Osteohistology of the Early Triassic ichthyopterygian reptile *Utatsusaurus hataii*: Implications for early ichthyosaur biology

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Ichthyosaurs were highly adapted to a marine lifestyle, as shown by their fish-like body shape and their assumed active swimming abilities and high metabolic rates. However, the processes of adaptation to an aquatic life in the early stages of this lineage remain poorly understood. Here, we present the first osteohistological data concerning the most basal ichthyopterygian yet known, *Utatsusaurus hataii*, from the Lower Triassic of Japan. The cancellous bone structure suggests adaptation to active swimming in an open marine environment. Moreover, the possible occurrence of rapidly deposited bone tissue, in a fibrolamellar complex, suggests a higher metabolic rate than in modern poikilothermic reptiles, and therefore a trend toward homeothermy. This basal ichthyosaur, with its elongate body, was already more adapted to an aquatic lifestyle than expected from its morphology, and the process of adaptation to a marine lifestyle was already well advanced by the Early Triassic.

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Introduction

The Ichthyopterygia (sensu Motani 1999, corresponding to the traditional Ichthyosauria sensu Sander 2000 and Maisch 2010) represents one of the most successful groups of Mesozoic marine reptiles and is known from the late Early Triassic to early Late Cretaceous (Motani 2005; Fig. 1A). Most of the derived forms, called "neoichthyosaurians", display a reduced pelvic girdle and a tuna-shaped body plan, with a spindle-shaped trunk, flippers, a dorsal fin, and a semilunate tail, suggesting a thunniform mode of swimming (Lingham-Soliar and Reif 1998; Fig. 1B). However, the earliest forms have a long, slender body with a rather straight (or weakly bent; see Motani 1999) long tail. They still retain a robust articulation of the pelvic girdle with the axial skeleton, and were probably anguilliform swimmers (Motani et al. 1996; Fig. 1C). Some Middle to Late Triassic forms, such as *Mixosaurus*, display an intermediate state between the basal ichthyopterygians and the neoichthyosaurians (Fig. 1D), and all these forms are considered to have been well adapted for a fully marine life.

Generally, the inner bone structure of secondarily aquatic amniotes displays either a compact or a cancellous structure, depending on the specific swimming style and habitat of the taxon (Ricqlès and Buffrénil 2001). One pattern corresponds to osteosclerosis; it is not morphologically observable, and might be associated with hyperplasy of the bone cortex, called pachyostosis, which results in a morphologically observable thickening of the bone (Houssaye 2009). It occurs in essentially or exclusively aquatic animals living in shallow waters (Houssaye 2009). The other pattern is osteoporotic. Interpreted as an especially advanced aquatic adaptation for improved locomotory performance (diving and accelerating capacities and/or manoeuvrability), it is characteristically found in highly aquatic tetrapods with good swimming abilities (Ricqlès and Buffrénil 2001; Houssaye 2009), such as extant cetaceans (Felts and Spurrel 1966; Buffrénil and Schoevaert 1988), the leatherback turtle (Rhodin 1985), some mosasaurs (Sheldon 1997), some plesiosaurs (Wiffen et al. 1995), and ichthyosaurs (Kiprijanov 1881; Seitz 1907; Gross 1934).
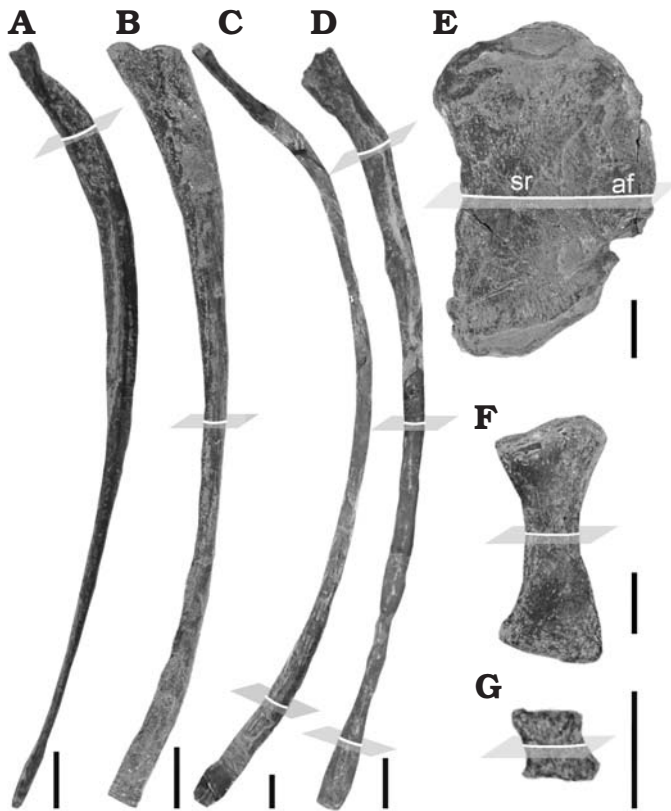
Fig. 1. Simplified view of the evolution of the Ichthyopterygia. **A**. Time-calibrated phylogenetic tree of the Ichthyopterygia. **B–D**. Schematic drawings (from McGowan and Motani 2003) showing the evolution of the body plan within the Ichthyopterygia (not to scale), between the basal ichthyopterygian *Utatsusaurus* (**B**), the basal ichthyosaurian *Mixosaurus* (**C**), and the scombrid fish-shaped neoichthyosaurian *Ophthalmosaurus* (**D**). Stratigraphic data compiled after McGowan and Motani (2003), Fernández (2003), Bennett et al. (2012), and Wiman (1910).

Limb bones, vertebrae, and/or ribs of the Triassic marine reptiles *Mixosaurus* (Reptilia; Ichthyosauria; Fig. 1C; Kolb et al. 2011) and *Omphalosaurus* (whose ichthyopterygian status is not fully established; Sander and Faber 1998, 2003 vs. Motani 2000), and a possible ichthyopterygian *Pessopteryx* (Houssaye et al., 2014), as well as those of the neoichthyosaurians *Ichthyosaurus*, *Stenopterygius* (Buffrénil and Mazin 1990; Houssaye et al. 2014), *Temnodontosaurus* (Kolb et al. 2011; Houssaye et al. 2014), *Mollesaurus* (Talevi and Fernández 2012), *Caypullisaurus* (Talevi et al. 2012), *Ophthalmosaurus* (Houssaye et al. 2014), and *Platypterygius* (Lopuchowycz and Massare 2002; Kiprijanov 1881), have been examined histologically. Limb bones and gastralia of *Mixosaurus* sp. and ribs of *Mollesaurus* show relatively high inner compactness (Kolb et al. 2011; Talevi and Fernández 2012), whereas all the other taxa display a cancellous inner skeletal organization (Buffrénil et al. 1987; Buffrénil and Mazin 1990; Kolb et al. 2011; Talevi et al. 2012; Houssaye et al. 2014).

Bone tissue types are defined by the orientation of the collagen fibres and the type of vascularization (e.g., type of canals, orientation, and density). This reflects various rates of bone deposition (Amprino 1947; Margerie et al. 2002; Montes et al. 2010) and has frequently been used to estimate the growth rates of extinct taxa (Padian et al. 2001, 2004). Bone depositional rate estimated by osteohistology indicates the basal metabolic rate of the animal (Padian et al. 2001). Indeed, poikilothermic reptiles generally have parallel-fibred bone with simple vascular canals, which is deposited slowly (e.g., Castanet et al. 1988). A characteristic feature of ichthyosaurian osteohistology is a quickly deposited bone tissue called the "fibrolamellar complex" (Buffrénil and Mazin 1990; Kolb et al. 2011; Houssaye et al. 2014), which corresponds to an association of a woven bone matrix and primary osteons (Francillon-Vieillot et al. 1990). The fibrolamellar complex is shared by modern rapidly growing mammals and birds, as well as by extinct synapsids, dinosaurs, and pterosaurs (e.g., Enlow and Brown 1956, 1957, 1958; Ricqlès

1976; Ray et al. 2004; Padian et al. 2001; Chinsamy et al. 2009). This suggests rapid growth and a relatively high metabolic rate (even possibly homeothermy) in the Ichthyosauria (Scheyer et al. 2010). A high metabolic rate and active swimming in the ichthyosaurians are also supported by previous biomechanical (e.g., Motani 2002) and isotopic (Bernard et al. 2010) studies.

On the other hand, the early history of ichthyosaurian biology remains poorly understood, partly because of insufficient information about the basal ichthyopterygians. Against this background, this study focuses on *Utatsusaurus hataii* Shikama, Kamei, and Murata, 1978, the most basal ichthyopterygian yet known. This animal is 2–3 m long, with a relatively slender body and an eel-like caudal fin. This transitional form retains both cranial and postcranial features of terrestrial amniotes to some degree (e.g., sacral ribs, equal length of the humerus and femur; Motani et al. 1998; Fig. 1C). This study presents for the first time histological data for this taxon and draws inferences about its growth, physiology, and ecology.

Institutional abbreviations.—IGPS, Institute of Geology and Paleontology, Tohoku University, Sendai, Japan; NSM, National Museum of Nature and Science, Tokyo, Japan; UHR, Hokkaido University Museum, Sapporo, Japan.

Other abbreviations.—HL, length of the humerus.

Material and methods

Utatsusaurus hataii was originally described based on 13 specimens from the Motoyoshi District, Miyagi Prefecture, Japan (Shikama et al. 1978), although only two incomplete specimens of the original referred specimens—the holotype (IGPS 95941) and a paratype (IGPS 95942)—are firmly recognized as *Utatsusaurus* (Motani 1997). Subsequently, two additional specimens from Ogatsu, Ishinomaki City, Miyagi

Fig. 2. Analysed skeletal elements of the ichthyopterygian *Utatsusaurus hataii* Shikama, Kamei, and Murata, 1978 (Osawa Formation, Spathian; Miyagi Prefecture, Japan). **A**, **D**. NSM-VP-21865, dorsal ribs. **B**, **C**. NSM-VP-20028, dorsal ribs. **E–G**. NSM-VP-21865, humerus (**E**), radius (**F**), and phalanx (**G**).The humerus shows a D-shaped outline, consisting of the anterior flange (af) and the shaft region (sr). Grey quadrangles and white lines show the sectional planes. Scale bars 10 mm.

Prefecture (NSM-VP-20028, UHR30691; Motani et al. 1998) and one from the Motoyoshi District (NSM-VP-21865; YN personal observations) have been collected.

In this study, samples were taken from two individuals of *Utatsusaurus* (NSM-VP-20028 and NSM-VP-21865). These specimens were discovered in the upper part of the Osawa Formation and are considered Late Olenekian (Spathian) in age (Kashiyama and Oji 2004). The length of the humerus (HL) of NSM-VP-21865 (52 mm) is approximately 90% that of the largest well-preserved humerus (IGPS 95942: 57 mm). The HL of NSM-VP-20028 is 61 mm, although this specimen does not seem to be as large as this value indicates, because it has experienced tectonic shearing deformation (Motani et al. 1998). Overall, it seems to be approximately the same size as IGPS95942. Therefore, these specimens are considered to be similar in size to the largest specimen.

We chose the mid-shaft as the preferred sampling location for the limb bones and ribs to ensure comparability with existing data; the mid-shaft has frequently been used for histological sampling in previous studies of the internal bone structure of aquatic tetrapods (e.g., Buffrénil et al. 2010; Canoville and Laurin 2010). Whenever possible, additional sections were taken from different positions to examine histological and microanatomical variations within the bone. Mid-shaft sections from two dorsal ribs (one from each individual), proximal sections from two dorsal ribs of NSM-VP-21865, and distal sections from two dorsal ribs (one from each individual) were taken (Fig. 2A–D). Mid-shaft sections from limb bones, including the humerus (Fig. 2E), radius (Fig. 2F), and phalange (Fig. 2G), were also taken. The humeral mid-shaft section crosses the well-marked tuberosity (Motani 1997), which shows apparent local periosteal hyperplasy within the shaft region. The histological sections were processed using standard procedures (see Kolb et al. 2011). Thin sections were analysed with Leica DMLP and Nikon Eclipse E600 microscopes under natural and polarized (with and without lambda compensator) lights. Photomicrographs were taken using Nikon Coolpix 5000 and Zeiss AxioCam MRc5. Although some histological features, notably the crystal orientation, were not always preserved, the observation and sampling of several bone elements with varying degrees of preservation allowed the description of bone histology.

Results

Rib.—In the rib proximal and mid-shaft sections, the cortical compactness varies between and within sections. It seems that there is no open medullary cavity or only a small one, but rather an extremely cancellous medullary region, with large cavities separated by only a few relatively thin trabeculae (Fig. 3A–C). The cortex appears to be highly vascularized with simple vascular canals (Fig. 4A, C) and it becomes progressively more cancellous from the periphery of the bone to its core, which already shows abundant erosional bays (Fig. 4A, C). The inner part of the cortex contains dense, irregularly organized globular cell lacunae (Fig. 4D) that are generally typical of fibrous bone (Francillon-Vieillot et al. 1990). Cross-polarized views of rib sections show nearly total monorefringence, which does not seem to reflect the original osteohistological state but to be a diagenetic artefact (Fig. 4A, B). A progressive decrease in density of cells and a morphological change from globular to spindle-shaped cells (Fig. 4D), typical of parallel-fibred or lamellar bone, is observed toward the periphery, which suggests that the peripheral cortex consists of parallel-fibred bone, despite its monorefringence under polarized light.

Rib distal sections suggest a relatively tubular structure, with a thin cortex and a relatively large inner medullary cavity (Fig. 3E, F) surrounded by endosteal bone (Fig. 4F). There is a change from the inner part of the cortex to the periphery, but contrary to the other sections, the change in the distal section is rather abrupt (Fig. 4F). The bone tissue and its preservational condition in each part of the cortex are similar to the proximal and mid-diaphyseal sections.

The microstructure of the rib sections is thus generally separable into three zones: (i) The perimedullary region with the osseous trabeculae or with a circumferential deposit,
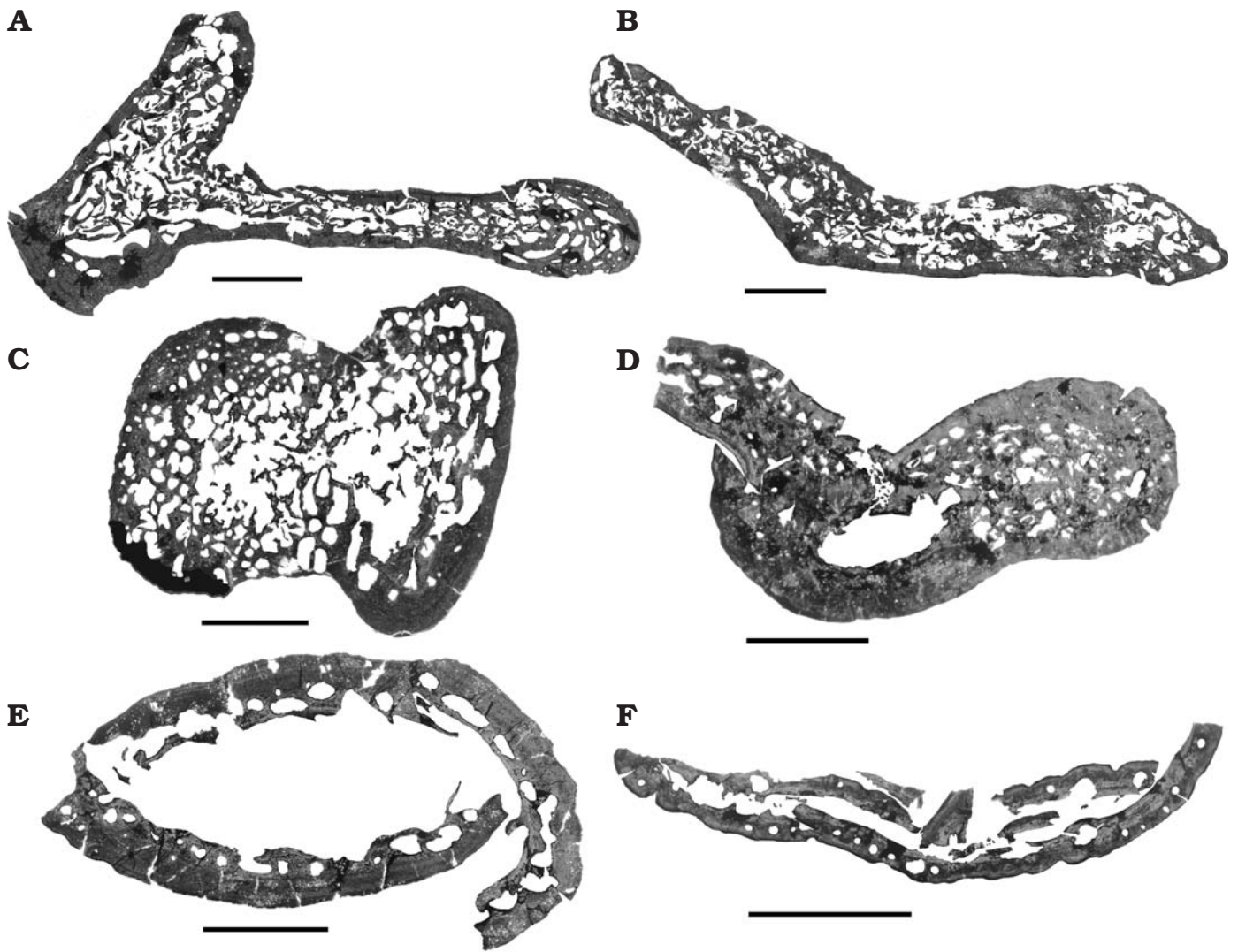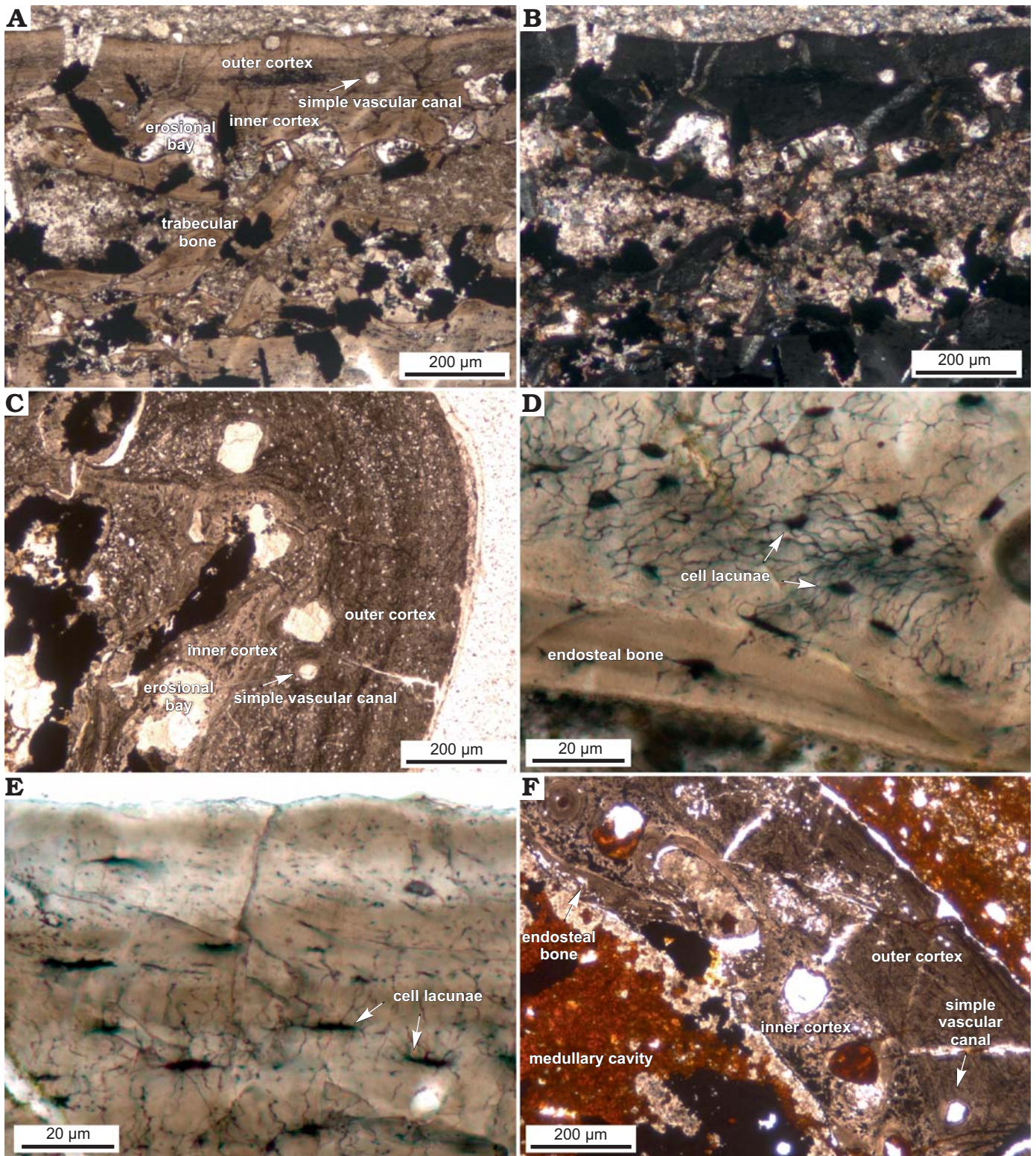
Fig. 3. Transverse sections of dorsal ribs of the ichthyopterygian *Utatsusaurus hataii* Shikama, Kamei, and Murata, 1978 (Osawa Formation, Spathian; Miyagi Prefecture, Japan). **A**, **B**. NSM-VP-21865, proximal sections; **A** (see Fig. 2A) and **B** (see Fig. 2D, upper section). **C**. NSM-VP-20028, mid-shaft section (see Fig. 2B). **D**. NSM-VP-21865, mid-shaft section (see Fig. 2D, middle section). **E**. NSM-VP-20028, distal section (see Fig. 2C). **F**. NSM-VP-21865, distal section (see Fig. 2D, lower section). Photographed under natural light. Scale bars 1 mm.

probably around an open medullary cavity; (ii) the internal cortex, whose cells evoke woven-fibred bone, rich in simple vascular canals and erosional bays; and (iii) the nearly avascular outer cortex, with spindle-shaped osetocyte lacunae evoking a parallel-fibred-like microstructure.

Appendicular skeleton.—A plate-like thin anterior expansion, called the "anterior flange" (e.g., Motani 1997) develops along the anterior margin of the humerus, making its outline D-shaped in dorsal and ventral views (Fig. 2E). As a consequence, the transverse sections of the humerus are antero-posteriorly elongated (Fig. 5A).

The cortex of the humerus is naturally thicker in the shaft region, especially at the position of the mid-shaft tuberosity, but much thinner in the anterior flange (Fig. 5A). Most of the cortical bone matrix in the shaft region is monorefringent under polarized light (Fig. 6A, B), perhaps reflecting the original random fibre arrangement or a preservational artefact. It con-

tains irregularly shaped osteocyte lacunae, which are irregularly distributed (Fig. 6C), evoking a woven bone matrix. Some parallel-fibred bone can also be observed locally, notably in the anterior flange (see below). The cortex of the shaft region is highly vascularized with longitudinally oriented primary osteons (Fig. 6D). This tissue seems thus to correspond to a standard fibrolamellar complex. The borders of the vascular canals undergo resorption during growth, so that the vascular canals evolve into large inter-trabecular spaces with irregular margins in the inner part of the sections (Fig. 6A, C), giving the bone a spongy inner organization with cancellousness increasing towards the core of the bone. Evidence of secondary bone deposition is not seen in the cortex region. In the shaft region the internal part located between the dorsal and ventral cortices is filled with collapsed thin trabeculae, consisting of endosteal lamellar bone (Fig. 6E).

The internal microstructure of the humeral shaft in trans-

Fig. 4. Details of dorsal rib microstructure of the ichthyopterygian *Utatsusaurus hataii* Shikama, Kamei, and Murata, 1978 (Osawa Formation, Spathian; Miyagi Prefecture, Japan). **A**, **B**. NSM-VP-21865, proximal section (see Fig. 2A). **C**. NSM-VP-20028, mid-diaphyseal section (see Fig. 2B). **D**, **E**. NSM-VP-21865, close-up views of cell lacunae in the inner (**D**) and outer (**E**) cortices of proximal dorsal rib (see Fig. 2D). **F**. NSM-VP-20028, distal section (Fig. 2C). Photographed under natural light (A, C–F) and polarized light without lambda compensator (B).

verse section is generally divided into three zones: (i) the trabecular bone in the inner part of the section, consisting of secondary lamellar bone; (ii) the inner cortex with, as a result of resorption, enlarged longitudinally oriented vascular canals; and (iii) the outer cortex, barely resorbed, with longitudinally oriented primary osteons.
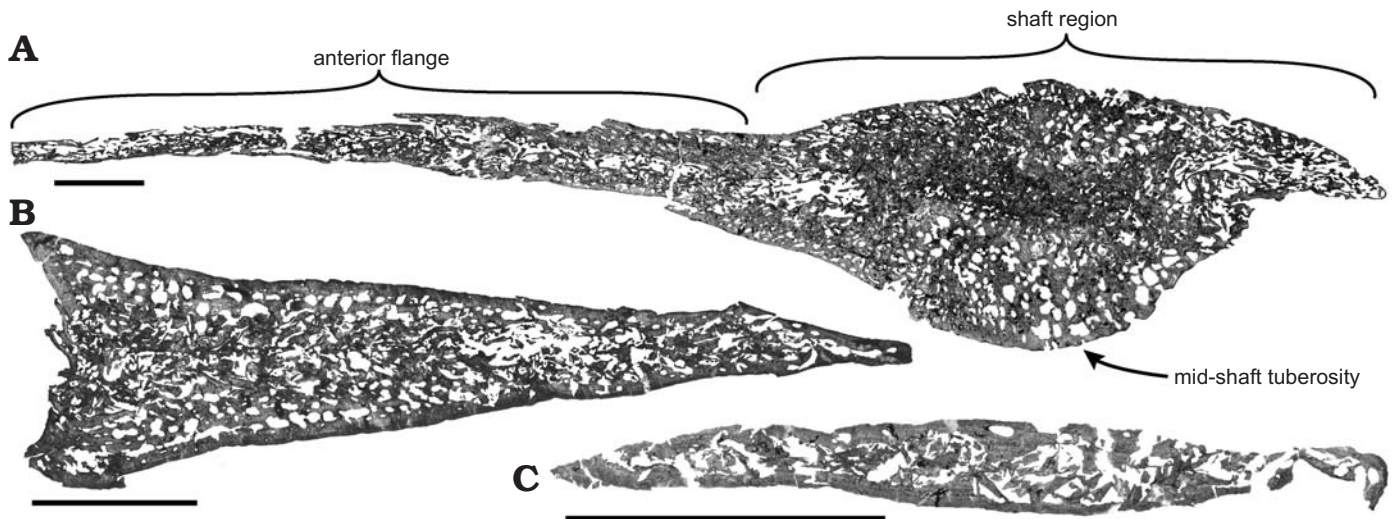
Fig. 5. Mid-diaphyseal sections of limb bones of the ichthyopterygian *Utatsusaurus hataii* Shikama, Kamei, and Murata, 1978, NSM-VP-21865 (Osawa Formation, Spathian; Miyagi Prefecture, Japan). **A**. Humerus (see Fig. 2E). **B**. Radius (see Fig. 2F). **C**. Phalange (see Fig. 2G). Photographed under natural light. Scale bars 2 mm.

The histological features in the humeral anterior flange are different from those observed in the shaft. A layer of endosteal parallel-fibred bone is deposited internal to the cortex. The inner cortical region of the anterior flange consists of woven-fibred bone and the periphery consists of parallel-fibred bone (Fig. 6F, G).

The mid-diaphyseal sections of the radius and the phalanx have an inner organization that is cancellous (Fig. 5B, C). In these bones, the preservational condition is the same as in the rib and humeral shaft region (Fig. 7A, B). However, a change can clearly be seen from globular-shaped, dense, randomly arranged cell lacunae, rich in canaliculi (which is typical of woven-fibred bone), in the inner part of the cortex to sparse, smaller, spindle-shaped cell lacunae, poor in canaliculi, at its periphery (Fig 7C–F). This change is accompanied by a significant change in the degree and type of vascularization in the radius, as shown by the abundant primary osteons in the inner part of cortex (Fig. 7A) and by the nearly avascular thin peripheral cortical layer with a few simple vascular canals (Fig. 7D). The cortex in the peripheral part the phalangeal section is barely vascularised (Fig. 7E).

Discussion

Implications for growth pattern and metabolic rate.— The bone histology of the examined bones in *Utatsusaurus* can be considered rather uniform. *Utatsusaurus* primary bone shows features of the cell lacunae and vascular canals evoking both fibrolamellar and parallel-fibred bone depending on the bone regions. It seems that fibrolamellar bone deposited in early stages and then parallel-fibred bone deposited at the end. The fibrolamellar complex is known to be deposited quickly, and suggests here a relatively rapid bone growth rate in *Utatsusaurus*, despite a later decrease in growth speed. However, no significant slowing of growth is observed in the humerus. Growth rate is known to vary between bones within a single specimen (Margerie et al. 2004). The humerus is the thickest and largest element studied, and it was probably growing faster than other examined elements at the time of death.

The fibrolamellar complex has also been observed in the humeri of *Mixosaurus* (Kolb et al. 2011). Its possible presence in *Utatsusaurus* suggests that both *Mixosaurus* and *Utatsusaurus*, the basal ichthyosaurian and the sister taxon to all other ichthyopterygians, had a relatively high growth rate, interpreted as a precondition for homeothermy. However, a reduction in the bone growth rate throughout ontogeny is observed in *Utatsusaurus* (except in the humerus), which suggests that this high growth rate did not persist for a long time during ontogeny, as was probably the case for *Mixosaurus* (Kolb et al. 2011).

The vascularization pattern in the ribs is similar to that described for hydropelvic mosasauroids (Houssaye and Bardet 2012; Houssaye et al. 2013), considered to be gigantothermic (Motani 2010). However, whereas mosasauroids essentially display parallel-fibered bone, *Utatsusaurus* displays a bone tissue comparable with the fibrolamellar complex, which changes into parallel-fibred bone only later in ontogeny. The rapid deposition of bone indicated by histology suggests a high metabolic rate in *Utatsusaurus*. Interestingly, this could mean that even a basal ichthyopterygian had a higher metabolic rate than the already large mosasaurs, which were highly adapted for an open-marine life (Houssaye and Bardet 2012).

At this point, it is not possible to infer when this higher metabolic rate was acquired in the process of secondary adaptation to the marine realm in the ichthyopterygian lineage, but this study suggests that it was acquired much earlier than previously thought (Motani 2010).

Cancellous bone and the inferred palaeoecology of *Utatsusaurus*.—The bones of *Utatsusaurus* are characterized by
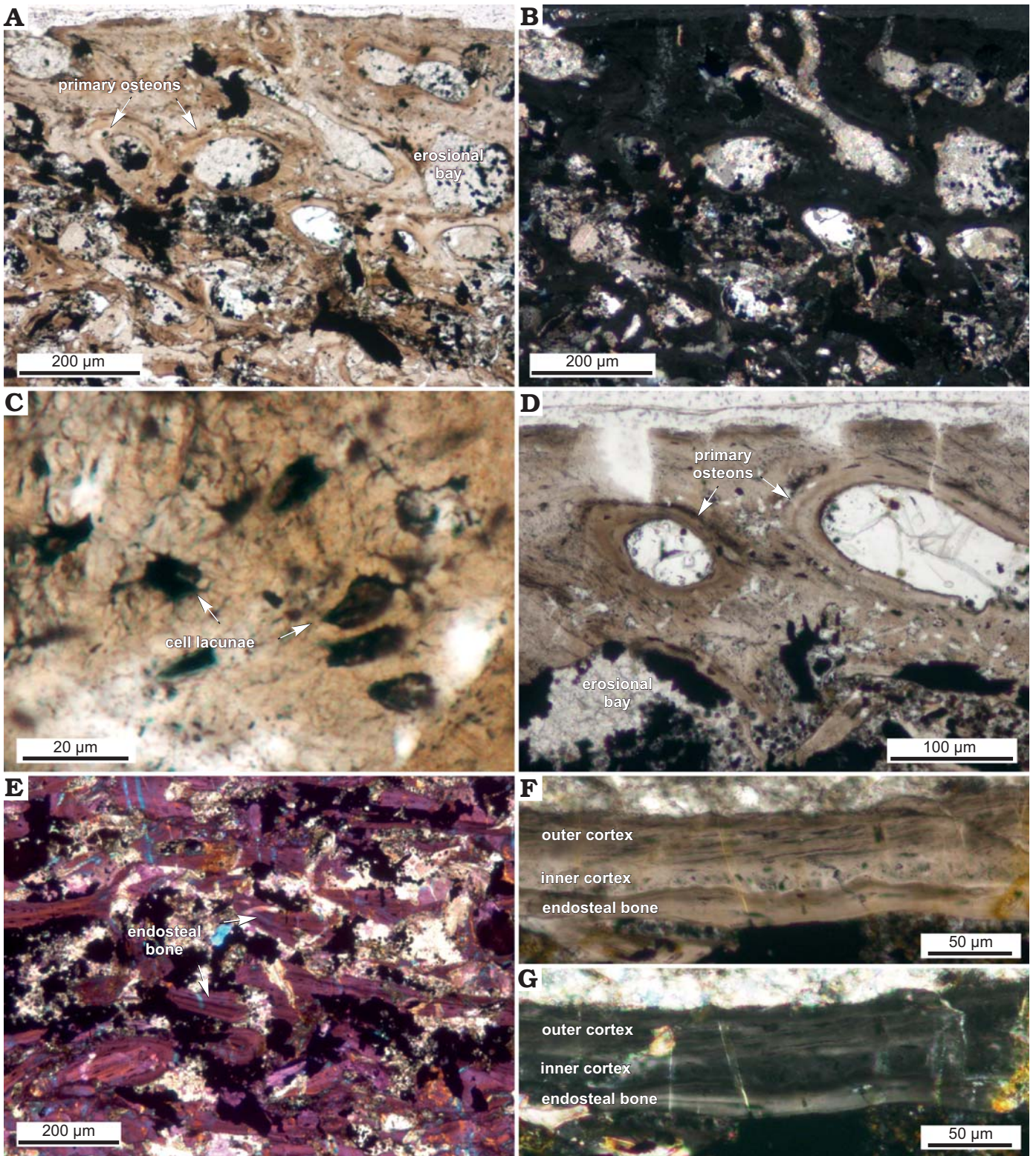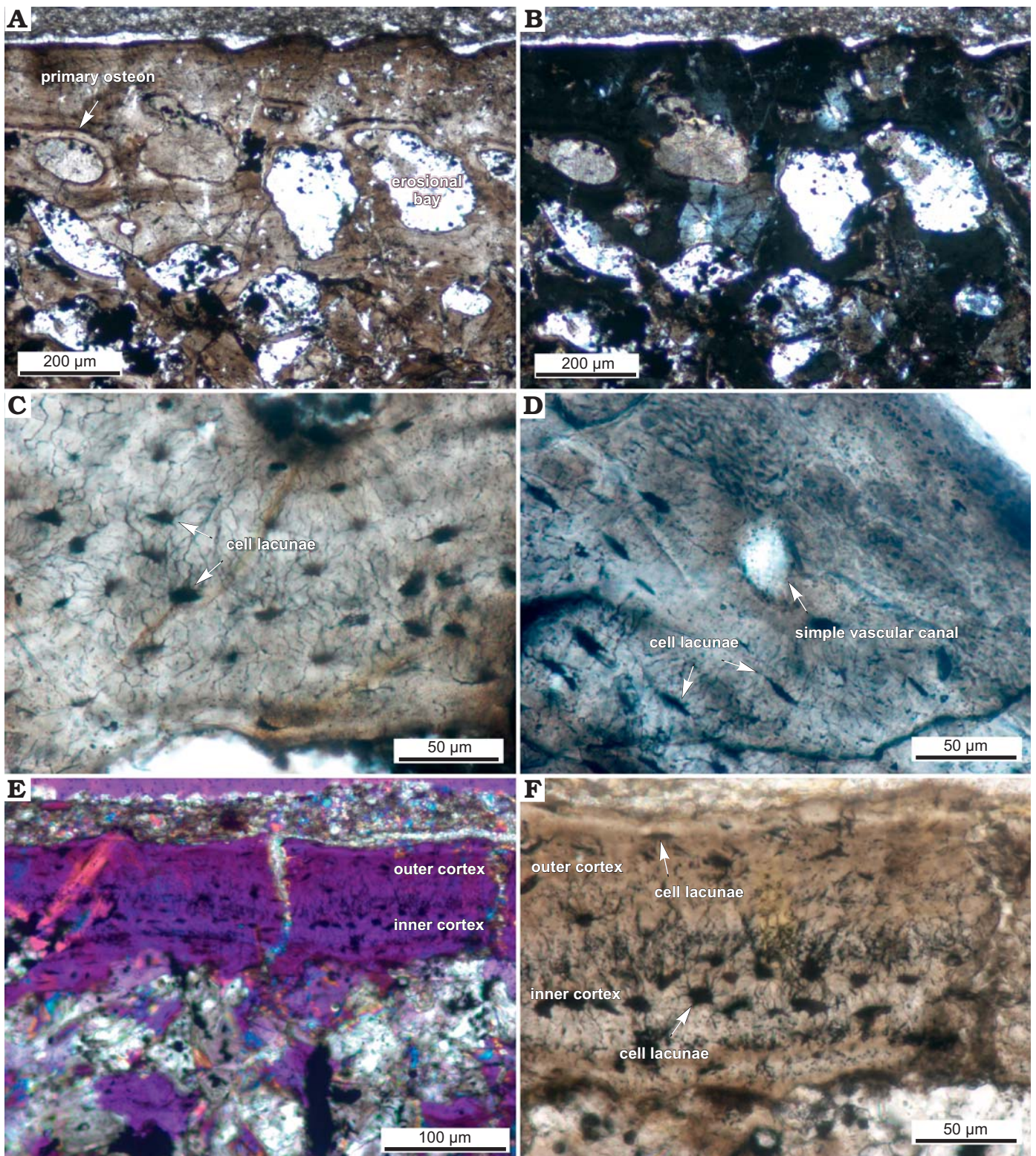
Fig. 6. Details of bone microstructure of a humerus (see Fig. 2E) of the ichthyopterygian *Utatsusaurus hataii* Shikama, Kamei, and Murata, 1978, NSM-VP-21865 (Osawa Formation, Spathian; Miyagi Prefecture, Japan). **A**, **B**. Periosteal cortex of mid-diaphyseal section. **C**. Close-up view of cell lacunae in the periosteal cortex. **D**. Close-up view of primary osteons in the primary periosteal bone. **E**. Mid-diaphyseal section, internal part of the shaft region. **F**, **G**. Cortex of the anterior flange. Photographed under natural light (A, C, D, F), polarized light without lambda compensator (B, G), and polarized light with lambda compensator (E).

imbalanced remodelling, during which active resorption—with the intense development of resorption bays—was only

followed by limited deposition of secondary bone (which mainly occurred in the inner part of the bone, so that many

Fig. 7. Details of limb bone microstructure of the ichthyopterygian *Utatsusaurus hataii* Shikama, Kamei, and Murata, 1978, NSM-VP-21865 (Osawa For-mation, Spathian; Miyagi Prefecture, Japan). **A**, **B**. Mid-diaphyseal section of the radius (see Fig. 2F). **C**, **D**. The same section as A, close-up views of inner (**C**) and outer (**D**) periosteal bone of the radius. **E**. Cortex of a phalanx (see Fig. 2G). **F**. The same section as E, close-up view of cortex. Photographed under natural light (A, C, D, F), polarized light without lambda compensator (B), and polarized light with lambda compensator (E).

thin bone trabeculae at the centre of several mid-shaft sections consist almost entirely of secondary lamellar bone). Con-sequently, all the bones studied display a cancellous inner organization. However, because of the relatively bad preser-vation of the bones, it is not possible to state whether the long bones of *Utatsusaurus* had a narrow open medullary cavity,

like those of *Omphalosaurus*, or no medullary cavity, as in *Stenopterygius*, *Ichthyosaurus*, and *Mixosaurus* (Buffrénil and Mazin 1990; Kolb et al. 2011). Although the thickness of the compact cortex cannot be precisely estimated, it appears rather low, so that most of the bone is spongy. A cancellous inner organization is also observed in the latest Early to Middle Triassic *Omphalosaurus* (Buffrénil et al. 1987), which might also represent a basal ichthyosaur (Sander and Faber 2003). Until now, this cancellous inner organization has never been observed in Early Triassic aquatic reptiles, except for *Omphalosaurus* (Buffrénil et al. 1987; Houssaye 2009). Therefore, among marine tetrapods, *Utatsusaurus* and *Omphalosaurus* are the earliest known examples of this specialization, which is advantageous for manoeuvrable swimming and cruising in a pelagic marine environment (Ricqlès and Buffrénil 2001). It is also noteworthy that the depositional environment of the Osawa Formation, which bears well associated *Utatsusaurus* skeletons, is considered to be a prodelta or a continental slope in a transgressive system (Kawakami and Kawamura 2002), which supports the hypothesis of a pelagic lifestyle for this taxon. This unexpected inferred lifestyle of the earliest ichthyosaur in the Early Triassic suggests a rapid ecological radiation of this lineage into the open marine environment after the Permian–Triassic mass extinction event.

Conclusions

The osteohistological analysis of the basal-most ichthyopterygian *Utatsusaurus hataii* has revealed a cancellous inner bone structure similar to that observed in extant open-sea-dwelling tetrapods. Histologically, the possible presence of the fibrolamellar complex at least early in development indicates a high metabolic rate and suggests that this taxon already displayed the precondition for homeothermy. *Utatsusaurus* is therefore interpreted as an active swimmer, inhabiting an open-marine environment. Despite the retention of some terrestrial characters in its morphology (Motani 1997; Motani et al. 1998), this taxon was already advanced in the process of secondary adaptation to the marine realm.

Acknowledgements

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