

Histological study of karaurids, the oldest known (stem) urodeles

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Little is known about the initial phases of lissamphibian history (before the Cretaceous), because their fossil record is quite scanty. Only the morphology of the earliest members has been investigated, although other sets of data, from bone microanatomy and histology, are known to yield valuable paleobiological information. In the present study, we provide the first histological and microanatomical data on the oldest known stem-urodeles, the karaurids, from the Middle Jurassic. Three humeri from the Upper Bathonian, Oxfordshire, referred to juvenile or subadult individuals of *Marmorerpeton* and to an unnamed caudate of undetermined (but obviously non-larval) ontogenetic stage, were sampled in order to shed new light on the habitat and ontogeny of these basal caudates. The great compactness of the three humeri suggests that these salamanders were aquatic. The presence of extensive amounts of calcified cartilage in the humeri greatly strengthens the case for the presence of neoteny in these taxa, a suggestion that had initially been made on the basis of a few morphological characters. This constitutes the oldest known occurrence of neoteny in lissamphibians. Finally, bone histology reveals that the growth of *Marmorerpeton* and the related unnamed caudate was fairly slow and cyclic, a characteristic of extant lissamphibians.

Keywords: Urodela; Caudata; Lissamphibia; paleohistology; neoteny; Jurassic

1. Introduction

Little is known about the earliest phases of lissamphibian history because the fossil record of this taxon is not as dense as that of many other vertebrates (Marjanović and Laurin 2008, 2013). Salientians first appear in the fossil record in the Early Triassic (Rage and Roček 1989; Evans and Borsuk-Biatynicka 2009), followed by Gymnophionomorpha in the Early Jurassic (Jenkins et al. 2007) and by Caudata in the Middle Jurassic (Evans et al. 1988; Averianov et al. 2008; Skutschas and Martin 2011). Of these earliest lissamphibians, only the morphology is known in any detail. Another set of biological data, unexploited so far in these taxa, bone histology, can yield valuable information on the growth pattern, gross physiology and ecologic adaptation of early amphibians. Below, we provide the first histological data on one of these clades, the karaurids, known from Central Asia (*Karaurus*, *Kokartus*: Averianov et al. 2008; Skutschas and Martin 2011), Britain (*Marmorerpeton*, Salamander A: Evans et al. 1988) and Portugal (cf. *Marmorerpeton*: Evans, personal observation). These data shed new light on the habitat and ontogeny of these oldest known stem-urodeles.

2. Materials and methods

The paleontological sample consists of three partial humeri from the Upper Bathonian (ca. 166–168 Ma), Middle

Jurassic of Kirtlington quarry, Oxfordshire, England. These are uncatalogued specimens, from University College London, curated by one of us (SE). Two of these bones, designated here for convenience as specimens I and II are referred to *Marmorerpeton* sp., which has been attributed to karaurids (Evans and Waldman 1996); the third bone (specimen III; i.e. salamander 'A' first mentioned by Evans and Milner 1991) is from an unnamed Caudata, possibly a karaurid, close in size to *Marmorerpeton*. Although lacking extensive parts of the diaphyseal region (specimen II) or of the epiphyses and metaphyses (specimens I and III), these bones were not submitted to severe diagenesis and are otherwise well preserved. Considering the diaphyseal diameter of the largest *Marmorerpeton* humerus found in Kirtlington quarry, 2.56 mm (Evans et al. 1988, Figure 9), our *Marmorerpeton* specimens should be considered as representative of late juveniles or subadults that had not reached their full size because their respective mean diameters are 1.04 mm (specimen I) and 0.93 mm (specimen II). For 'salamander A', the ontogenetic stage is uncertain, although the bone (specimen III) has a smaller diameter (0.74 mm), all specimens of that taxon are apparently of about the same size (SE, personal observation), and could well be subadults or adults. One transverse thin section was made as close as possible to the middle of the diaphysis in each of these bones (Figure 1). The sections from specimens I and III can be considered as

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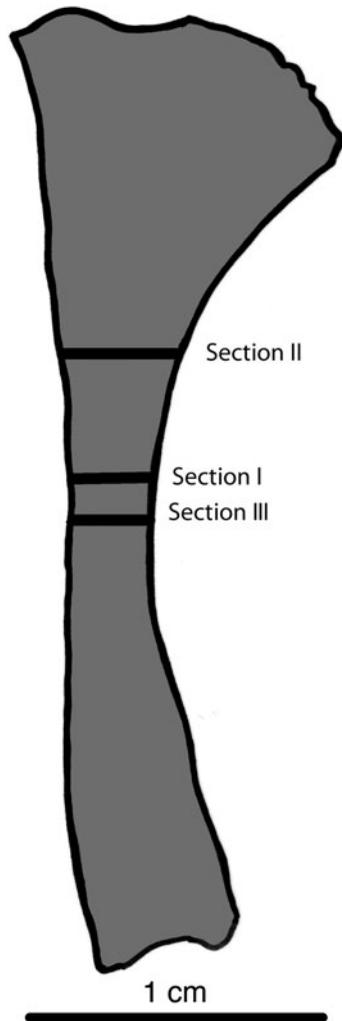


Figure 1. Sketch of a *Marmorerpeton* humerus showing the location and orientation of the sections made in the specimens (one section per specimen). Note that the section of specimen III belongs to ‘salamander A’; the other two sections (of specimens I and II) belong to *Marmorerpeton*.

located at mid-diaphysis, whereas that from specimen II is somewhat shifted towards the metaphyseal region. The technical processing of the sections was based on the standard procedures described by Padian and Lamm (2013). The final sections were examined with an optical microscope in transmitted ordinary or polarised light. Assessment of bone compactness and other measurements made on the sections were performed on binarised images with the software Image J (National Institute of Health, Bethesda, MD, USA).

3. Histological observations

The section from specimen I shows that the humeral shaft is entirely amedullar (Figure 2(A)). At this level, broad erosion bays (23 of these bays can be observed) randomly

colonise the whole sectional area. Due to the occurrence of Howship’s lacunae (Figure 2(A),(B)), the outlines of the bays are irregular and notched, an aspect that characteristically results from the action of clastic cells (osteoclasts and chondroclasts). This section displays two distinct tissues.

A small disc (mean diameter of 190 μm , i.e. 18.3% of mean sectional diameter) composed of the intercellular matrix of calcified cartilage is located in the medullar territory of the shaft (Figure 2(A)). It represents 3.7% of the whole sectional area (Table 1). At the level of this section, the cartilage matrix looks amorphous, with only some unidentifiable dark granules. The calcified cartilage is partly eroded (up to 12% is eroded) by two broad resorption bays, one of which extends to the neighbouring part of the cortex.

The rest of the section consists of typical bone tissue of periosteal origin that forms the cortex of the humeral diaphysis. The cortex is proportionally thick (it occupies 81.7% of mean sectional diameter) and stratified into distinct growth cycles, clearly revealed by polarised light (Figure 2(A)). Each of these cycles includes two parts: (1) a thick monorefringent basal part, or *zone* (cf. nomenclature from Francillon-Vieillot et al. 1990), in which osteocyte lacunae display variable aspects due to their uneven orientation in the sectional plane (Figure 2(B)). This osseous tissue is akin to the woven-fibred type. (2) A narrower peripheral part, or *annulus*, displays a mass birefringence (no subdivision into lamellae) and spindle-like cell lacunae oriented parallel to each other and to the outer limit of the bone cortex (Figure 2(B)). This represents parallel-fibred bone. In the section of specimen I, there are two concentric growth cycles. In all extant tetrapods for which the periodicity of growth cycles was specifically studied, it was shown to be basically annual: one zone + one annulus are formed each year (review in e.g. Baglinière et al. 1992). Therefore, this *Marmorerpeton* humerus (I) is probably from an individual that died by the end of its second year, and possibly during the unfavourable season. In both the *zone* and *annuli*, cell lacunae have no or poorly developed canaliculi. The bone cortex looks devoid of inner vascular canals, with the possible exception of a short radial canal some 50 μm in diameter (this canal could also be an oblong resorption bay). However, two deep indentations on the outer cortical margin (Figure 2(A),(B)) suggest that some of the blood vessels of the periosteum were incorporated into the cortex by the time the animal died; cortical growth was therefore still proceeding at this stage. This is consistent with the modest size of this bone (maximal section diameter of 1040 μm), i.e. 41% of the largest *Marmorerpeton* humerus collected by Evans et al. (1988).

The section from humerus II (Figure 2(C)) is basically comparable to the section described above but with four notable differences. (1) The relative area occupied by the

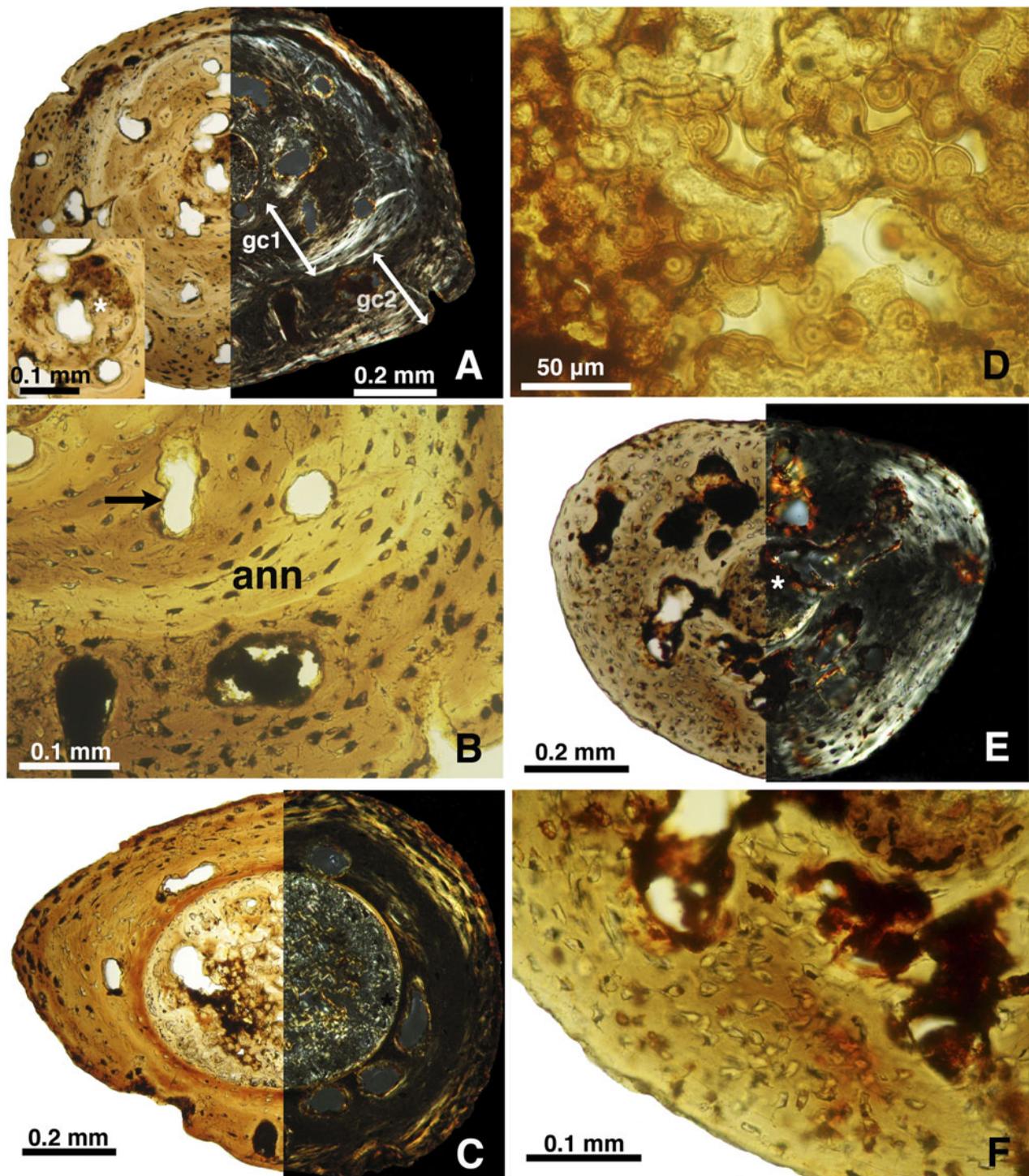


Figure 2. (Colour online) Bone histology of specimens I and II (*Marmorerpeton*) and of 'salamander A' (III). (A) General view of the section from specimen I (mid-diaphyseal region), with detail of the calcified cartilage matrix occupying the centre of the section (asterisk). Left half: ordinary transmitted light; right half: polarised transmitted light. The bone is completely amedullar and its periosteal cortex is stratified into two growth cycles (gc 1 and gc 2). (B) Periosteal cortex in the section from specimen I. The arrow points to a resorption bay showing Howship's lacunae. Cell morphology in the annulus (ann.) is more slender than in the neighbouring zones. (C) General view of the section from specimen II (metaphyseal level). The medullary area, entirely occupied by calcified cartilage, is much broader than in section I, and the periosteal cortex displays only one growth cycle. (D) *Globuli ossei* in the calcified cartilage in section II. (E) General view of section III (mid-diaphyseal level). Same symbol as for Figure 2(A). (F) Detail of the periosteal cortex showing the morphology of cell lacunae in specimen III (compare with Figure 2(B)).

Table 1. Basic histomorphometric parameters of the three humeral sections.

	Sec. Area (mm ²)	Mean Diam. (mm)	Glob. Comp. (%)	R. Ar. Calc. Cart. (%)
Section I	0.755	1.04	93.03	3.7
Section II	0.636	0.93	95.06	30.8
Section III	0.446	0.74	81.30	5.3

Notes: Sec. Area: total sectional area; Mean Diam.: mean sectional diameter; Glob. Comp. global sectional compactness (i.e. total area of mineralized tissues/total sectional area); R. Ar. Calc. Cart.: relative area of the calcified cartilage (i.e. total area of the medullary cartilage/total sectional area).

calcified cartilage (30.8% of whole sectional area) is much greater in this section, and the periosteal cortex is proportionally thinner (it contributes to 36% of mean sectional diameter). This structure is logically related to the position of this section, i.e. closer to the metaphysis; it thus naturally contains a larger volume of calcified cartilage. (2) The calcified cartilage matrix (Figure 2(D)) is not amorphous; instead, it displays well-characterised *globuli ossei* (Francillon-Vieillot et al. 1990, p. 513). The latter represents chondrocyte lacunae secondarily filled by endosteal bone deposits. (3) Bone cortex in the section from specimen II contains only one growth mark. This could indicate either that humerus II comes from a younger individual than humerus I (this is unlikely because of the relatively small difference in mean diameter between these bones) or that the metaphyseal region, being ontogenetically younger than the middle part of the diaphysis, naturally recorded fewer growth marks. (4) Inner bone resorption in the cortex and in the medullary calcified cartilage was less active in the section from specimen II, as evidenced by the lower number of resorption bays (11 vs. 23). As a consequence, the global compactness (cortex + medulla) of the humerus in this section is slightly more elevated than in the section of specimen I (95% vs. 93%; Table 1).

The bone structure displayed by the section from specimen III (salamander A; Figure 2(E)) is roughly similar to that observed on specimen I (Figure 2(A),(B)). In both cases, the humeral shaft is non-tubular and its medullary region is occupied by amorphous calcified cartilage matrix. This tissue represents 5.3% of the total area in this section. As in the two previous sections, several (12) broad erosion bays colonise the cortex and the medullary region of the bone, and are responsible for the lower global compactness of this section (81.3%). The cortex of the humeral shaft is completely avascular at the level of this section, and it displays no peripheral notching that could result from the incorporation of periosteal blood vessels. Two main differences distinguish specimen III ('salamander A') from specimen I (*Marmorerpeton*). First, bone cortex in specimen III displays only one zone and a much broader annulus composed of

birefringent parallel-fibred tissue (Figure 2(E)). Second, osteocyte lacunae in the annulus of specimen III are roughly spindle-like and lack canaliculi (Figure 2(F)), but they are much less slender (more globular) than in the annuli of specimen I.

4. Discussion

The presence of extensive amounts of unresorbed calcified cartilage in our three humeri is a prominent skeletal peculiarity of *Marmorerpeton* and of 'salamander A', an undetermined Jurassic urodele (but possibly a karaurid). In the long bones of tetrapods, the persistence of calcified cartilage at a distance from the epiphyses in relatively late ontogenetic stages reveals a delay or inhibition of endochondral osteogenesis (de Ricqlès 1964, 1975). This trait is viewed as typical evidence of neoteny involving the skeleton, the so-called 'skeletal neoteny' (de Ricqlès 1975). In lissamphibians, skeletal neoteny is most often accompanied with a variable set of other neotenic traits, such as permanent aquatic habits, persistence of gills or gill slits in adults (de Ricqlès 1975).

Given that our three humeri are from juveniles or subadults, it is important to assess whether the persistence of calcified cartilages in their bone diaphyses reflects a real delay or inhibition in endochondral ossification, or is merely consistent with the developmental stage that they had reached by the time they died. Inferences of this nature are best based on comparative data. In this case, we use the most detailed description of skeletal maturation in any urodele that we know of, that of *Pleurodeles waltl* provided by de Ricqlès (1964, 1965). *Pleurodeles waltl* is an extant urodele displaying an adult size relatively close to that of *Marmorerpeton*. The maximal length of the latter is estimated to 40 cm (Evans et al. 1988), whereas the former reaches 30 cm in total length (3/4 of *Marmorerpeton* size). The general histological structure observed in our three humeri typically occurs in very early larval stages of *Pleurodeles waltl*. Indeed, in this taxon, the presence of large amounts of calcified cartilage in the mid-diaphyseal region of the humerus, and the onset of broad resorption lacunae in the basal cortex and medullary calcified cartilage, occur at development stage 55C, according to Gallien and Durocher's (1957) classification, when the humerus is 5.4 mm in length and 273 µm in diaphyseal diameter (de Ricqlès 1964). Considering the size difference between *Pleurodeles* and *Marmorerpeton*, and assuming that standardised body size is an adequate proxy of developmental stage in urodeles (Laurin and Germain 2011), it can be inferred that a qualitative development stage equivalent to 55C occurred in *Marmorerpeton* humeri that had a diameter of some 364 µm (273 × 4/3), a dimension far below the actual diameters of our three specimens. Therefore, the latter can

be considered as maintaining early larval features in relatively late juveniles or in subadult individuals. This condition presumably reflects skeletal neoteny in our *Marmorerpeton* and ‘salamander A’ specimens. This conclusion confirms Evans et al.’s (1988) interpretation of the neotenic significance of various cranial peculiarities of *Marmorerpeton*.

As for other neotenic features, skeletal neoteny can be variably characterised, and the delay in endochondral ossification can be unevenly expressed among taxa (de Ricqlès 1975). In our specimens, there is no trace of endochondral ossification, i.e. the process resulting in the substitution of endosteal bone tissue from pre-existing calcified cartilage. This suggests that the general neotenic process that occurred in these taxa was pronounced. It is, of course, impossible to know from our data how complete this neoteny was, and whether it involved other morphological characters than those mentioned by Evans et al. (1988), such as the numerous pits on the surfaces of vertebral centra, probably reflecting dense vascularisation, the vomerine tooth row parallel to the marginal dentition and the weak pedicel, and possibly, the absence of a crista dorsalis on the humerus for insertion of the muscle latissimus dorsi. In particular, we cannot assess whether external gills were retained, as in several species of *Ambystoma*, or whether the gills were lost, as in cryptobranchids (Duellman and Trueb 1986, p. 191), or even whether gill slits were retained (see review in Wiens et al. 2005). In extant urodele taxa, the extent of delay in somatic development related to neoteny is suspected to be tissue or organ-specific (Wakahara 1996). Neoteny in karaurids, the oldest known heterochrony documented so far in lissamphibians, to our knowledge, probably evolved independently from that of more recent, crown-urodeles. The fact that karaurid taxa (including the two sampled here) are neotenic and aquatic does not necessarily imply that this is primitive for Caudata because the fossil record is known to be strongly biased towards aquatic taxa (Shipman 1981). The individuals of *Marmorerpeton* sampled here, as well as ‘salamander A’, were probably aquatic because a great compactness (absence of a free medullary cavity) and the presence of multiple resorption cavities in long bones is a characteristic of aquatic lissamphibians (Laurin et al. 2004, 2009, 2011; Canoville and Laurin 2009). Unfortunately, we could not apply the inference models that we proposed earlier (e.g. Canoville and Laurin 2009) to assess the habitat of our specimens because these models were based on adult individuals, and the sections described above appear to be from individuals that were still in an active phase of growth. They possibly had lifestyles similar to those of extant neotenic urodeles, such as cryptobranchids, sirenids, proteids or *Amphiuma*. In any case, aquatic habits of *Marmorerpeton* and ‘salamander A’ are also suggested by faunal association:

all abundant taxa in Kirtlington, such as crocodylians, turtles, choristoderans and amiids (Evans and Milner 1991), are aquatic.

Bone histology suggests that the growth of *Marmorerpeton* and of ‘salamander A’ was fairly slow, in absolute terms, and that cyclic decreases in the growth rate occurred as is the characteristic of lissamphibians in general (Castanet 2002). However, in the two taxa studied here, the cyclic fluctuation of growth was relatively mild, as compared to numerous extant caudates (e.g. Miaud and Guillaume 2005), and resulted only in annuli (that reflect slow growth), not in ‘lines of arrested growth’ (complete growth stop). This suggests that the seasons were not strongly marked, which is consistent with the associated faunal and the paleoenvironment that has been inferred for Kirtlington, namely a near-shore lagoon (Evans and Waldman 1996).

Despite the similarities between *Marmorerpeton* and ‘salamander A’ that appear to reflect similar life-history attributes, the histological differences between sections of specimens I and II (*Marmorerpeton*) on the one hand and the section of specimen III (‘salamander A’) on the other hand give additional support for the taxonomic distinctness of ‘salamander A’. However, this histological study is not the proper place to name this taxon.

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