meunier, 1987; Meunier & Huysseune, 1992).

IV. PHYLOGENETIC ORIGIN AND EVOLUTION OF ACELLULAR BONE

(1) Ancestral character state reconstruction

For our entire data set of 677 fossil and extant actinopterygians, we scored the presence of cellular or acellular bone (Table S1; scales scored separately). When bone lacks osteocytes only in certain skeletal elements (e.g. teleosts with cellular bone but acellular scales) or ontogenetic stages (e.g. in larval anguilliforms) we scored its status as 'cellular'.

This data set was mapped onto three time-calibrated trees stemming from three recent multi-locus or phylogenomic studies of actinopterygian intra-relationships. Topology #1 (T1) was obtained in an analysis of nine nuclear protein-coding loci including 232 taxa, all extant (Near *et al.*, 2012); Topology #2 (T2) is based on an analysis (Betancur-R. *et al.*, 2013) of 20 nuclear and one mitochondrial loci including 1582 extant taxa, to which 240 fossil taxa were added based on previously argued phylogenetic placements (Betancur-R., Ortí & Pyron, 2015); Topology #3 (T3) was obtained from a transcriptomic analysis of 1721 exons (Hughes *et al.*, 2018). In order to achieve consistency in clade names, we relied on the phylogenetic classification proposed by Betancur-R. *et al.* (2017), itself based on the molecular phylogeny that yielded T2.

All three topologies mostly differ at the level of the first dichotomies within Euteleostei. They all recover an Osmeriformes + Stomiiformes clade (Stomiati) and a Salmoniformes + Esociformes clade, but Galaxiiformes are sister to Neoteleostei *sensu stricto* in T1, to Salmoniformes + Esociformes in T2, and to Stomiati in T3. Similarly, Argentiniformes are sister to Salmoniformes + Esociformes in T1, to this clade + Galaxiiformes in T2, and to Galaxiiformes + Stomiati in T3.

We used a sub-sample of taxa that are included in both our cellularity data set and at least one of the topologies. When two different species of the same genus were used in two different data sets, we considered the genus as a whole, since no case of variability of cell type between species of the same genus is known. This sub-sample retains 100 extant taxa for T1, 292 taxa including 26 fossils for T2, and 121 extant taxa for T3. Every major actinopterygian lineage is present in the resulting trees with a few exceptions for which osteohistological data are lacking entirely, such as the salamanderfish (Lepidogalaxiiformes) and the jellynose fishes (Ateleopodiformes). Ancestral states at the nodes were reconstructed with the *ace* function of the APE package in R (Paradis, Claude & Strimmer, 2004). Two models of ancestral character state estimations were tested: an 'all rates different' (ARD) model (that allows transitions from cellular to acellular and from acellular to cellular to have different frequencies) and a 'symmetrical' model (that constrains transition frequencies to be equal). The difference between the transition frequencies was very low even with the ARD model, but the Akaike information criterion (AIC) very slightly favoured the symmetrical model, leading us to apply the latter to our analyses.

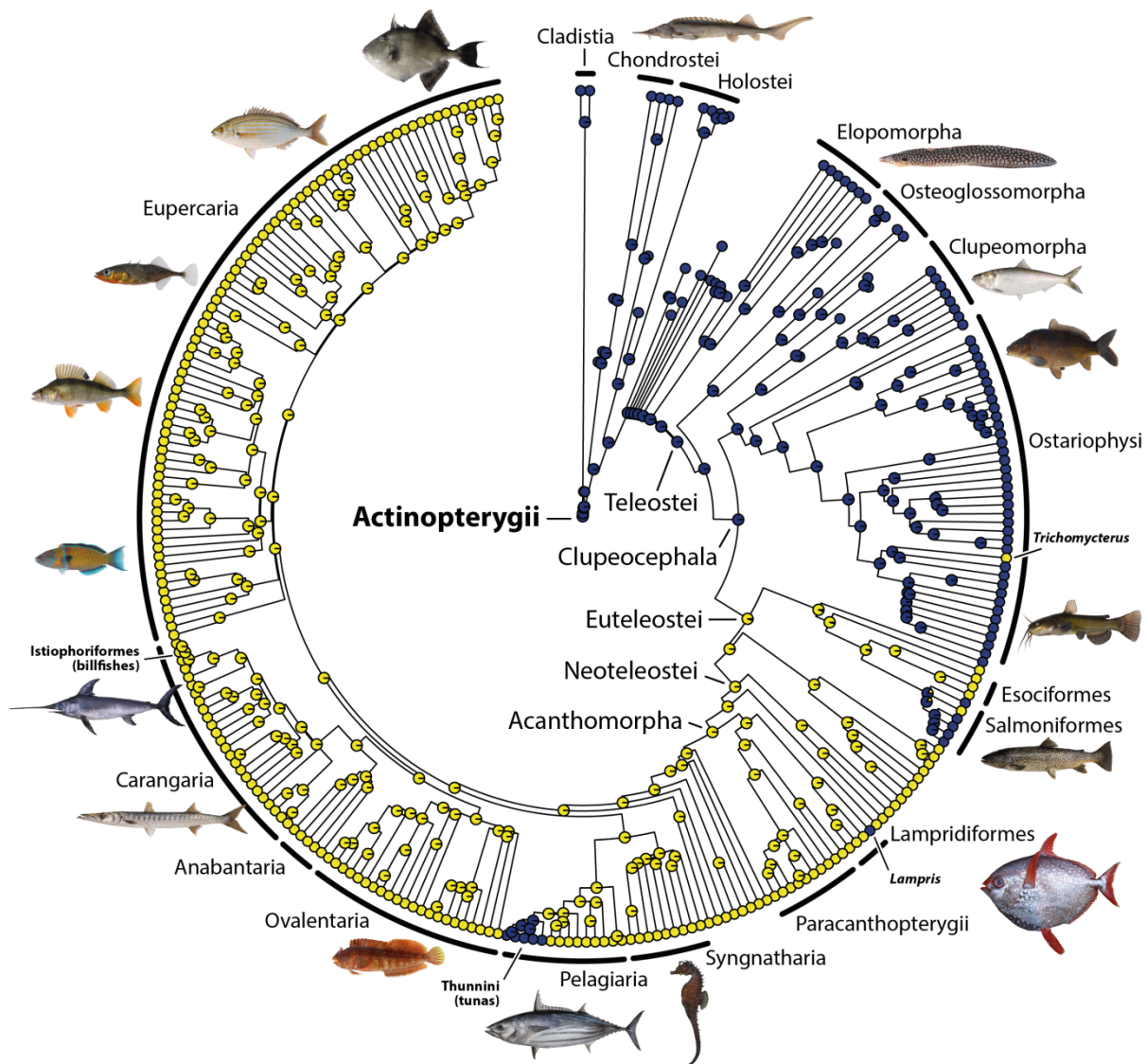


Figure 3. Time-calibrated multilocus tree of actinopterygians (ray-finned fishes), obtained from the optimisation of the character states 'cellular bone' (in dark blue) and 'acellular bone' in (yellow) on the topology T2 (Betancur-R. *et al.*, 2015). Character states for coded species are at the tips, and the reconstructed ancestral states at the nodes. A few key taxa, discussed in the text, are signalled in bold case. Taxon pictures are from Iglésias (2014b).

(2) Reconstructed origin of acellular bone

Results from all topologies recover cellular bone as the plesiomorphic state for actinopterygians, teleosts and every other node outside of Euteleostei, with a very high likelihood of 0.99 (Figs 3, S1–S3). T2 includes fossil taxa but they did not affect the ancestral state reconstructions, since those that were sampled all possess cellular bone in a region of the tree where it is also found in extant taxa (Fig. 3).

The reconstructed ancestral state for Euteleostei is ambiguous and varies from one topology to the other. With T1, the ancestral state for Euteleostei is equivocal. The likelihoods of the ancestral state being 'cellular' or 'acellular' are between 0.45 and 0.55

for three clades: Euteleostei, Argentiniformes + (Esociformes + Salmoniformes) and Esociformes + Salmoniformes (Figs 4, S1). In this scenario, whether cellular bone in argentiniforms and salmoniforms is a secondary reacquisition or the retention of an ancestral state is unclear.

With T2, the ancestral state for Euteleostei is acellular bone with a very high likelihood of 0.95 (Figs 3, 4, S2), implying that argentiniforms and salmoniforms both reacquired cellular bone secondarily and separately. T3 also implies an ancestral acellular bone for Euteleostei (and a secondary reacquisition of cellular bone in argentiniforms and salmoniforms), albeit with a slightly lower likelihood of 0.89 (Figs. 4, S3).

T1 and T2 were both produced with similar methods involving multi-locus molecular data sets adequately covering actinopterygian diversity, and it is difficult to establish whether one is more credible than the other. Phylogenetic resolution at the base of the euteleost tree is poor due to conflict between molecular markers and sparse taxon sampling, and remains a point of contention in the literature (Campbell *et al.*, 2017; Straube *et al.*, 2018; Hughes *et al.*, 2018).

Bone histology of the salamanderfish *Lepidogalaxias* has never been studied, but could be critical to accurately reconstruct the ancestral euteleostean state, since it is consistently recovered by molecular studies as the sister group to all other euteleosts (Li *et al.*, 2010; Near *et al.*, 2012; Betancur-R. *et al.*, 2013; Campbell *et al.*, 2017; Straube *et al.*, 2018; Hughes *et al.*, 2018). As long as the phylogeny of euteleosts is not stabilised, and the osteohistology of more taxa not sampled (e.g. other argentiniforms and galaxiids, *Lepidogalaxias*), ambiguity concerning the exact phylogenetic origin of acellular bone will remain. Certain early fossil euteleosts, such as the Late Cretaceous †*Spaniodon* (that has cellular bone) could also potentially play a key role in elucidating this character's evolution. However, their usefulness is hampered by even greater phylogenetic uncertainty than that for living lineages. For example †*Spaniodon* was included in a clade grouping esociforms, salmoniforms and osmeriforms in a phylogenetic analysis (Taverne & Filleul, 2003), but this topology is rejected by modern molecular phylogenies, leaving the position of this fossil taxon unknown. The megadiverse Neoteleostei (more than 18,000 extant species) are reconstructed as having acellular bone ancestrally with all three topologies (likelihood = 0.99; Figs 3, 4, S1–S3). Two distinct neoteleost lineages are reconstructed as having reacquired cellular bone independently: (1) the 'true' tunas *Auxis*, *Katsuwonus*, *Euthynnus* and *Thunnus*, forming the probably monophyletic tribe Thunnini within Scombridae; (2) the opah *Lampris* in the monotypic Lamprididae (Fig. 3).

In conclusion, the clade in which acellular bone appears is equivocal with our ancestral state reconstructions. T2 and T3 clearly support that acellular bone appears in Euteleostei, while the ancestral state for Euteleostei is equivocal with T1, leaving open the possibility of an independent appearance of acellular bone in Esociformes and in the clade that unites Stomiati, Galaxiiformes and Neoteleostei (Figs 4, S1–S3).

In any case, acellular bone is almost entirely absent outside of Euteleostei, being notably described in: (1) some larval anguilliforms, (2) the clupeiform *Anchoviella* sp., (3) certain cranial dermal bones of the cypriniform *Danio rerio*, and (4) the siluriform *Trichomycterus* sp. (see Section III.2; Fig. 3). It is noteworthy that all these occurrences correspond to either larvae or to taxa with characteristically small adult body sizes. A size-related explanation for the absence of osteocytes cannot be excluded in this context (see Section III.3a for an exploration in the case of *D. rerio*).

Acellular bone seems to appear phylogenetically earlier in scales than in the rest of the skeleton (Meunier, 1987; Meunier & Huysseune, 1992). Since acellular scales are described in clupeomorphs, ostariophysans and every euteleost with the exception of tunas (Table S1), we hypothesise that acellular scales are a character state of the clade Clupeocephala (i.e. all modern teleosts but elopomorphs and osteoglossomorphs). A systematic review of the histology of teleost scales is needed to test this hypothesis suitably. The nature of the external layer of teleost scales is controversial, and some authors have proposed that it has a different evolutionary origin to bone (e.g. Sire *et al.*, 2009), potentially explaining why cellularity is lost earlier in this tissue than in ‘true’ bone.

(3) Secondary reacquisition of cellular bone

(a) Probable occurrence in salmoniforms

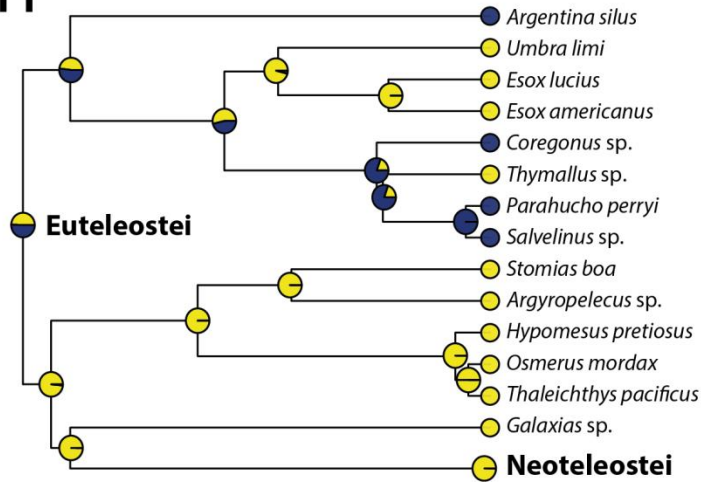
Our ancestral state reconstructions suggest that cellular bone was secondarily reacquired in salmon, trouts and their relatives (Salmoniformes), but this is equivocal due to topological uncertainty at the base of the euteleost tree (Fig. 4). The same reconstructions also equivocally support a separate secondary reacquisition of osteocytes in argentiniforms. However, since our data only rely on one species (*Argentina silus*) and the phylogenetic position of argentiniforms is highly uncertain, we refrain from commenting until more observations are available.

As described above, cellular bone does not seem to be distributed uniformly within salmoniforms, according to our SR μ CT data (Tables 1, S1): (1) in the grayling *Thymallus thymallus* (Thymallinae), we did not observe osteocytes conclusively; (2) in the shortnose cisco *Coregonus reighardi* (Coregoninae), osteocytes are present, but sparsely distributed within bone; (3) in *Stenodus leucichthys* (Coregoninae) and all observed Salmoninae, osteocytes are present, and uniformly distributed inside bone. Moss (1965) already noted that osteocyte abundance varies within bone elements in at least some salmoniforms, which has been interpreted by Parenti (1986) as a possible ‘intermediate’ stage between cellular and acellular bone.

Salmoniform phylogeny is currently disputed, particularly in the relationships between thymallines, coregonines and salmonines. Recent molecular studies have recovered three different topologies: Coregoninae + Salmoninae (Alexandrou *et al.*, 2013; Horreo, 2017), Thymallinae + Salmoninae (Near *et al.*, 2012; Crête-Lafrenière, Weir & Bernatchez, 2012; Betancur-R. *et al.*, 2013), and Coregoninae + Thymallinae (Campbell *et al.*, 2013; Macqueen & Johnston, 2014; Hughes *et al.*, 2018), also affecting the three topologies we used in our analyses. These competing phylogenies mean that the pattern of evolution of cellular bone in salmoniforms as a whole is uncertain.

Many salmoniforms are anadromous, meaning that sexually mature individuals migrate upstream over sometimes long distances. This behaviour involves intense and sustained swimming activity, which is likely to affect physiology and metabolism. How it influences bone growth and structure is not fully understood, but it appears that bone responds adaptively to the anadromous lifestyle. In the Atlantic salmon (*Salmo salar*), bones undergo halastasis (a diffuse demineralisation without degradation of the organic matrix) during spawning migration (Kacem & Meunier, 2003, 2009). In addition, *S. salar* shows a prominent increase in the volume of osteocyte lacunae in adult specimens compared to juveniles, which is probably explained by osteocytic osteolysis (Kacem & Meunier, 2000). Moreover, bone in salmon exposed to sustained swimming shows increases in

T1



Argentiniformes



Esociformes



Salmoniformes



Stomiiformes

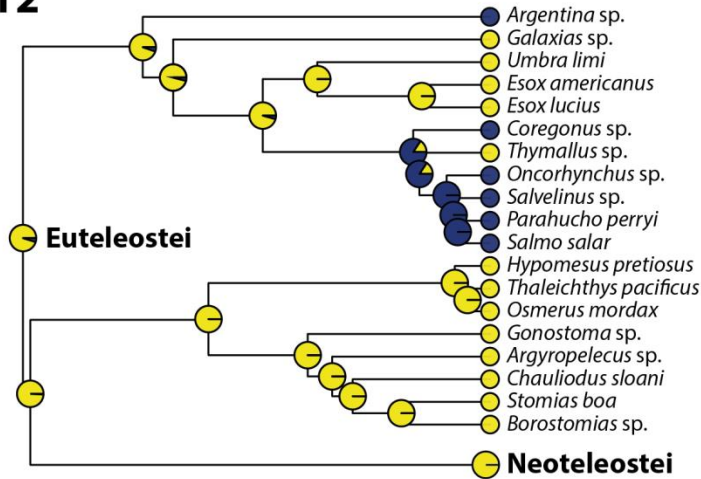


Osmeriformes



Galaxiiformes

T2



Argentiniformes
Galaxiiformes



Esociformes



Salmoniformes



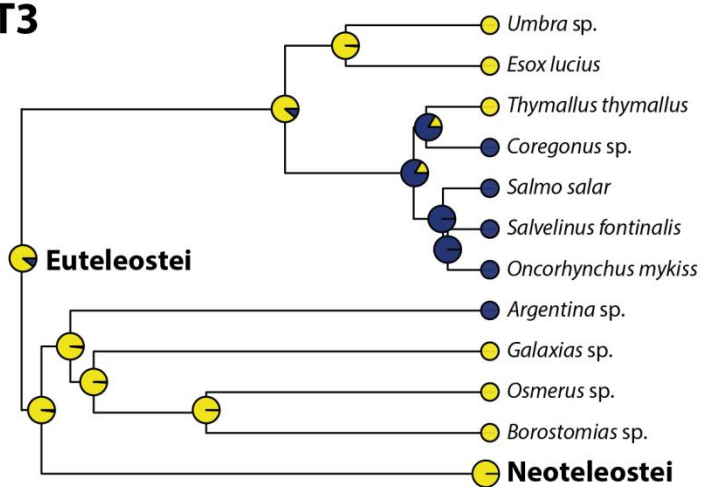
Osmeriformes



Stomiiformes



T3



Esociformes



Salmoniformes



Argentiniformes



Galaxiiformes

Osmeriformes



Stomiiformes



Figure 4. Sections of the time-calibrated multilocus trees obtained from the optimisation of the character states ‘cellular bone’ (in dark blue) and ‘acellular bone’ in (yellow) on topologies T1 (Near *et al.*, 2012), T2 (Betancur-R. *et al.*, 2015) and T3 (Hughes *et al.*, 2018), highlighting divergences at the level of the euteleost clade. Character states for coded species are at the tips, and the reconstructed ancestral states at the nodes. Taxon pictures are from Iglésias (2014b).

osteocyte abundance (Totland *et al.*, 2011). These observations support the hypothesis that osteocytes play an important role in resorbing salmon bone during anadromous migration.

Anadromy is likely to be a trait that evolved multiple times in various lineages within salmoniforms from strictly freshwater ancestors (McDowall, 1997, 2001; Alexandrou *et al.*, 2013). Anadromy is widespread in salmonines (especially in the clade formed by *Salmo*, *Oncorhynchus* and *Salvelinus*), and in most species of *Coregonus* (Alexandrou *et al.*, 2013). Osteocytes are also observed in all of these taxa, while they seem to be absent in the non-migrating freshwater *Thymallus* and in esociforms, the probable sister group to salmoniforms. The occurrence of cellular bone then roughly follows that of anadromy in this particular teleost clade. A notable exception occurs in the genus *Prosopium*, a non-migrating taxon that possesses cellular bone. Nevertheless, it is possible that the hypothesised reacquisition of cellular bone in at least some salmoniforms would have allowed or facilitated the evolution of anadromy in these animals, using a combination of halastasis and osteocytic osteolysis to function as a source of calcium and/or phosphorus for metabolism and muscle activity. A more extensive survey of bone histology in salmoniforms, especially for taxa that have not been studied so far (such as the non-migrating salmonines *Hucho* and *Brachymystax*), and in anadromous euteleosts outside of salmoniforms, is necessary to investigate the potential coevolution of bone cellularity with anadromous habits.

(b) Convergent occurrences in red-muscle endotherms

Unlike salmoniforms, there is no ambiguity that osteocytes were reacquired secondarily in two acanthomorph lineages (Figs 3, 5A): tunas and the opah (Davesne *et al.*, 2018). Tunas are scombrids, a family that molecular analyses place reliably into the clade Pelagiaria, itself included in the ultradiverse acanthomorph clade Percomorpha (Betancur-R. *et al.*, 2013, 2017; Near *et al.*, 2013; Miya *et al.*, 2013; Alfaro *et al.*, 2018). The ‘true’ tunas (Thunnini) consist of five genera (*Allothunnus*, *Auxis*, *Euthynnus*, *Katsuwonus* and *Thunnus*), and their monophyly is supported by morphological (Collette *et al.*, 1984; Carpenter, Collette & Russo, 1995) and most molecular phylogenies (Block *et al.*, 1993; Betancur-R. *et al.*, 2013; Miya *et al.*, 2013). The opah (*Lampris* sp.) is a lampridiform, a clade whose phylogenetic position within acanthomorphs is uncertain, but that branches outside of Percomorpha in any case (Betancur-R. *et al.*, 2013; Near *et al.*, 2013; Davesne *et al.*, 2014, 2016; Alfaro *et al.*, 2018). There is then clear evidence that the secondary reacquisition of osteocytes occurred independently in both lineages (Davesne *et al.*, 2018).

While cellular bone has long been known in tunas (Kölliker, 1859; Stéphan, 1900; Amprino & Godina, 1956; Moss, 1961b), fewer data were available on other scombrid taxa and acellular bone was known only from the Atlantic mackerel *Scomber scombrus* and the Spanish mackerel *Scomberomorus maculatus* (Kölliker, 1859; Amprino & Godina, 1956; Moss, 1961b). Our SR μ CT data allow us to confirm the absence of

osteocytes from the ribs of a larger sample of scombrids: the butterfly kingfish *Gasterochisma melampus*, the blue mackerel *Scomber australasicus*, the wahoo *Acanthocybium solandri*, the bonito *Sarda orientalis* and the dogtooth ‘tuna’ *Gymnosarda unicolor* (Table 1, Fig. 5C, D). *Sarda* and *Gymnosarda* are particularly relevant because they probably constitute the sister group to Thunnini (Collette *et al.*, 1984; Block *et al.*, 1993; Miya *et al.*, 2013). All of these taxa are outside of Thunnini, supporting that ‘true’ tunas are the only scombrids with cellular bone (Fig. 5A, E). Within lampridiforms, acellular bone has been described in the ribbonfishes *Trachipterus trachipterus* and *Zu cristatus* (Kölliker, 1859), in the oarfish *Regalecus russelii* (Paig-Tran *et al.*, 2016) and in the veliferid *Velifer hypselopterus* (Davesne *et al.*, 2018). Our SR μ CT data show that the veliferid *Metavelifer multiradiatus* also lacks osteocytes (Table 1), and veliferids are probably sister to all other lampridiforms (Olney, Johnson & Baldwin, 1993; Wiley, Johnson & Dimmick, 1998; Davesne *et al.*, 2014). The absence of osteocytes in veliferids, and in the Cretaceous stem lampridiform †*Aipichthys* *velifer* supports that acellular bone is plesiomorphic for lampridiforms (Davesne *et al.*, 2018). Thus, the opah is secondarily cellular within lampridiforms, akin to ‘true’ tunas within scombrids (Fig. 5A).

Tunas and the opah share many life-history traits, to which the reappearance of osteocytes could potentially be imputed. However, a closer examination of these traits across acanthomorph diversity reveals that most do not correlate with the presence of osteocytes. (1) Sustained, active swimming is also found in other large-bodied pelagic predators with acellular bone, such as carangids (Smith-Vaniz *et al.*, 1995), the dolphinfish *Coryphaena hippurus* (Moss, 1961*b*), billfishes (Kölliker, 1859; Amprino & Godina, 1956; Moss, 1961*b*; Atkins *et al.*, 2014) and several scombrids outside of ‘true’ tunas (Fig. 5A, C, D). (2) A large body size does not seem to be a factor either: within scombrids, the osteocytic bullet tuna *Auxis rochei* rarely exceeds 350 mm in total length as an adult (Collette & Nauen, 1983), while the dogtooth ‘tuna’ *Gymnosarda unicolor* and wahoo *Acanthocybium solandri* both commonly exceed 1000 mm in total length (Collette & Nauen, 1983) and are anosteocytic (Fig. 5D). Other very large pelagic acanthomorphs such as the oarfish *Regalecus* sp., billfishes, or the oceanic sunfish *Mola mola* (Kölliker, 1859) all have acellular bone as well. (3) Finally, the reacquisition of osteocytes does not seem to be linked with structural homeostasis: bone in tunas, opah and billfishes appears to have active, intense and sustained resorption and remodelling activities (Fig. 5B, D, E) evidenced by the extensive presence of secondary bone (Amprino & Godina, 1956; Poplin *et al.*, 1976; Castanet & Ricqlès, 1986; Atkins *et al.*, 2014; Davesne *et al.*, 2018). However, bone in billfishes is acellular (Fig. 5B), confirming that this intense remodelling activity does not require the presence of osteocytes (Atkins *et al.*, 2014; Currey *et al.*, 2017).

Conversely, a correlation between cellular bone and endothermy in acanthomorphs appears to be more substantiated (Meunier, 1987; Ricqlès *et al.*, 1991; Meunier & Huyseune, 1992; Davesne *et al.*, 2018). Our new SR μ CT data confirm that cellular bone co-occurs with a modification in the distribution and position of the lateral aerobic red muscles (Fig. 5A), that concentrate in the anterior portion of the body and become internalised within myotomes, coming closer to the axial skeleton; this configuration is unique to ‘true’ tunas amongst scombrids (Graham, Koehn, & Dickson, 1983; Block *et al.*, 1993; Graham & Dickson, 2000, 2004).

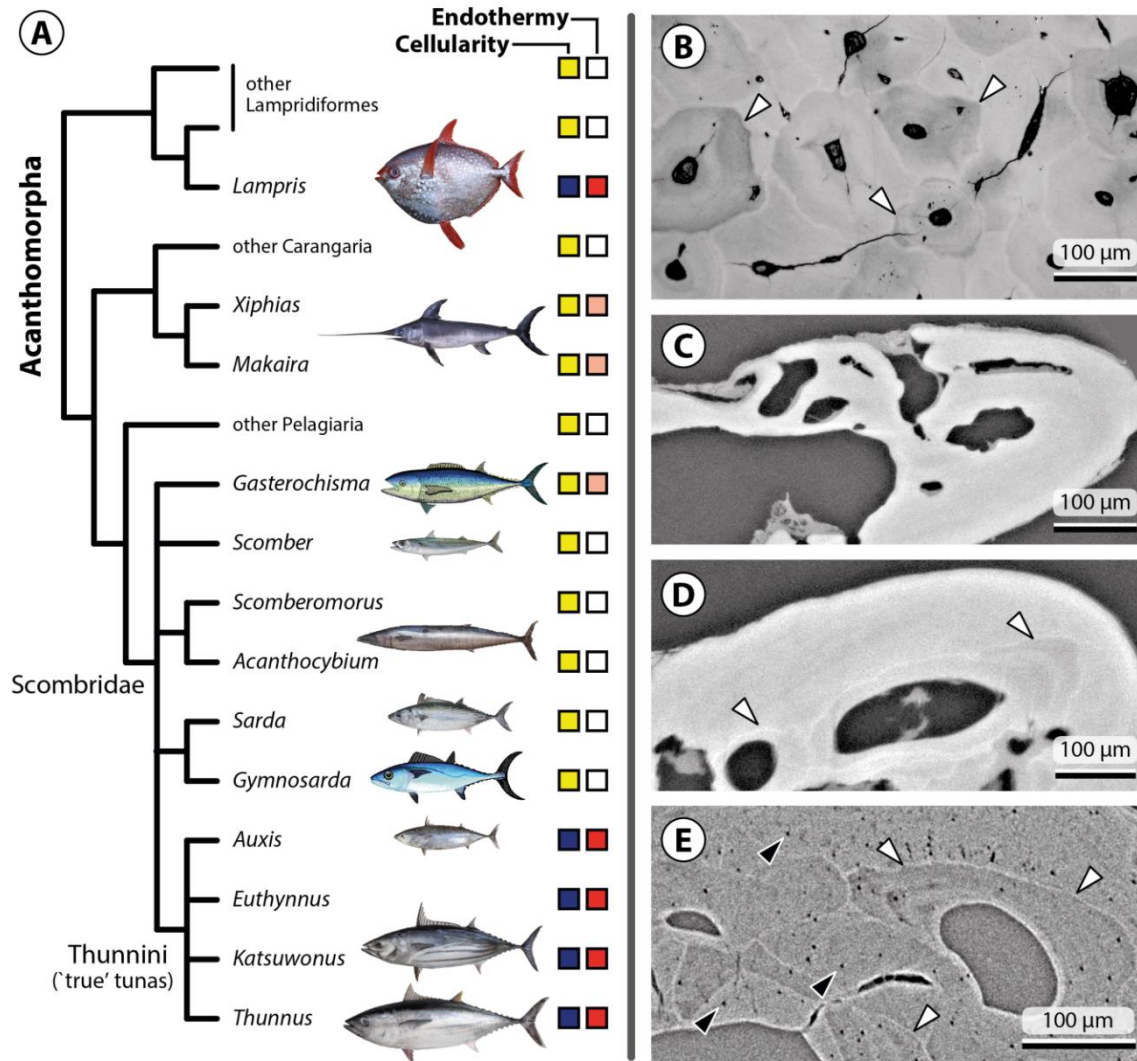


Figure 5. (A) Phylogenetic distribution of bone type in endothermic acanthomorph teleosts and their close relatives (modified from Davesne *et al.*, 2018). The squares represent bone type (acellular in yellow, cellular in dark blue) and thermal physiology (ectothermy in white, cranial endothermy in salmon pink, red-muscle endothermy in red). Taxon pictures from Iglésias (2014b) and R. N. Cada (www.fishbase.org). (B) Thin section through the rostrum of the marlin *Makaira nigricans*, a billfish. Bone is acellular, but shows secondary osteons delimited by resorption lines (white arrowheads). Photograph courtesy of A. Atkins. (C) Synchrotron tomographic slice in a rib of the butterfly kingfish *Gasterochisma melampus*, a scombrid (AMNH I-93480 SD). Bone is acellular. (D) Synchrotron tomographic slice in a rib of the dogtooth 'tuna' *Gymnosarda unicolor*, a scombrid (MNHN.ICOS.00492). Bone is acellular. Note secondary bone deposition around the blood vessels, delimited by resorption lines (white arrowheads). (E) Synchrotron tomographic slice in a rib of the 'true' tuna *Euthynnus affinis* (AMNH I-56274 SD). Bone is cellular (osteocytes marked with black arrowheads), with extensive deposition of secondary bone delimited by resorption lines (white arrowheads). (C–E) Images produced by D. Davesne.

This configuration is thought to be associated with heat production and retention (i.e. endothermy): the heat that is produced by muscle activity during swimming is insulated from the exterior and retained within the body due to a network of specialised blood vessels, named retia (Graham *et al.*, 1983; Graham & Dickson, 2001; Katz, 2002). This peculiar configuration has been called 'red-muscle endothermy' by various authors

(Block *et al.*, 1993; Dickson & Graham, 2004; Watanabe *et al.*, 2015). The opah developed a distinct form of red-muscle endothermy in which the red pectoral-fin muscles produce most of the heat, are insulated from the outside by a thick fatty layer, and the heat is kept and redistributed *via* retia located within the gills (Wegner *et al.*, 2015). A form of endothermy is also found in two other acanthomorph lineages: billfishes and the butterfly kingfish *Gasterochisma melampus*, a non-Thunnini scombrid (Fig. 5A). In these cases, heat is produced by specialised modified ocular muscles (the superior rectus in billfishes and the lateral rectus in *G. melampus*) that lost their contractile activity and cycle calcium ions between the cytoplasm and sarcoplasmic reticulum (Carey, 1982; Block, 1986, 1994; Dickson & Graham, 2004). Since it only warms the brain and the eyes, this configuration is often called ‘cranial endothermy’ (Dickson & Graham, 2004). Osteocytes are absent in the bill and ribs of billfishes (Atkins *et al.*, 2014), and our SR μ CT data failed to find them in a rib of *G. melampus* (Table 1, Fig. 5B,C), implying that cranial endotherms, unlike red-muscle endotherms, have acellular bone. We also observe acellular bone in the sclerotic ossicles of *G. melampus* and of the billfishes *Kajikia albida* and *Xiphias gladius* (Table 1), confirming that the cellularity of a bone is not affected by its proximity to the heat-generating muscles. In the opah, the sclerotic ossicles have cellular bone like the rest of the skeleton (Table 1). Heat production by red muscles involved in swimming (rather than modified ocular muscles) and redistribution in a large proportion of the body (rather than in the brain region only) is the key distinction between red-muscle and cranial endothermy. Given that both acanthomorph lineages that developed red-muscle endothermy are also the only ones that reacquired osteocytes, a correlation between these characters is likely (Davesne *et al.*, 2018). As for salmoniforms, we can hypothesise that the correlation stems from an intense muscular activity associated with sustained swimming. The latter is necessary both to hunt prey and to produce heat *via* the myotomal or pectoral red muscles. Since muscles are important consumers of calcium, an element primarily found in bony tissues, osteolytic osteolysis potentially played an important role in the appearance of red-muscle endothermic strategies. Whether the reacquisition of osteocytes facilitated the evolution of red-muscle endothermy, or both characters coevolved under a common selective pressure is unclear.

(c) Structural evidence for a re-acquisition in salmoniforms, tunas and opahs

While osteocyte morphology is very diverse in vertebrate bone in general, two main morphologies seem to occur in teleost cellular bone (Fig. 6). In the first type, osteocytes have a rounded or irregular cell body, and show numerous, thin cytoplasmic processes that branch into canaliculi in all directions. This gives these osteocytes a typically ‘star-shaped’ morphology (Fig. 6A). In the second type, osteocytes are much more elongate (‘spindle-shaped’) and orientate in a preferential direction, presumably following the collagen lamellae of the extracellular matrix (Kerschnitzki *et al.*, 2011). Their cell bodies are more regular in shape, and they have only two cytoplasmic processes that are located at the extremities of the cell body, aligning with its long axis. They also have very few, non-branching canaliculi, that tend to orientate in preferential directions (Fig. 6B, C). Both osteocyte types seem to coexist within teleost cellular bone, for example in *D. rerio* (Weigle & Franz-Odenaal, 2016). Conversely, in the bone of salmoniforms and ‘true’ tunas (Stéphan, 1900; Meunier & Huysseune, 1992; Totland *et al.*, 2011; Davesne *et al.*, 2018), the spindle-shaped osteocytes seem to be the only type that is present (Fig.

6B,C). In the opah, osteocytes are close to the ‘spindle-shaped’ morphology, since they have very few cytoplasmic processes and canaliculi that all orientate in a preferential direction, but they are not located at the extremities of the cell body like in tunas and salmoniforms (Fig. 6D). It is not clear whether this second type of osteocytes forms a connected canalicular system; at least in salmonids they might not be connected to each other at all (Totland *et al.*, 2011). Moreover, their morphology does not seem to change significantly between primary and remodelled bone, for example in the opah (Davesne *et al.*, 2018).

It appears that the three lineages that have in common an inferred or likely reacquisition of cellular bone share these structural similarities in osteocyte morphology. This suggests that their peculiar morphology might be linked with the evolutionary reacquisition of osteocytes from an ancestral acellular bone. Weigle & Franz-Odenaal (2016) proposed that these types of osteocytes have different developmental origins, and that the elongate, spindle-shaped osteocytes are derived from the elongate ‘osteoblast-like’ cells that line the bone. It is possible that all secondarily reacquired osteocytes share this unique developmental origin, and that the other, ‘typical’ osteocytes derive from a mode of formation that does not occur in salmoniforms, tunas and opahs and was possibly lost at the euteleost node. Structural similarities in osteocyte morphology appear further to support that their reacquisition is underlined by shared, and not fully understood, mechanisms.

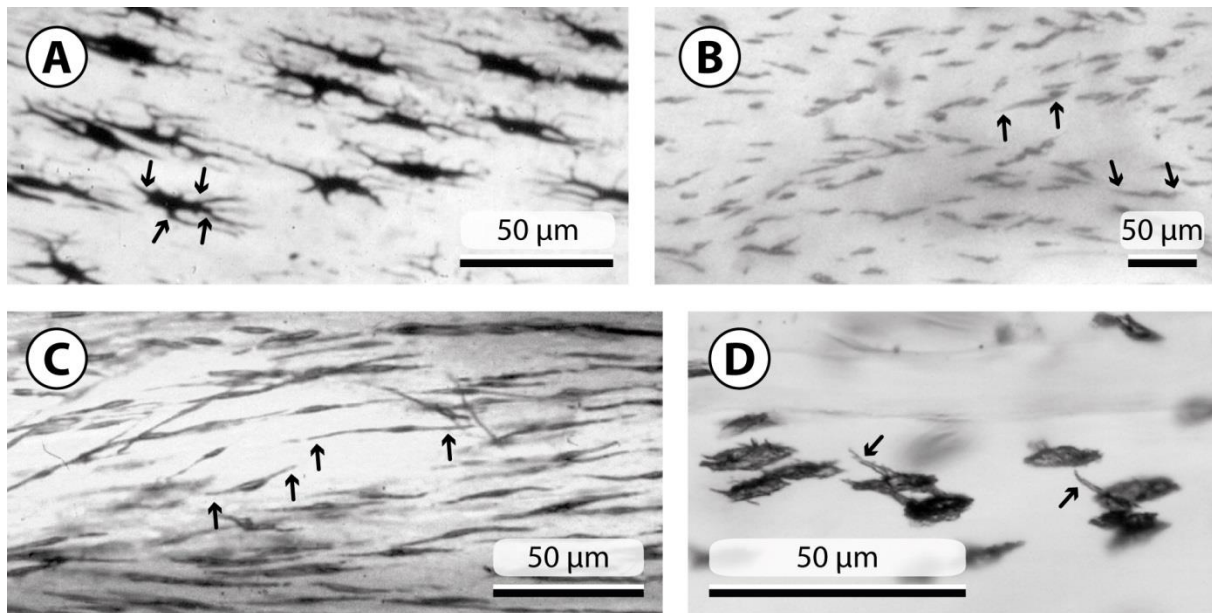


Figure 6. Osteocyte morphology in taxa that retain the ancestral cellular bone (A) or that secondarily reacquired it from acellular ancestors (B–D). (A) ‘Star-shaped’ osteocytes in the dorsal-fin spine of the carp *Cyprinus carpio*, an ostariophysan. Note the irregular shape of the lacunae, and the numerous cytoplasmic processes ending in canaliculi branching in all directions (arrows). Modified from Meunier & Huyseune (1992). (B) ‘Spindle-shaped’ osteocytes in the coracoid of the salmon *Salmo salar* (NHMUK, uncatalogued), a salmoniform. Note the two cytoplasmic processes located at both extremities of the cell axis (arrows). Photograph by D. Davesne and A. D. Schmitt. (C) ‘Spindle-shaped’ osteocytes in the dorsal-fin spine of the tuna *Katsuwonus pelamis*, an acanthomorph. Note the two cytoplasmic processes located at both extremities of the cell axis (arrows). Photograph by F. J. Meunier. (D) ‘Spindle-shaped’

osteocytes in the rib of the opah *Lampris* sp. (MNHN-ZA-AC-A-7506), an acanthomorph. Note the few canaliculi, all pointing in the same direction (arrows). Modified from Davesne *et al.* (2018).

V. THE ROLE OF MINERAL HOMEOSTASIS IN THE LOSS AND REACQUISITION OF OSTEOCYTES

Of the main functions of bone, those related to mechanical homeostasis (e.g. strain detection and bone remodelling) function in the absence of osteocytes (see Section II.2). Mineral homeostasis, on the other hand, relies on a variety of mechanisms including halastasis, i.e. a diffuse demineralisation of the bone without affecting its organic matrix (Lopez, 1976; Kacem & Meunier, 2003; Sbaihi *et al.*, 2007), osteoblast-mediated bone resorption (Francillon-Vieillot *et al.*, 1990; Ricqlès *et al.*, 1991), and osteocyte-mediated bone resorption (osteocytic osteolysis). Halastasis has only been observed so far in taxa with cellular bone, and evidently osteocytic osteolysis is lacking in acellular bone. This suggests that acellular bone is less efficient than cellular bone in regulating mineral content in the body. In aquatic animals like teleosts, however, it is likely that enough calcium and phosphorus is available from the diet and ambient water to compensate the less-efficient mineral homeostasis (Witten & Huysseune, 2009; Cohen *et al.*, 2012; Shahar & Dean, 2013; Doherty *et al.*, 2015). Therefore, it is possible that osteocytes are not required either for mechanical or mineral homeostasis in teleosts because both functions can be achieved by other means (Dean & Shahar, 2012). In that context, the disappearance of osteocytes in at least some euteleosts could be hypothesised to be due to a relaxed selective pressure that does not compensate the cost of maintaining them (Shahar & Dean, 2013; Doherty *et al.*, 2015). However, this hypothesis alone clearly does not explain the phylogenetic distribution of acellular bone: if a low selective pressure was not preventing the loss of osteocytes, we would expect this phenomenon to be widespread in teleosts and other aquatic vertebrates. Our data support the contrary: probably just a single main disappearance of cellular bone, potentially in euteleosts, along with other, extremely rare losses in species-poor lineages (at least in *Trichomycterus* sp. and some bones of *Danio rerio*) that could be size-related (see Section III.3a). Other mechanisms may have been involved, such as developmental heterochrony (e.g. Parenti, 1986).

Tunas, opahs and potentially salmoniforms all reacquired osteocytes secondarily (see Section IV.3). They also share specific adaptations that lead to increased and sustained muscular activity: an anadromous migrating behaviour in salmoniforms, and specialised red muscles involved in heat production in tunas and opahs. At least in these taxa, the main function of osteocytes could be that of osteocytic osteolysis, as has been proposed for teleosts as a whole by previous authors (e.g. Cohen *et al.*, 2012). Reacquiring osteocytes would allow the use of bone as a major source of calcium and phosphorus, which would constitute a key adaptive advantage in an organism experiencing increased pressure on maintaining efficient muscle activity. Mineral homeostasis is then proposed to have played a major role in the evolution of acellular bone in teleost fishes.

VI. CONCLUSION

(1) According to our ancestral state reconstructions (Figs. 3,4), acellular bone is a synapomorphy of either Euteleostei (as supported by two out of three tree topologies), or of a smaller clade consisting of Stomiati, Galaxiiformes and Neoteleostei (as supported by one tree topology). New analyses incorporating histological information on more

euteleost taxa (e.g. *Lepidogalaxias*, more argentiniforms and galaxiiforms) including early fossil representatives, and a stabilisation of the euteleost phylogeny, are both necessary to clarify the ambiguity on the exact clade in which acellular bone evolved. Given the equivocal support for the euteleost ancestral state in T1 (Fig. 4), and considering other lines of evidence (such as structural similarities between osteocytes in salmoniforms and tunas; Fig. 6), we consider it to be more likely that osteocytes were lost in Euteleostei, with a secondary reacquisition in salmoniforms.

(2) Scales became acellular earlier than the rest of the skeleton in teleost phylogeny, probably in the clade Clupeocephala, which includes clupeomorphs, ostariophysans and euteleosts. More comparative data are needed to confirm this hypothesis.

(3) Scales aside, acellular bone appears to be almost absent outside of Euteleostei (Fig. 3). We reject its occurrence in the bonefish *Albula vulpes* and the mooneyes *Hiodon* sp. The catfish *Trichomycterus* sp. appears to be acellular and the zebrafish *Danio rerio* has both cellular and acellular bone in its cranial skeleton, but the occurrence of acellular bone in both may be explained by their small adult body sizes. More comparative data encompassing multiple bones in multiple teleost species will be necessary to support whether these are isolated or more widespread occurrences.

(4) Within spiny-rayed teleosts (Acanthomorpha), osteocytes have been secondarily reacquired in tunas (Thunnini) and in the opah *Lampris* sp. The exact co-occurrence of osteocytes with that of an endothermic physiology based on red muscle activity (Fig. 5) strongly suggests that these traits are correlated in acanthomorph teleosts. Other traits shared by tunas and the opah are also present in some acanthomorphs with acellular bone (e.g. large body size, cranial endothermy, intense bone remodelling), and so are less plausible as explanations of the evolutionary reacquisition of osteocytes.

(5) Acellular teleost bone can perform every structural and mechanical function of cellular bone (e.g. detection of strains and constraints, adaptive remodelling) and both have very similar mechanical properties. However, acellular bone seems to be less efficient in terms of mineral homeostasis, probably because it lacks the possibility to perform osteocytic osteolysis. Osteocytes are secondarily reacquired in lineages that may have increased requirements for minerals, mostly to support an intense and sustained muscular activity: the red-muscle endotherms and (potentially) the anadromous salmoniforms. This pattern seems to support the hypothesis that the most fundamental role of osteocytes in teleost bone physiology is that of mineral, rather than mechanical homeostasis.

(6) Our review of the available evidence with the addition of new data allowed us to establish for the first time a detailed phylogenetic hypothesis for the evolution of osteocytes in teleosts. Acellular bone is a fundamental model to understand bone function, because it lacks a cell type that is classically thought to play a major role in the structure and maintenance of bony tissues. This review highlights the need to use large-scale comparative histological data, backed by a rigorous phylogenetic framework, to address fundamental questions on the interplay of bone structure, function and physiology.

VII. ACKNOWLEDGEMENTS

We thank the colleagues, curators and collection managers that granted or facilitated access to specimens: R. Arrindell and B. Brown (AMNH), A. López-Arbarello (LMU Munich), G. Clément, D. Germain and P. Béarez (MNHN), E. Bernard and Z. Johanson

(NHMUK), H. Ketchum, J. Hay, M. Carnall and E. Westwig (OUMNH), H. Furrer and C. Klug (PIMUZ), D. Nelson (UMMZ). R. Betancur-R. (University of Oklahoma) provided the tree files critical for the T2 ancestral state reconstruction analysis. A. Bailleul (Chinese Academy of Sciences) is thanked for discussion on acellular bone in tetrapods. H. Middleton and C. Nicklin are thanked for providing fossil specimens for the study, and A. Atkins (BINA), S. Iglésias (MNHN) and N. Tamura for providing illustrations. J. Wells and O. Green (University of Oxford) are thanked for preparing histological sections and for help with their imaging. ESRF beam time was obtained *via* two proposals (LS2614, LS2758). The authors thank S. Sanchez (Uppsala University), V. Fernandez (NHMUK) and P. Tafforeau (ESRF) for their crucial help during the synchrotron experiments, image treatment and reconstruction, as well as for scientific discussion. Two anonymous reviewers provided valuable comment that improved a previous version of the manuscript. This work was supported by the Leverhulme Trust (RPG-2016-168) and by a Junior Research Fellowship at Wolfson College, University of Oxford (D.D.).

REFERENCES

References marked with an asterisk are cited only within the supporting information.

- ALEXANDROU, M.A., SWARTZ, B.A., MATZKE, N.J. & OAKLEY, T.H. (2013). Genome duplication and multiple evolutionary origins of complex migratory behavior in Salmonidae. *Molecular Phylogenetics and Evolution* **69**, 514–523.
- ALFARO, M.E., FAIRCLOTH, B.C., HARRINGTON, R.C., SORENSON, L., FRIEDMAN, M., THACKER, C.E., OLIVEROS, C.H., ČERNÝ, D. & NEAR, T.J. (2018). Explosive diversification of marine fishes at the Cretaceous–Palaeogene boundary. *Nature Ecology & Evolution* **2**, 688–696.
- AMPRINO, R. & GODINA, G. (1956). Osservazioni sul rinnovamento strutturale dell'osso in Pesci Teleostei. *Pubblicazioni della Stazione Zoologica di Napoli* **28**, 62–71.
- ARRATIA, G. (2015). Complexities of early Teleostei and the evolution of particular morphological structures through time. *Copeia* **103**, 999–1025.
- ATKINS, A., DEAN, M.N., HABEGGER, M.L., MOTTA, P.J., OFER, L., REPP, F., SHIPOV, A., WEINER, S., CURREY, J.D. & SHAHAR, R. (2014). Remodeling in bone without osteocytes: billfish challenge bone structure–function paradigms. *Proceedings of the National Academy of Sciences* **111**, 16047–16052.
- ATKINS, A., MILGRAM, J., WEINER, S. & SHAHAR, R. (2015a). The response of anosteocytic bone to controlled loading. *Journal of Experimental Biology* **218**, 3559–3569.
- ATKINS, A., REZNIKOV, N., OFER, L., MASIC, A., WEINER, S. & SHAHAR, R. (2015b). The three-dimensional structure of anosteocytic lamellated bone of fish. *Acta Biomaterialia* **13**, 311–323.
- BAILLEUL, A.M. & HORNER, J.R. (2016). Comparative histology of some craniofacial sutures and skull-base synchondroses in non-avian dinosaurs and their extant phylogenetic bracket. *Journal of Anatomy* **229**, 252–285.
- BEAREZ, P., MEUNIER, F.J. & KACEM, A. (2005). Description morphologique et histologique de l'hyperostose vertébrale chez la thonine noire, *Euthynnus lineatus* (Teleostei: Perciformes: Scombridae). *Cahiers de Biologie Marine* **46**, 21–28.
- BERG, L.S. (1947). *Classification of fishes both Recent and fossil*. J. W. Edwards, Ann Arbor.
- BETANCUR-R., R., BROUGHTON, R.E., WILEY, E.O., CARPENTER, K., LOPEZ, J.A., LI, C.,

- HOLCROFT, N.I., ARCILA, D., SANCIANGCO, M., CURETON, J.C., ZHANG, F., BUSER, T., CAMPBELL, M.A., BALLESTEROS, J.A., ROA-VARON, A., *ET AL.* (2013). The tree of life and a new classification of bony fishes. *PLoS Currents Tree of Life* **2013 Apr 1**, DOI: 10.1371/currents.tol.53ba26640df0ccaee75bb165c8c26288.
- BETANCUR-R., R., ORTI, G. & PYRON, R.A. (2015). Fossil-based comparative analyses reveal ancient marine ancestry erased by extinction in ray-finned fishes. *Ecology Letters* **18**, 441–450.
- BETANCUR-R., R., WILEY, E.O., ARRATIA, G., ACERO, A., BAILLY, N., MIYA, M., LECOINTRE, G. & ORTÍ, G. (2017). Phylogenetic classification of bony fishes. *BMC Evolutionary Biology* **17**, 162.
- BLANC, M. (1953). Contribution à l'étude de l'ostéogénèse chez les Poissons Téléostéens. *Mémoires du Muséum national d'Histoire naturelle, Série A* **7**, 1–146.
- BLOCK, B.A. (1986). Structure of the brain and eye heater tissue in marlins, sailfish, and spearfishes. *Journal of Morphology* **190**, 169–189.
- BLOCK, B.A. (1994). Thermogenesis in muscle. *Annual Review of Physiology* **56**, 535–577.
- BLOCK, B.A., FINNERTY, J.R., STEWART, A.F.R. & KIDD, J. (1993). Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. *Science* **260**, 210–214.
- BONEWALD, L.F. (2011). The amazing osteocyte. *Journal of Bone and Mineral Research* **26**, 229–238.
- BRAZEAU, M.D. & FRIEDMAN, M. (2014). The characters of Palaeozoic jawed vertebrates. *Zoological Journal of the Linnean Society* **170**, 779–821.
- *BRITO, P.M., ALVARADO-ORTEGA, J. & MEUNIER, F.J. (2017). Earliest known lepisosteoid extends the range of anatomically modern gars to the Late Jurassic. *Scientific Reports* **7**, 17830.
- BRITO, P.M. & MEUNIER, F.J. (2000). The morphology and histology of the scales of Aspidorhynchidae (Actinopterygii, Halecostomi). *Geobios* **33**, 105–111.
- BRITO, P.M., MEUNIER, F.J. & GAYET, M. (2000). The morphology and histology of the scales of the Cretaceous gar *Obaichthys* (Actinopterygii, Lepisosteidae): phylogenetic implications. *Comptes Rendus de l'Académie des Sciences, Paris, Sciences de la Terre et des Planètes* **331**, 823–829.
- *BRUNEEL, B. & WITTEN, P.E. (2015). Power and challenges of using zebrafish as a model for skeletal tissue imaging. *Connective Tissue Research* **56**, 161–173.
- BUFFRÉNIL, V. DE, CLARAC, F., CANOVILLE, A. & LAURIN, M. (2016). Comparative data on the differentiation and growth of bone ornamentation in Gnathostomes (Chordata: Vertebrata). *Journal of Morphology* **277**, 634–670.
- CAMPBELL, M.A., ALFARO, M.E., BELASCO, M. & LOPEZ, J.A. (2017). Early-branching euteleost relationships : areas of congruence between concatenation and coalescent model inferences. *PeerJ* **5**, e3548.
- CAMPBELL, M.A., LÓPEZ, J.A., SADO, T. & MIYA, M. (2013). Pike and salmon as sister taxa: detailed intraclade resolution and divergence time estimation of Esociformes + Salmoniformes based on whole mitochondrial genome sequences. *Gene* **530**, 57–65.
- CAO, L., MORIISHI, T., MIYAZAKI, T., IIMURA, T., HAMAGAKI, M., NAKANE, A., TAMAMURA, Y., KOMORI, T. & YAMAGUCHI, A. (2011). Comparative morphology of the osteocyte lacunocanalicular system in various vertebrates. *Journal of Bone and Mineral*

- Metabolism* **29**, 662–670.
- CAREY, F.G. (1982). A brain heater in the swordfish. *Science* **216**, 1327–1329.
- CARPENTER, K.E., COLLETTE, B.B. & RUSSO, J.L. (1995). Unstable and stable classifications of scombroid fishes. *Bulletin of Marine Science* **56**, 379–405.
- CASTANET, J. & RICQLES, A. DE (1986). Sur la relativité de la notion d'ostéones primaires et secondaires et de tissus osseux primaire et secondaire en général. *Annales des Sciences naturelles, Zoologie, Paris* **8**, 103–109.
- CHEVRINAIS, M., SIRE, J. & CLOUTIER, R. (2017). From body scale ontogeny to species ontogeny: Histological and morphological assessment of the Late Devonian acanthodian *Triazeugacanthus affinis* from Miguasha, Canada. *PLoS ONE* **12**, e0174655.
- COATES, M.I., SEQUEIRA, S.E.K., SANSOM, I.J. & SMITH, M.M. (1998). Spine and tissues of ancient sharks. *Nature* **396**, 729–730.
- COHEN, L., DEAN, M.N., SHIPOV, A., ATKINS, A., MONSONEGO-ORNAN, E. & SHAHAR, R. (2012). Comparison of structural, architectural and mechanical aspects of cellular and acellular bone in two teleost fish. *The Journal of Experimental Biology* **215**, 1983–1993.
- COLLETTE, B.B. & NAUEN, C.E. (1983). *FAO Species Catalogue, Vol. 2: Scombrids of the World. An Annotated and Illustrated Catalogue of Tunas, Mackerels, Bonitos and Related Species Known to Date*. Food and Agriculture Organization of the United Nations, Rome.
- COLLETTE, B.B., POTTHOFF, T., RICHARDS, W.J., UEYANAGI, S., RUSSO, J.L. & NISHIKAWA, Y. (1984). Scombroidei: development and relationships. In *Ontogeny and Systematics of Fishes, Special Publication Number 1, American Society of Ichthyologists and Herpetologists* (eds H.G. MOSER, W.J. RICHARDS, D.M. COHEN, M.P. FAHAY, A.W. KENDALL & S.L. RICHARDSON), pp. 591–620. Allen Press, Lawrence, Kansas.
- CRÊTE-LAFRENIÈRE, A., WEIR, L.K. & BERNATCHEZ, L. (2012). Framing the Salmonidae family phylogenetic portrait: a more complete picture from increased taxon sampling. *PLoS ONE* **7**, e46662.
- CURREY, J.D., DEAN, M.N. & SHAHAR, R. (2017) Revisiting the links between bone remodelling and osteocytes: insights from across phyla. *Biological Reviews* **92**, 1702–1719.
- CURREY, J.D. & SHAHAR, R. (2013). Cavities in the compact bone in tetrapods and fish and their effect on mechanical properties. *Journal of Structural Biology* **183**, 107–122.
- DAGET, J., GAYET, M., MEUNIER, F.J. & SIRE, J.Y. (2001). Major discoveries on the dermal skeleton of fossil and recent polypteriforms: a review. *Fish and Fisheries* **2**, 113–124.
- DAVESNE, D., FRIEDMAN, M., BARRIEL, V., LECOINTRE, G., JANVIER, P., GALLUT, C. & OTERO, O. (2014). Early fossils illuminate character evolution and interrelationships of Lampridiformes (Teleostei, Acanthomorpha). *Zoological Journal of the Linnean Society* **172**, 475–498.
- DAVESNE, D., GALLUT, C., BARRIEL, V., JANVIER, P., LECOINTRE, G. & OTERO, O. (2016). The phylogenetic intrarelationships of spiny-rayed fishes (Acanthomorpha, Teleostei, Actinopterygii): fossil taxa increase the congruence of morphology with molecular data. *Frontiers in Ecology and Evolution* **4**, 129.

- DAVESNE, D., MEUNIER, F.J., FRIEDMAN, M., BENSON, R.B.J. & OTERO, O. (2018). Histology of the endothermic opah (*Lampris* sp.) suggests a new structure–function relationship in teleost fish bone. *Biology Letters* **14**, 20180270.
- DEAN, M.N. & SHAHAR, R. (2012). The structure-mechanics relationship and the response to load of the acellular bone of neoteleost fish: a review. *Journal of Applied Ichthyology* **28**, 320–329.
- DENISON, R.H. (1963). The early history of the vertebrate calcified skeleton. *Clinical Orthopaedics and Related Research* **31**, 141–152.
- DESSE, G., MEUNIER, F.J., PERON, M. & LAROCHE, J. (1981). Hyperostose vertébrale chez l'animal. *Rhumatologie* **33**, 105–119.
- DICKSON, K.A. & GRAHAM, J.B. (2004). Evolution and consequences of endothermy in fishes. *Physiological and Biochemical Zoology* **77**, 998–1018.
- DOHERTY, A.H., GHALAMBOR, C.K. & DONAHUE, S.W. (2015). Evolutionary physiology of bone: bone metabolism in changing environments. *Physiology* **30**, 17–29.
- DONOGHUE, P.C.J. & SANSOM, I.J. (2002). Origin and early evolution of vertebrate skeletonization. *Microscopy Research and Technique* **59**, 352–372.
- DOWNS, J.P. & DONOGHUE, P.C.J. (2009). Skeletal histology of *Bothriolepis canadensis* (Placodermi, Antiarchi) and evolution of the skeleton at the origin of jawed vertebrates. *Journal of Morphology* **270**, 1364–1380.
- EKANAYAKE, S. & HALL, B.K. (1987). The development of acellularity of the vertebral bone of the Japanese medaka, *Oryzias latipes* (Teleostei; Cyprinodontidae). *Journal of Morphology* **193**, 253–261.
- EKANAYAKE, S. & HALL, B.K. (1988). Ultrastructure of the osteogenesis of acellular vertebral bone in the Japanese medaka, *Oryzias latipes* (Teleostei, Cyprinodontidae). *The American Journal of Anatomy* **182**, 241–249.
- ENLOW, D.H. & BROWN, S.O. (1956). A comparative histological study of fossil and recent bone tissues. Part I. *The Texas Journal of Science* **8**, 405–443.
- ESCHMEYER, W.N., FRICKE, R. & VAN DER LAAN, R. (2018). Catalog of Fishes: genera, species, references. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> [accessed 19 March 2018].
- *ESTÊVÃO, M.D., SILVA, N., REDRUELLO, B., COSTA, R., GREGÓRIO, S., CANÁRIO, A.V.M. & POWER, D.M. (2011). Cellular morphology and markers of cartilage and bone in the marine teleost *Sparus auratus*. *Cell and Tissue Research* **343**, 619–635.
- FIAZ, A.W., VAN LEEUWEN, J.L. & KRANENBARG, S. (2010). Phenotypic plasticity and mechano-transduction in the teleost skeleton. *Journal of Applied Ichthyology* **26**, 289–293.
- FRANCILLON-VIEILLOT, H., BUFFRENIL, V. DE, CASTANET, J., GERAUDIE, J., MEUNIER, F.J., SIRE, J.-Y., ZYLBERBERG, L. & RICQLES, A. DE (1990). Microstructure and mineralization of vertebrate skeletal tissues. In *Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends. Volume I* (ed J.G. CARTER), pp. 471–530. Van Nostrand Reinhold, New York.
- FRANZ-ODENDAAL, T.A., HALL, B.K. & WITTEN, P.E. (2006). Buried alive: how osteoblasts become osteocytes. *Developmental Dynamics* **235**, 176–190.
- *FROESE, R. & PAULY, D. (2019). FishBase. www.fishbase.org [accessed 29 January 2019].
- *GAUDANT, J. & MEUNIER, F.J. (1996). Etude d'un cas de pachyostose chez un Clupeidae

- fossile du Miocène terminal de l'ouest algérien, *Sardina? crassa* (Sauvage). *Cybium* **20**, 169–183.
- GAUDANT, J. & MEUNIER, F.J. (2004). Un test pour déterminer la position systématique du genre *Thaumaturus* Reuss 1844 (poisson téléostéen) : l'approche paléohistologique. *Courier Forschung-Institut Senckenberg* **252**, 79–93.
- GAYET, M. & MEUNIER, F.J. (1992). Polyptéroides (Pisces, Cladistia) du Maastrichtien et du Paléocène de Bolivie. *Geobios* **14**, 159–168.
- GEGENBAUR, C., KÖLLIKER, A. & MÜLLER, H. (1853). Bericht über einige im Herbst 1852 in Messina angestellte vergleichend-anatomische Untersuchungen. *Zeitschrift für wissenschaftliche Zoologie* **4**, 299–373.
- GÉRAUDIE, J. & MEUNIER, F.J. (1984). Structure and comparative morphology of camptotrichia of lungfish fins. *Tissue and Cell* **16**, 217–236.
- GERMAIN, D., SCHNELL, N.K. & MEUNIER, F.J. (in press). Some histological data on bone and teeth in the barbeled dragonfishes *Borostomias panamensis* Regan & Trewavas, 1929 and *Stomias boa* Reinhardt 1842 (Stomiidae; Stomiiformes). *Cybium*.
- GILES, S., RÜCKLIN, M. & DONOGHUE, P.C.J. (2013). Histology of “placoderm” dermal skeletons: implications for the nature of the ancestral gnathostome. *Journal of Morphology* **274**, 627–644.
- GLOWACKI, J., COX, K.A., O’SULLIVAN, J., WILKIE, D. & DEFTOS, L.J. (1986). Osteoclasts can be induced in fish having an acellular bony skeleton. *Proceedings of the National Academy of Sciences* **83**, 4104–4107.
- GOODRICH, E.S. (1907). On the scales of fish, living and extinct, and their importance in classification. *Proceeding of the Zoological Society, London* **2**, 751–774.
- GOODWIN, M.B. & HORNER, J.R. (2004). Cranial histology of pachycephalosaurs (Ornithischia: Marginocephalia) reveals transitory structures inconsistent with head-butting behavior. *Paleobiology* **30**, 253–267.
- GRAHAM, J.B. & DICKSON, K.A. (2000). The evolution of thunniform locomotion and heat conservation in scombrid fishes: new insights based on the morphology of *Allothunnus fallai*. *Zoological Journal of the Linnean Society* **129**, 419–466.
- GRAHAM, J.B. & DICKSON, K.A. (2001). Anatomical and physiological specialization for endothermy. In *Fish Physiology. Volume 19: Tuna: Physiology, Ecology, and Evolution* pp. 121–165.
- GRAHAM, J.B. & DICKSON, K.A. (2004). Tuna comparative physiology. *Journal of Experimental Biology* **207**, 4015–4024.
- GRAHAM, J.B., KOEHRN, F.J. & DICKSON, K.A. (1983). Distribution and relative proportions of red muscle in scombrid fishes: consequences of body size and relationships to locomotion and endothermy. *Canadian Journal of Zoology* **61**, 2087–2096.
- GROSS, W. (1930). Die Fische des mittleren Old Red Südlivlands. *Geologische und Palaeontologische Abhandlungen* **18**, 123–156.
- HALL, B.K. (2015). *Bones and Cartilage, Developmental and Evolutionary Skeletal Biology - Second Edition*. Academic Press.
- HALSTEAD, L.B. (1963). Aspidin: The precursor of bone. *Nature* **199**, 46–48.
- HALSTEAD, L.B. (1969). Calcified tissues in the earliest vertebrates. *Calcified Tissue Research* **3**, 107–124.
- HORREO, J.L. (2017). Revisiting the mitogenomic phylogeny of Salmoninae: new insights thanks to recent sequencing advances. *PeerJ* **5**, e3828.

- HORTON, J.M. & SUMMERS, A.P. (2009). The material properties of acellular bone in a teleost fish. *Journal of Experimental Biology* **212**, 1413–1420.
- HUGHES, D.R., BASSETT, J.R. & MOFFAT, L.A. (1994). Histological identification of osteocytes in the allegedly acellular bone of the sea breams *Acanthopagrus australis*, *Pagrus auratus* and *Rhabdosargus sarba* (Sparidae, Perciformes, Teleostei). *Anatomy and Embryology* **190**, 163–179.
- HUGHES, L.C., ORTÍ, G., HUANG, Y., SUN, Y., BALDWIN, C.C., THOMPSON, A.W., ARCILA, D., BETANCUR-R., R., LI, C., BECKER, L., BELLORA, N., ZHAO, X., LI, X., WANG, M., FANG, C., ET AL. (2018). Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. *Proceedings of the National Academy of Sciences* **115**, 6249–6254.
- *HUYSEUNE, A. (1986). Late skeletal development at the articulation between upper pharyngeal jaws and neurocranial base in the fish, *Astatotilapia elegans*, with the participation of a chondroid form of bone. *The American Journal of Anatomy* **177**, 119–137.
- HUYSEUNE, A. (2000). Skeletal system. In *The Laboratory Fish* (ed G. OSTRANDER), pp. 307–317. Academic Press.
- IGLÉSIAS, S.P. (2014a). Handbook of the marine fishes of Europe and adjacent waters (a natural classification based on collection specimens, with DNA barcodes and standardized photographs) - Volume I (Chondrichthyans and Cyclostomata) - Provisional version 08. <http://iccanam.mnhn.fr>
- IGLÉSIAS, S.P. (2014b). Handbook of the marine fishes of Europe and adjacent waters (a natural classification based on collection specimens, with DNA barcodes and standardized photographs) - Volume II (Actinopterygians) - Provisional version 10. <http://iccanam.mnhn.fr>
- JERVE, A., QU, Q., SANCHEZ, S., BLOM, H. & AHLBERG, P.E. (2016). Three-dimensional paleohistology of the scale and median fin spine of *Lophosteus superbus* (Pander 1856). *PeerJ* **4**, e2521.
- JOHNSON, G.D. & PATTERSON, C. (1996). Relationships of lower euteleostean fishes. In *Interrelationships of Fishes* (eds M.L.J. STIASSNY, L.R. PARENTI & G.D. JOHNSON), pp. 251–317. Academic Press, San Diego.
- KACEM, A. & MEUNIER, F.J. (2000). Mise en évidence de l'ostéolyse périostéocytaire vertébrale chez le saumon Atlantique *Salmo salar* (Salmonidae, Teleostei), au cours de sa migration anadrome. *Cybium* **24**, 105–112.
- KACEM, A. & MEUNIER, F.J. (2003). Halastatic demineralization in the vertebrae of Atlantic salmon, during their spawning migration. *Journal of Fish Biology* **63**, 1122–1130.
- KACEM, A. & MEUNIER, F.J. (2009). Transformations of the texture and the mineralization of the dentary bone in the Atlantic salmon, *Salmo salar* L. (Salmonidae), during anadromous migration. *Cybium* **33**, 61–72.
- KATZ, S.L. (2002). Design of heterothermic muscle in fish. *The Journal of Experimental Biology* **205**, 2251–2266.
- KEATING, J.N. & DONOGHUE, P.C.J. (2016). Histology and affinity of anaspids, and the early evolution of the vertebrate dermal skeleton. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20152917.
- KEATING, J.N., MARQUART, C.L., MARONE, F. & DONOGHUE, P.C.J. (2018). The nature of aspidin and the evolutionary origin of bone. *Nature Ecology & Evolution* **2**, 1501–1506.

- KERSCHNITZKI, M., WAGERMAIER, W., ROSCHGER, P., SETO, J., SHAHAR, R., DUDA, G.N., MUNDLOS, S. & FRATZL, P. (2011). The organization of the osteocyte network mirrors the extracellular matrix orientation in bone. *Journal of Structural Biology* **173**, 303–311.
- *KHEMIRI, S., MEUNIER, F.J., LAURIN, M. & ZYLBERBERG, L. (2001). Morphology and structure of the scales in the Gadiformes (Actinopterygii: Teleostei: Paracanthopterygii) and a comparison to the elasmoid scales of other Teleostei. *Cahiers de Biologie Marine* **42**, 345–362.
- KÖLLIKER, A. (1859). On the different types in the microscopic structure of the skeleton of osseous fishes. *Proceedings of the Royal Society of London* **9**, 656–668.
- KRANENBARG, S., VAN CLEYNENBREUGEL, T., SCHIPPER, H. & VAN LEEUWEN, J. (2005). Adaptive bone formation in acellular vertebrae of sea bass (*Dicentrarchus labrax* L.). *The Journal of Experimental Biology* **208**, 3493–3502.
- LAVOUÉ, S., MIYA, M., POULSEN, J.Y., MØLLER, P.R. & NISHIDA, M. (2008). Monophyly, phylogenetic position and inter-familial relationships of the Alepocephaliformes (Teleostei) based on whole mitogenome sequences. *Molecular Phylogenetics and Evolution* **47**, 1111–1121.
- *LECOMTE, F., MEUNIER, F.J. & ROJAS-BELTRAN, R. (1989). Some data on the growth of *Arius proops* (Ariidae, Siluriforme) in the estuaries of French Guyana. *Aquatic Living Resources* **2**, 63–38.
- LEPRÉVOST, A., AZAÏS, T., TRICHET, M. & SIRE, J.-Y. (2017). Vertebral development and ossification in the Siberian sturgeon (*Acipenser baerii*), with new insights on bone histology and ultrastructure of vertebral elements and scutes. *The Anatomical Record* **300**, 437–449.
- LI, J., XIA, R., MCDOWALL, R.M., LÓPEZ, J.A., LEI, G. & FU, C. (2010). Phylogenetic position of the enigmatic *Lepidogalaxias salamandroides* with comment on the orders of lower euteleostean fishes. *Molecular Phylogenetics and Evolution* **57**, 932–936.
- LISTON, J., NEWBREY, M.G., CHALLANDS, T.J. & ADAMS, C.E. (2013). Growth, age and size of the Jurassic pachycormid *Leedsichthys problematicus* (Osteichthyes: Actinopterygii). In *Mesozoic Fishes 5 - Global Diversity and Evolution* (eds G. ARRATIA, H.-P. SCHULTZE & M.V.H. WILSON), pp. 145–175. Verlag Dr. Friedrich Pfeil, Munich.
- LOPEZ, E. (1970). L'os cellulaire d'un poisson téléostéen *Anguilla anguilla* L. I. Etude histocytologique et histophysique. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **109**, 552–565.
- LOPEZ, E. (1976). Effects of calcitonin and ultimobranchialectomy (UBX) on calcium and bone metabolism in the eel, *Anguilla anguilla* L. *Calcified Tissue Research* **20**, 173–186.
- MACQUEEN, D.J. & JOHNSTON, I.A. (2014). A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20132881.
- MAYRINCK, D., BRITO, P.M., MEUNIER, F.J., ALVARADO-ORTEGA, J. & OTERO, O. (2017). †*Sorbinicharax verraesi*: an unexpected case of a benthic fish outside Acanthomorpha in the Upper Cretaceous of the Tethyan Sea. *PLoS ONE* **12**, e0183879.

- 1319 McDOWALL, R.M. (1997). The evolution of diadromy in fishes (revisited) and its place in
1320 phylogenetic analysis. *Reviews in Fish Biology and Fisheries* **7**, 443–462.
- 1321 McDOWALL, R.M. (2001). The origin of salmonid fishes: Marine, freshwater... or neither?
1322 *Reviews in Fish Biology and Fisheries* **11**, 171–179.
- 1323 METTENHEIMER, C. (1854). Anatomisch-histologische Untersuchungen über den
1324 *Tetragonurus Cuvieri* Risso. *Abhandlungen der Senckenbergischen*
1325 *Naturforschenden Gesellschaft* **1**, 214–257.
- 1326 MEUNIER, F.J. (1984a). Etude de la minéralisation de l'os chez les téléostéens à l'aide de
1327 la microradiographie quantitative: résultats préliminaires. *Cybium* **8**, 43–49.
- 1328 MEUNIER, F.J. (1984b). Spatial organization and mineralization of the basal plate of
1329 elasmoid scales in osteichthyans. *American Zoologist* **24**, 953–964.
- 1330 MEUNIER, F.J. (1987). Os cellulaire, os acellulaire et tissus dérivés chez les
1331 Ostéichthyens: les phénomènes de l'acellularisation et de la perte de minéralisation.
1332 *L'Année Biologique* **26**, 201–233.
- 1333 MEUNIER, F.J. (1989). The acellularisation process in osteichthyan bone. In *Trends in*
1334 *Vertebrate Morphology: Proceedings of the 2nd International Symposium on*
1335 *Vertebrate Morphology*, Vienna, 1986 pp. 443–446.
- 1336 *MEUNIER, F.J. (2009). Structure and mineralization of the scales in the clown trigger-fish
1337 *Balistoides conspicillum* (Teleostei: Tetraodontiformes: Balistidae). *Cahiers de*
1338 *Biologie Marine* **50**, 47–56.
- 1339 *MEUNIER, F.J. (2011). The Osteichthyes, from the Paleozoic to the extant time, through
1340 histology and palaeohistology of bony tissues. *Comptes Rendus Palevol* **10**, 347–
1341 355.
- 1342 MEUNIER, F.J. & ARNULF, I. (2018). Some histological data of bone and teeth in the Rift
1343 Eelpout, *Thermarces cerberus* (Zoarcidae). *Cybium* **42**, 83–86.
- 1344 MEUNIER, F.J. & BÉAREZ, P. (2019). Histological study of the cutaneous bony scutes in
1345 the John dory, *Zeus faber* Linnaeus, 1758 (Teleostei; Zeiformes; Zeidae). *Cahiers*
1346 *de Biologie Marine* **60**. DOI : 10.21411/CBM.A.260F6487
- 1347 MEUNIER, F.J., BEAREZ, P. & FRANCILLON-VIEILLOT, H. (1999). Some morphological and
1348 histological aspects of hyperostosis in the Eastern Pacific marine fish *Prionotus*
1349 *stephanophrys* Lockington, 1880 (Triglidae). In *Proceedings of the 5th Indo-Pacific*
1350 *Fish Conference, Nouméa, 1997* (eds B. SÉRET & J.-Y. SIRE), pp. 125–133. Société
1351 Française d'Ichtyologie, Paris.
- 1352 MEUNIER, F.J. & BRITO, P.M. (2004). Histology and morphology of the scales in some
1353 extinct and extant teleosts. *Cybium* **28**, 225–235.
- 1354 MEUNIER, F.J., BRITO, P.M. & LEAL, M.-E.C. (2013a). Morphological and histological data
1355 on the structure of the lingual toothplate of *Arapaima gigas* (Osteoglossidae;
1356 Teleostei). *Cybium* **37**, 263–271.
- 1357 MEUNIER, F.J., CUPELLO, C.D. & CLÉMENT, G. (2019). The skeleton and the mineralized
1358 tissues of the living coelacanth. *Bulletin of Kitakyushu Museum of Natural History*
1359 *and Human History* **17**, 37–48.
- 1360 MEUNIER, F.J., DESCHAMPS, M.-H., LECOMTE, F. & KACEM, A. (2008a). Le squelette des
1361 poissons téléostéens : structure, développement, physiologie, pathologie. *Bulletin*
1362 *de la Société Zoologique de France* **133**, 9–32.
- 1363 MEUNIER, F.J. & DESSE, G. (1986). Les hyperostoses chez les Téléostéens: description,
1364 histologie et problèmes étiologiques. *Ichthyophysiological Acta* **10**, 130–141.
- 1365 MEUNIER, F.J. & DESSE, J. (1994). Histological structure of hyperostotic cranial remains

- of *Pomadasys hasta* (Osteichthyes, Perciformes, Haemulidae) from archaeological sites of the Arabian Gulf and the Indian Ocean. *Annalen - Koninklijk Museum voor Midden-Afrika - Zoologische Wetenschappen* **274**, 47–53.
- MEUNIER, F.J., DUTHEIL, D.B. & BRITO, P.M. (2013*b*). Histological study of the median lingual dental plate of the Cretaceous fish †*Palaeonotopterus greenwoodi* (Teleostei: Osteoglossomorpha) from the Kem-Kem beds, Morocco. *Cybium* **37**, 121–125.
- MEUNIER, F.J., ERDMANN, M. V., FERMON, Y. & CALDWELL, R.L. (2008*b*). Can the comparative study of the morphology and histology of the scales of *Latimeria menadoensis* and *L. chalumnae* (Sarcopterygii: Actinistia, Coelacanthidae) bring new insight on the taxonomy and the biogeography of recent coelacanthids? *Geological Society, London, Special Publications* **295**, 351–360.
- MEUNIER, F.J., EUSTACHE, R.-P., DUTHEIL, D. & CAVIN, L. (2016). Histology of ganoid scales from the early Late Cretaceous of the Kem Kem beds, SE Morocco: systematic and evolutionary implications. *Cybium* **40**, 121–132.
- *MEUNIER, F.J. & FRANCILLON-VIEILLOT, H. (1999). Histological structure of the caudal spine of the surgeonfish *Ctenochaetus striatus* (Teleostei: Acanthuridae). In *Proceedings of the 5th Indo-Pacific Fish Conference, Nouméa, 1997* (eds B. SÉRET & J.-Y. SIRE), pp. 117–124. Société Française d'Ichtyologie, Paris.
- MEUNIER, F.J., FRANÇOIS, Y. & CASTANET, J. (1978). Etude histologique et microradiographique des écailles de quelques Actinoptérygiens primitifs actuels. *Bulletin de la Société Zoologique de France* **103**, 309–318.
- MEUNIER, F.J., GAUDANT, J. & BONELLI, E. (2010). Morphological and histological study of the hyperostoses of *Lepidopus albyi* (Sauvage, 1870), a fossil Trichiuridae from the Tortonian (Upper Miocene) of Piedmont (Italy). *Cybium* **34**, 293–301.
- *MEUNIER, F.J. & GAYET, M. (1992). Nouveau remaniement de la ganoïne chez un Semionotidae du Crétacé supérieur de Bolivie : intérêt paléobiologique. *Geobios* **25**, 767–774.
- *MEUNIER, F.J. & GAYET, M. (1996). A new polypteriform from the Late Cretaceous and the middle Paleocene of South America. In *Mesozoic Fishes - Systematics and Paleoecology* (eds G. ARRATIA & G. VIOHL), pp. 95–103. Verlag Dr. Friedrich Pfeil, Munich.
- *MEUNIER, F.J. & GERMAIN, D. (2018). The histological structure of teeth in the northern wolffish *Anarhichas denticulatus* (Teleostei: Perciformes: Anarhichadidae). *Cahiers de Biologie Marine* **59**, 217–224.
- *MEUNIER, F.J., GERMAIN, D. & OTERO, O. (2018*a*). A histological study of the lingual molariform teeth in *Hyperopisus bebe* (Mormyridae; Osteoglossomorpha). *Cybium* **42**, 87–90.
- MEUNIER, F.J. & HERBIN, M. (2014). La collection de préparations histologiques effectuées par Paul Gervais (1816-1879) sur le squelette des 'poissons'. *Cybium* **38**, 23–42.
- MEUNIER, F.J. & HUYSEUNE, A. (1992). The concept of bone tissue in Osteichthyes. *Netherlands Journal of Zoology* **42**, 445–458.
- *MEUNIER, F.J., JOURNIAC, N., LAVOUE, S. & RABET, N. (2002). Caractéristiques histologiques des marques de croissance squelettique chez l'atipa, *Hoplosternum littorale* (Hancock, 1828) (Teleostei, Siluriformes) dans le marais de Kaw (Guyane Française). *Bulletin Français de la Pêche et de la Pisciculture* **364**, 71–86.

- *MEUNIER, F.J., LECOMTE, F. & DUHAMEL, G. (2018b). Some histological data on bone and teeth in the grey notothen (*Lepidonotothen squamifrons*) and in the mackerel icefish (*Champsocephalus gunnari*) (Notothenioidei; Perciformes; Teleostei). *Cybium* **42**, 91–97.
- MEUNIER, F.J., OTERO, O. & LAURIN, M. (2018c). Histological study of the jaw teeth in the Devonian actinopterygian †*Cheirolepis canadensis* (Whiteaves). *Cybium* **42**, 67–74.
- MEUNIER, F.J. & POPLIN, C. (1995). Paleohistological study of the scales of *Amia robusta* Priem, 1901, Amiidae from the Thanetian (Paleocene) of Cernay (France). *Geobios* **19**, 39–43.
- *MEUNIER, F.J. & SAUR, F. (2007). Étude morphologique et structurale des écailles de *Tetragonurus cuvieri* (Tetragonuridae) et de *Cleidopus gloriamaris* (Monocentridae). *Cybium* **31**, 123–132.
- MEUNIER, F.J. & SIRE, J. (1981). Sur la structure et la minéralisation des écailles de germon, *Thunnus alalunga* (Téléostéen, Perciforme, Thunnidae). *Bulletin de la Société Zoologique de France* **106**, 327–336.
- MEUNIER, F.J., SORBA, L. & BEAREZ, P. (2004). Presence of vascularized acellular bone in the elasmoid scales of *Micropogonias altipinnis* (Osteichthyes, Perciformes, Sciaenidae). *Cybium* **28**, 25–31.
- MIYA, M., FRIEDMAN, M., SATOH, T.P., TAKESHIMA, H., SADO, T., IWASAKI, W., YAMANOE, Y., NAKATANI, M., MABUCHI, K., INOUE, J.G., POULSEN, J.Y., FUKUNAGA, T., SATO, Y. & NISHIDA, M. (2013). Evolutionary origin of the Scombridae (tunas and mackerels): members of a Paleogene adaptive radiation with 14 other pelagic fish families. *PLoS ONE* **8**, e73535.
- MOSS, M.L. (1961a). Osteogenesis of acellular teleost fish bone. *American Journal of Anatomy* **108**, 99–110.
- MOSS, M.L. (1961b). Studies of the acellular bone of teleost fish. I. Morphological and systematic variations. *Acta Anatomica* **46**, 343–462.
- MOSS, M.L. (1962). Studies of the acellular bone of teleost fish. II. Response to fracture under normal and acalcemic conditions. *Acta Anatomica* **48**, 46–60.
- MOSS, M.L. (1963). The biology of acellular teleost bone. *Annals of the New York Academy of Sciences* **109**, 337–350.
- MOSS, M.L. (1965). Studies of the acellular bone of teleost fish. V. Histology and mineral homeostasis of fresh-water species. *Acta Anatomica* **60**, 262–276.
- MOSS, M.L. & FREILICH, M. (1963). Studies of the acellular bone of teleost fish. IV. Inorganic content of calcified tissues. *Acta Anatomica* **55**, 1–8.
- MOSS, M.L. & POSNER, A.S. (1960). X-ray diffraction study of acellular teleost bone. *Nature* **188**, 1037–1038.
- NEAR, T.J., DORNBURG, A., EYTAN, R.I., KECK, B.P., SMITH, W.L., KUHN, K.L., MOORE, J.A., PRICE, S.A., BURBRINK, F.T., FRIEDMAN, M. & WAINWRIGHT, P.C. (2013). Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proceedings of the National Academy of Sciences* **110**, 12738–12743.
- NEAR, T.J., EYTAN, R.I., DORNBURG, A., KUHN, K.L., MOORE, J.A., DAVIS, M.P., WAINWRIGHT, P.C., FRIEDMAN, M. & SMITH, W.L. (2012). Resolution of ray-finned fish phylogeny and timing of diversification. *Proceedings of the National Academy of Sciences* **109**, 13698–13703.
- NEMOTO, Y., HIGUCHI, K., BABA, O., KUDO, A. & TAKANO, Y. (2007). Multinucleate osteoclasts in medaka as evidence of active bone remodeling. *Bone* **40**, 399–408.

- OLNEY, J.E., JOHNSON, G.D. & BALDWIN, C.C. (1993). Phylogeny of lampridiform fishes. *Bulletin of Marine Science* **52**, 137–169.
- ØRVIG, T. (1951). Histologic studies of placoderms and fossil elasmobranchs. 1- The endoskeleton, with remarks on the hard tissues of lower vertebrates in general. *Arkiv för Zoologi* **2**, 321–454.
- ØRVIG, T. (1967). Phylogeny of tooth tissues: evolution of some calcified tissues in early vertebrates. In *Structural and Chemical Organization of Teeth, Vol. I* pp. 45–105. Academic Press, New York.
- ØRVIG, T. (1978). Microstructure and growth of the dermal skeleton in fossil actinopterygian fishes: *Birgeria* and *Scanilepis*. *Zoologica Scripta* **7**, 33–56.
- PAIG-TRAN, E.W.M., BARRIOS, A.S. & FERRY, L.A. (2016). Presence of repeating hyperostotic bones in dorsal pterygiophores of the oarfish, *Regalecus russellii*. *Journal of Anatomy* **229**, 560–567.
- PARADIS, E., CLAUDE, J. & STRIMMER, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290.
- PARENTI, L.R. (1986). The phylogenetic significance of bone types in euteleost fishes. *Zoological Journal of the Linnean Society* **87**, 37–51.
- PATTERSON, C. (1977). Cartilage bones, dermal bones and membrane bones, or the exoskeleton versus the endoskeleton. In *Problems in Vertebrate Evolution. Linnean Society Symposium Series, No. 4* (eds S. MAHALA ANDREWS, R.S. MILES & A.D. WALKER), pp. 77–121. Academic Press, London.
- POPLIN, C., POPLIN, F. & RICQLÈS, A. DE (1976). Quelques particularités anatomiques et histologiques du rostre de l'espadon (*Xiphias gladius* L.). *Comptes Rendus de l'Académie des sciences, Paris, série D* **282**, 1105–1108.
- QU, Q., SANCHEZ, S., ZHU, M., BLOM, H. & AHLBERG, P.E. (2017). The origin of novel features by changes in developmental mechanisms: ontogeny and three-dimensional microanatomy of polyodontode scales of two early osteichthyans. *Biological Reviews* **92**, 1189–1212.
- QUEKETT, J. (1855). *Descriptive and Illustrated Catalogue of the Histological Series Contained in the Museum of The Royal College of Surgeons of England. Vol. II. Structure of the Skeleton of Vertebrate Animals*. Taylor & Francis, London.
- RAMSDEN, S.D., BRINKMANN, H., HAWRYSHYN, C.W. & TAYLOR, J.S. (2003). Mitogenomics and the sister of Salmonidae. *Trends in Ecology and Evolution* **18**, 605–607.
- RICHTER, M. & SMITH, M.M. (1995). A microstructural study of the ganoine tissue of selected lower vertebrates. *Zoological Journal of the Linnean Society* **114**, 173–212.
- RICQLÈS, A. DE, MEUNIER, F.J., CASTANET, J. & FRANCILLON-VIEILLOT, H. (1991). Comparative microstructure of bone. In *Bone: A Treatise, Volume III* (ed B.K. HALL), pp. 1–78. CRC Press.
- ROCHFERT, G.Y., PALLU, S. & BENHAMOU, C.L. (2010). Osteocyte: the unrecognized side of bone tissue. *Osteoporosis International* **21**, 1457–1469.
- *ROLVIEN, T., NAGEL, F., MILOVANOVIC, P., WUERTZ, S., MARSHALL, R.P., JESCHKE, A., SCHMIDT, F.N., HAHN, M., WITTEN, P.E., AMLING, M. & BUSSE, B. (2016). How the European eel (*Anguilla anguilla*) loses its skeletal framework across lifetime. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20161550.
- ROSEN, D.E. (1973). Interrelationships of higher euteleostean fishes. In *Interrelationships of Fishes* (eds P.H. GREENWOOD, R.S. MILES & C. PATTERSON), pp. 397–513. Academic Press, London.

- ROSEN, D.E. (1985). An essay on euteleostean classification. *American Museum Novitates* **2827**, 1–57.
- SANSOM, I.J., HAINES, P.W., ANDREEV, P. & NICOLL, R.S. (2013). A new pteraspidomorph from the Nihil Formation (Katian, Late Ordovician) of the Canning Basin, Western Australia. *Journal of Vertebrate Paleontology* **33**, 764–769.
- SANTAMARIA, N., BELLO, G., PASSANTINO, L., DI COMITE, M., ZUPA, R., POUSIS, C., VASSALLO-AGIUS, R., CICIRELLI, V., BASILONE, G., MANGANO, S. & CORRIERO, A. (2018). Micro-anatomical structure of the first spine of the dorsal fin of Atlantic bluefin tuna, *Thunnus thynnus* (Osteichthyes: Scombridae). *Annals of Anatomy* **219**, 1–7.
- SBAIHI, M., KACEM, A., AROUA, S., BALOCHE, S., ROUSSEAU, K., LOPEZ, E., MEUNIER, F. & DUFOUR, S. (2007). Thyroid hormone-induced demineralisation of the vertebral skeleton of the eel, *Anguilla anguilla*. *General and Comparative Endocrinology* **151**, 98–107.
- SCHAEFFER, B. (1977). The dermal skeleton in fishes. In *Problems in Vertebrate Evolution. Linnean Society Symposium Series, No. 4* (eds S. MAHALA ANDREWS, R.S. MILES & A.D. WALKER), pp. 25–52. Academic Press, London.
- SCHEYER, T.M., SCHMID, L., FURRER, H. & SÁNCHEZ-VILLAGRA, M.R. (2014). An assessment of age determination in fossil fish: the case of the opercula in the Mesozoic actinopterygian *Saurichthys*. *Swiss Journal of Palaeontology* **133**, 243–257.
- SCHULTZE, H.-P. (2016). Scales, enamel, cosmine, ganoine, and early osteichthyans. *Comptes Rendus Palevol* **15**, 83–102.
- SHAHAR, R. & DEAN, M.N. (2013). The enigmas of bone without osteocytes. *BoneKEY Reports* **2**, 343.
- SIMMONS, D.J., SIMMONS, N.B. & MARSHALL, J.H. (1970). The uptake of calcium-45 in the acellular-boned toadfish. *Calcified Tissue Research* **5**, 206–221.
- SIRE, J.-Y., DONOGHUE, P.C.J. & VICKARYOUS, M.K. (2009). Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *Journal of Anatomy* **214**, 409–440.
- *SIRE, J. & MEUNIER, F.J. (1993). Ornementation superficielle et structure des plaques osseuses dermiques de quelques Siluriformes cuirassés (Loricariidae, Callichthyidae, Doradidae). *Annales des Sciences naturelles, Zoologie, Paris* **14**, 101–123.
- SIRE, J.-Y. & MEUNIER, F.J. (1994). The canaliculi of Williamson in holostean bone (Osteichthyes, Actinopterygii): a structural and ultrastructural study. *Acta Zoologica* **75**, 235–247.
- SIRE, J.-Y. & MEUNIER, F.J. (2017). Typical tubules in the acellular bone of gilthead sea bream *Sparus aurata* (Teleostei: Perciformes: Sparidae). *Cahiers de Biologie Marine* **58**, 467–474.
- SIRE, J.Y., HUYSSEUNE, A. & MEUNIER, F.J. (1990). Osteoclasts in teleost fish: light-and electron-microscopical observations. *Cell and Tissue Research* **260**, 85–94.
- SMITH, M.M. & HALL, B.K. (1990). Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. *Biological Reviews* **65**, 277–373.
- SMITH, M.M., HOBDELL, M.H. & MILLER, W.A. (1972). The structure of the scales of *Latimeria chalumnae*. *Journal of Zoology* **167**, 501–509.
- SMITH-VANIZ, W.F., KAUFMAN, L.S. & GLOWACKI, J. (1995). Species-specific patterns of

- hyperostosis in marine teleost fishes. *Marine Biology* **121**, 573–580.
- SPENCE, R., GERLACH, G., LAWRENCE, C. & SMITH, C. (2008). The behaviour and ecology of the zebrafish, *Danio rerio*. *Biological Reviews* **83**, 13–34.
- STENSIÖ, E. (1958). Les Cyclostomes fossiles ou Ostracodermes. In *Traité de Zoologie Tome XIII : Agnathes et Poissons vol. 3* pp. 173–425. Masson, Paris.
- STEPHAN, P. (1900). Recherches histologiques sur la structure du tissu osseux des poissons. *Bulletin des Sciences de France et de la Belgique* **33**, 281–429.
- STRAUBE, N., LI, C., MERTZEN, M., YUAN, H. & MORITZ, T. (2018). A phylogenomic approach to reconstruct interrelationships of main clupecocephalan lineages with a critical discussion of morphological apomorphies. *BMC Evolutionary Biology* **18**, 158.
- TAKAGI, Y. & YAMADA, J. (1992). Effects of calcium deprivation on the metabolism of acellular bone in tilapia, *Oreochromis niloticus*. *Comparative Biochemistry and Physiology A* **102**, 481–485.
- TAVERNE, L. & FILLEUL, A. (2003). Osteology and relationships of the genus *Spaniodon* (Teleostei, Salmoniformes) from the Santonian (Upper Cretaceous) of Lebanon. *Palaeontology* **46**, 927–944.
- TOTLAND, G.K., FJELLDAL, P.G., KRYVI, H., LØKKA, G., WARGELIUS, A., SAGSTAD, A., HANSEN, T. & GROTMOL, S. (2011). Sustained swimming increases the mineral content and osteocyte density of salmon vertebral bone. *Journal of Anatomy* **219**, 490–501.
- WAINWRIGHT, D.K., INGERSOLL, S. & LAUDER, G. V. (2018). Scale diversity in bigeye tuna (*Thunnus obesus*): Fat-filled trabecular scales made of cellular bone. *Journal of Morphology* **279**, 828–840.
- WATANABE, Y.Y., GOLDMAN, K.J., CASELLE, J.E., CHAPMAN, D.D. & PAPASTAMATIOU, Y.P. (2015). Comparative analyses of animal-tracking data reveal ecological significance of endothermy in fishes. *Proceedings of the National Academy of Sciences* **112**, 6104–6109.
- WEGNER, N.C., SNODGRASS, O.E., DEWAR, H. & HYDE, J.R. (2015). Whole-body endothermy in a mesopelagic fish, the opah, *Lampris guttatus*. *Science* **348**, 786–790.
- WEIGELE, J. & FRANZ-ODENDAAL, T.A. (2016). Functional bone histology of zebrafish reveals two types of endochondral ossification, different types of osteoblast clusters and a new bone type. *Journal of Anatomy* **229**, 92–103.
- WEISS, R.E. & WATABE, N. (1979). Studies on the biology of fish bone. III. Ultrastructure of osteogenesis and resorption in osteocytic (cellular) and anosteocytic (acellular) bones. *Calcified Tissue International* **28**, 43–56.
- WILEY, E.O., JOHNSON, G.D. & DIMMICK, W.W. (1998). The phylogenetic relationships of lampridiform fishes (Teleostei: Acanthomorpha), based on a total-evidence analysis of morphological and molecular data. *Molecular Phylogenetics and Evolution* **10**, 417–425.
- WILLIAMSON, W.C. (1849). On the microscopic structure of the scales and dermal teeth of some ganoid and placoid fish. *Philosophical Transactions of the Royal Society of London. Series B* **139**, 435–475.
- WILLIAMSON, W.C. (1851). Investigations into the structure and development of the scales and bones of fishes. *Philosophical Transactions of the Royal Society of London* **141**, 643–702.

- WILSON, M.V.H. & WILLIAMS, R.R.G. (2010). Salmoniform fishes: key fossils, supertree, and possible morphological synapomorphies. In *Origin and Phylogenetic Interrelationships of Teleosts* (eds J.S. NELSON, H.-P. SCHULTZE & M.V.H. WILSON), pp. 379–409. Verlag Dr. Friedrich Pfeil, Munich.
- WITTEN, P.E. (1997). Enzyme histochemical characteristics of osteoblasts and mononucleated osteoclasts in a teleost fish with acellular bone (*Oreochromis niloticus*, Cichlidae). *Cell and Tissue Research* **287**, 591–599.
- WITTEN, P.E., FJELLDAL, P.G., HUYSEUNE, A., MCGURK, C., OBACH, A. & OWEN, M.A.G. (2018). Bone without minerals and its secondary mineralization in Atlantic salmon (*Salmo salar*): the recovery from phosphorus deficiency. *Journal of Experimental Biology*, jeb.188763.
- WITTEN, P.E. & HALL, B.K. (2002). Differentiation and growth of kype skeletal tissues in anadromous male Atlantic salmon (*Salmo salar*). *International Journal of Developmental Biology* **46**, 719–730.
- WITTEN, P.E. & HALL, B.K. (2003). Seasonal changes in the lower jaw skeleton in male Atlantic salmon (*Salmo salar* L.): remodelling and regression of the kype after spawning. *Journal of Anatomy* **203**, 435–450.
- WITTEN, P.E., HANSEN, A. & HALL, B.K. (2001). Features of mono- and multinucleated bone resorbing cells of the zebrafish *Danio rerio* and their contribution to skeletal development, remodeling, and growth. *Journal of Morphology* **250**, 197–207.
- WITTEN, P.E. & HUYSEUNE, A. (2009). A comparative view on mechanisms and functions of skeletal remodelling in teleost fish, with special emphasis on osteoclasts and their function. *Biological Reviews* **84**, 315–346.
- WITTEN, P.E. & HUYSEUNE, A. (2010). The unobtrusive majority: mononucleated bone resorbing cells in teleost fish and mammals. *Journal of Applied Ichthyology* **26**, 225–229.
- WITTEN, P.E., HUYSEUNE, A., FRANZ-ODENDAAL, T.A., FEDAK, T., VICKARYOUS, M.K., COLE, A. & HALL, B.K. (2004). Acellular teleost bone: dead or alive, primitive or derived? *The Palaeontological Association Newsletter* **55**, 37–41.
- WITTEN, P.E., OWEN, M.A.G., FONTANILLAS, R., SOENENS, M., MCGURK, C. & OBACH, A. (2016). A primary phosphorus-deficient skeletal phenotype in juvenile Atlantic salmon *Salmo salar*. The uncoupling of bone formation and mineralization. *Journal of Fish Biology* **88**, 690–708.
- WITTEN, P.E. & VILLWOCK, W. (1997). Growth requires bone resorption at particular skeletal elements in a teleost fish with acellular bone (*Oreochromis niloticus*, Teleostei: Cichlidae). *Journal of Applied Ichthyology* **13**, 149–158.
- WYSOLMERSKI, J.J. (2012). Osteocytic osteolysis: time for a second look? *BoneKEY Reports* **1**, 229.
- ZYLBERBERG, L., MEUNIER, F.J. & LAURIN, M. (2010). A microanatomical and histological study of the postcranial dermal skeleton in the Devonian sarcopterygian *Eusthenopteron foordi*. *Acta Palaeontologica Polonica* **55**, 459–470.
- ZYLBERBERG, L., MEUNIER, F.J. & LAURIN, M. (2016). A microanatomical and histological study of the postcranial dermal skeleton in the Devonian actinopterygian *Cheirolepis canadensis*. *Acta Palaeontologica Polonica* **61**, 363–376.

IX. SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Table S1. Complete list of actinopterygian (ray-finned fish) taxa surveyed by our literature review, including additional species obtained with our synchrotron microtomography (SR μ CT) data.

The supra-specific taxonomy follows Betancur-R. *et al.* (2017). In the case of older literature, species names were often outdated. We used FishBase (Froese & Pauly, 2019) and the Catalog of Fishes (Eschmeyer *et al.*, 2019) to identify the corresponding valid names.

Key for the 'Notes' column: ¹leptocephalus larva; ²possible error in identifying bone type; ³localised cellular bone in hyperostoses; ⁴acellular hyperostotic bone; ⁵alleged osteocytes in cytoplasmic tubules.

Fig. S1. Time-calibrated multilocus tree of extant actinopterygians (ray-finned fishes), obtained from the optimisation of the character states 'cellular bone' (in dark blue) and 'acellular bone' (in yellow) on the topology T1 (Near *et al.*, 2012).

Fig. S2. Time-calibrated multilocus tree of extant and fossil actinopterygians (ray-finned fishes), obtained from the optimisation of the character states 'cellular bone' (in dark blue) and 'acellular bone' (in yellow) on the topology T2 (Betancur-R. *et al.*, 2015).

Fig. S3. Time-calibrated multilocus tree of extant actinopterygians (ray-finned fishes), obtained from the optimisation of the character states 'cellular bone' (in dark blue) and 'acellular bone' (in yellow) on the topology T3 (Hughes *et al.*, 2018).

Table 1. Bone type (presence or absence of osteocytes) in the taxa surveyed by our synchrotron microtomography (SR- μ CT) data. C = cellular bone; A = acellular bone. ¹Bones sampled for *Cyprinus carpio*: frontal, maxilla, dentary, pharyngobranchial, opercle, abdominal vertebra, rib, dorsal-fin spine, cleithrum, pelvic bone. Specimens were obtained from private collectors and from the following natural history collections: American Museum of Natural History, New York City, USA (AMNH); Muséum national d'Histoire naturelle, Paris, France (MNHN); Natural History Museum, London, UK (NHMUK); Oxford University Museum of Natural History, Oxford, UK (OUMNH); Paleontological Institute and Museum, Zurich, Switzerland (PIMUZ); University of Michigan Museum of Paleontology (UMMP) and of Zoology (UMMZ); Université de Poitiers, France.

Taxon		Species name	Vernacular name	Type	Bone sampled	Specimen used
†Cheirolepidiformes	†Cheirolepididae	† <i>Cheirolepis canadensis</i>	-	C	dentary	UMMP 3453
Polypteriformes	Polypteridae	<i>Erpetoichthys calabaricus</i>	reedfish	C	dentary	Université de Poitiers, uncat.
		<i>Polypterus delhezi</i>	barred bichir	C	dentary	Université de Poitiers, uncat.
<i>Incertae sedis</i>	<i>Incertae sedis</i>	† <i>Birgeria stensioei</i>	-	C	dentary	PIMUZ T2188
Chondrostei	†Chondrosteidae	† <i>Chondrosteus acipenseroides</i>	-	C	dentary	NHMUK PV P 2261a
	Aipenseridae	<i>Acipenser gueldenstaedtii</i>	Danube sturgeon	C	dentary	MNHN.ICOS.01529
†Pycnodontiformes	†Pycnodontidae	† <i>Proscinetes elegans</i>	-	C	dentary	NHMUK PV P 1626
†Dapediiformes	†Dapediidae	† <i>Dapedium</i> sp.	-	C	dentary	OUMNH J.3041
Holostei	<i>Incertae sedis</i>	† <i>Eoeugnathus megalepis</i>	-	C	dentary	PIMUZ T344
		† <i>Heterolepidotus dorsalis</i>	-	C	dentary	NHMUK PV P 10290
		† <i>Hulettia americana</i>	-	C	dentary	UMMP 11217
	Amiidae	<i>Amia calva</i>	bowfin	C	dentary	OUMNH 21648
	†Caturidae	† <i>Caturus furcatus</i>	-	C	dentary	private collection
	Lepisosteidae	<i>Atractosteus tropicus</i>	tropical gar	C	dentary	MNHN.ICOS. PB-901
		<i>Lepisosteus oculatus</i>	spotted gar	C	dentary	UMMZ 178806/S
	†Semionotidae	† <i>Semionotus elegans</i>	-	C	dentary	UMMP 13664
	†Aspidorhynchiformes	† <i>Aspidorhynchus cf. eodus</i>	-	C	dentary	private collection
		† <i>Vinctifer comptoni</i>	-	C	dentary	UMMP 101950
†Pachycormiformes	†Pachycormidae	† <i>Euthynotus incognitus</i>	-	C	dentary, rib	NHMUK PV P 2044
		† <i>Hypsocormus</i> sp.	-	C	dentary	private collection
		† <i>Leedsichthys problematicus</i>	-	C	gill raker	private collection
		† <i>Pachycormus macropterus</i>	-	C	dentary	MNHN.F.JRE87
†Pholidophoriformes	<i>Incertae sedis</i>	† <i>Pholidophoroides crenulata</i>	-	C	dentary	NHMUK PV OR 36313

		† <i>Pholidophoropsis caudalis</i>	-	C	dentary	OUMNH J.3363
†Dorsetichthyiformes	†Dorsetichthyidae	† <i>Dorsetichthys bechei</i>	-	C	dentary	OUMNH J.3369
†Leptolepidiformes	†Ascalaboidae	† <i>Ascalabos voithii</i>	-	C	dentary	NHMUK PV P 3673a
		† <i>Tharsis dubius</i>	-	C	dentary	NHMUK PV OR 37852b
	†Leptolepididae	† <i>Leptolepis macrophthalmus</i>	-	C	dentary	private collection
†Ichthyodectiformes	<i>Incertae sedis</i>	† <i>Allothrissops regleyi</i>	-	C	dentary, rib	NHMUK PV P 921
		† <i>Pachythrissops laevis</i>	-	C	dentary	NHMUK PV P 41859
		† <i>Thrissops formosus</i>	-	C	dentary, rib	NHMUK PV OR 35013
	†Ichthyodectidae	† <i>Ichthyodectes cf. ctenodon</i>	-	C	dentary	UMMP V56318
		† <i>Xiphactinus cf. audax</i>	-	C	dentary	UMMP 11003
†Crossognathiiformes	†Crossognathidae	† <i>Rhacolepis buccalis</i>	-	C	dentary	UMMP 101952
Elopomorpha	<i>Incertae sedis</i>	† <i>Osmeroides</i> sp.	-	C	dentary	OUMNH K.64151
		† <i>Urenchelys germanus</i>	-	C	dentary	NHMUK PV P 62726
	Albulidae	<i>Albula vulpes</i>	bonefish	C	rib, opercle, ceratobranchial	UMMZ 186965/S
		† <i>Istieus grandis</i>	-	C	dentary	NHMUK PV P 3886
	Elopidae	† <i>Anaethalion angustus</i>	-	C	dentary, rib	NHMUK PV OR 37926
		† <i>Davichthys gardineri</i>	-	C	dentary	NHMUK PV P 63231
		<i>Elops saurus</i>	ladyfish	C	dentary, rib	UMMZ 189366/S
	Megalopidae	† <i>Flindersichthys denmaedi</i>	-	C	dentary	NHMUK PV P 59694
		<i>Megalops cyprinoides</i>	Indo-Pacific tarpon	C	dentary, rib	MNHN.ICOS.00987
	Anguillidae	<i>Anguilla anguilla</i>	European eel	C	dentary	MNHN.ICOS. D-35
	Congridae	<i>Conger conger</i>	European conger	C	dentary, rib	MNHN.ICOS.SP-24
	Muraenesocidae	<i>Muraenesox cinereus</i>	daggertooth pike-conger	C	dentary	MNHN.ICOS.00286
	Muraenidae	<i>Gymnothorax moringa</i>	spotted moray	C	dentary, rib	UMMZ 173403/S
		<i>Muraena helena</i>	Mediterranean moray	C	dentary	MNHN.ICOS.01039
Osteoglossomorpha	Hiodontidae	† <i>Eohiodon falcatus</i>	-	C	dentary	NHMUK PV P 61245
		<i>Hiodon alosoides</i>	goldeye	C	dentary	UMMZ 189540/S
		<i>Hiodon tergisus</i>	mooneye	C	rib	UMMZ 180315/S
	Notopteridae	<i>Chitala chitala</i>	giant featherback	C	dentary, rib	UMMZ 193675/S

	Osteoglossidae	<i>Arapaima gigas</i>	arapaima	C	dentary, rib	MNHN.ICOS.00557
		† <i>Brychaetus muelleri</i>	-	C	dentary	NHMUK PV OR 28424
		<i>Osteoglossum bicirrhosum</i>	silver arowana	C	dentary, rib	MNHN.ICOS.00630
		† <i>Phareodus encaustus</i>	-	C	dentary	NHMUK PV P 64636I
Clupeomorpha	<i>Incertae sedis</i>	† <i>Knightia</i> sp.	-	C	dentary	UMMP Tmp-1008
	†Armigatidae	† <i>Armigatus namourensis</i>	-	C	dentary, rib	NHMUK PV P 63151a
	†Ellimmichthyidae	† <i>Ellimmichthys longicostatus</i>	-	C	dentary, rib	NHMUK PV P 9855
	Chirocentridae	<i>Chirocentrus dorab</i>	wolf-herring	C	dentary, rib	UMMZ 220543/S
	Clupeidae	<i>Alosa pseudoharengus</i>	alewife	C	dentary, rib	UMMZ 187300/S
		<i>Sardinops sagax</i>	Pacific sardine	C	dentary	MNHN.ICOS.5036
Gonorhynchiformes	Chanidae	† <i>Tharrias araripes</i>	-	C	dentary, rib	NHMUK PV P 54675b
Cypriniformes	Catostomidae	<i>Catostomus commersonii</i>	white sucker	C	dentary, rib	UMMZ 178869/S
	Leuciscidae	<i>Abramis brama</i>	freshwater bream	C	dentary	MNHN.ICOS.00756
	Cyprinidae	<i>Cyprinus carpio</i>	common carp	C	various bones ¹	MNHN.ICOS.00610
	Tincidae	<i>Tinca tinca</i>	tench	C	dentary	MNHN.ICOS.00585
Characiformes	Cynodontidae	<i>Hydrolycus scomberoides</i>	payara	C	dentary	MNHN.ICOS.01021
	Erythrinidae	<i>Hoplias malabaricus</i>	trahira	C	dentary, rib	MNHN.ICOS.00631
	Serrasalminidae	<i>Serrasalmus spilopleura</i>	speckled piranha	C	dentary, rib	MNHN.ICOS.01027
Siluriformes	Ariidae	<i>Ariopsis felis</i>	hardhead sea catfish	C	dentary, rib	UMMZ 223241/S
		<i>Galeichthys feliceps</i>	white barbel	C	dentary	MNHN.ICOS.00875
	Heptapteridae	<i>Pimelodella gracilis</i>	graceful pimelodella	C	dentary, rib	UMMZ 204550/S
	Trichomycteridae	<i>Trichomycterus</i> sp.	pencil catfish	A	dentary	MNHN.ICOS.00887
Gymnotiformes	Gymnotidae	<i>Gymnotus carapo</i>	banded knifefish	C	dentary, rib	UMMZ 207893/S
Euteleostei	<i>Incertae sedis</i>	† <i>Spaniodon elongatus</i>	-	C	dentary, rib	NHMUK PV OR 44831
Salmoniformes	Salmonidae	<i>Coregonus reighardi</i>	shortnose cisco	C	dentary, rib	UMMZ 172476/S
		<i>Oncorhynchus clarkii</i>	cutthroat trout	C	dentary	UMMZ 191615/S
		† <i>Oncorhynchus lacustris</i>	-	C	dentary	UMMP 47839
		<i>Oncorhynchus mykiss</i>	rainbow trout	C	dentary	UMMZ uncat.
		<i>Oncorhynchus tshawytscha</i>	Chinook salmon	C	dentary	UMMZ uncat.

Acanthomorpha		† <i>Paleolox larsoni</i>	-	C	dentary	UMMP 50352
		<i>Parahucho perryi</i>	Japanese huchen	C	dentary	UMMZ 187612
		† <i>Prosopium prolixus</i>	-	C	dentary	UMMP 21728
		<i>Prosopium williamsoni</i>	mountain whitefish	C	dentary	UMMZ 182503/S
		<i>Salmo salar</i>	Atlantic salmon	C	dentary	MNHN.ICOS.00619
		<i>Salmo trutta</i>	sea trout	C	dentary	UMMZ uncat.
		<i>Salvelinus confluentus</i>	bull trout	C	dentary	UMMZ uncat.
		<i>Salvelinus fontinalis</i>	brook trout	C	dentary	UMMZ uncat.
		<i>Salvelinus namaycush</i>	lake trout	C	dentary	UMMZ 177542
		<i>Stenodus leucichthys</i>	inconnu	C	dentary, rib	UMMZ 187119/S
		<i>Thymallus thymallus</i>	grayling	A	rib	MNHN.ICOS.00626
	Veliferidae	<i>Metavelifer multiradiatus</i>	spinyfin velifer	A	rib	AMNH I-91798 SD
	Lamprididae	<i>Lampris</i> sp.	opah	C	sclerotic ossicle	AMNH I-21766 SD
	Polymixiidae	<i>Polymixia nobilis</i>	stout beardedfish	A	rib	AMNH I-210677 SD
	Coryphaenidae	<i>Coryphaena hippurus</i>	common dolphinfish	A	rib	MNHN.ICOS.00189
	Carangidae	<i>Trachurus trachurus</i>	horse mackerel	A	rib	MNHN.ICOS.A-14
	Xiphiidae	<i>Xiphias gladius</i>	swordfish	A	rib, sclerotic ossicle	MNHN.ICOS.6988, AMNH I-15658 SD
	Istiophoridae	<i>Kajikia albida</i>	Atlantic white marlin	A	rib, sclerotic ossicle	UMMZ 198674/S
	Moronidae	<i>Dicentrarchus labrax</i>	European seabass	A	rib	private collection
	Scaridae	<i>Chlorurus microrhinos</i>	steephead parrotfish	A	rib	MNHN.ICOS.00912
	Scombridae	<i>Acanthocybium solandri</i>	wahoo	A	rib	MNHN.ICOS.01010
		<i>Euthynnus affinis</i>	little tunny	C	rib	AMNH I-56274 SD
		<i>Gasterochisma melampus</i>	butterfly kingfish	A	rib, sclerotic ossicle	AMNH I-93480 SD
		<i>Gymnosarda unicolor</i>	dogtooth 'tuna'	A	rib	MNHN.ICOS.00492
		<i>Sarda orientalis</i>	striped bonito	A	rib	MNHN.ICOS.00954
		<i>Scomber australasicus</i>	blue mackerel	A	rib	MNHN.ICOS.00254
		<i>Thunnus obesus</i>	bigeye tuna	C	rib	MNHN.ICOS.00374