Salamander-like development in a seymouriamorph revealed by palaeohistology

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The amniotes generally lay eggs on land and are thereby differentiated from lissamphibians (salamanders, frogs and caecilians) by their developmental pattern. Although a number of 330–300-Myr old fossils are regarded as early tetrapods placed close to amniotes on the basis of anatomical data, we still do not know whether their developmental pattern was more similar to those of lissamphibians or amniotes. Here we report palaeohistological and skeletochronological evidence supporting a salamander-like development in the seymouriamorph *Discosauriscus*. Its long-bone growth pattern, slow diaphyseal growth rate and delayed sexual maturity (at more than 10 years old) are more comparable with growth features of extant salamanders rather than extant amniotes, even though they are mostly hypothesized to be phylogenetically closer to living amniotes than salamanders.

Keywords: Amniota; salamanders; *Discosauriscus*; growth evolution; skeletochronology

1. INTRODUCTION

The appearance of amniotes is a major evolutionary event in vertebrate history (Ahlberg & Milner 1994) allowing tetrapods to become largely independent of external water for reproduction and development (Sumida & Martin 1997). Knowledge of the ontogeny allowing tetrapods to become largely independent of water can illuminate evolutionary problems, such as those that surround the origin of amniotes.

2. MATERIAL AND METHODS

In order to understand the evolution of strategies in Palaeozoic tetrapods, this study focus on the seymouriamorph *Discosauriscus australis* (Makowsky 1876), generally more closely related to extant amniotes than salamanders (e.g. Anderson 2007; Ruta & Coates 2007). Long bones of 19 well-preserved specimens (Klembara 1997; Klembara & Bartik 2000), from the Lower Permian (Czech Republic), are studied (c.f. electronic supplementary material). Developmental stages of this growth series have been determined according to anatomical features, and exemplars from all stages were selected (Klembara 1998). Histological and growth patterns in *Discosauriscus* are compared with that of extant morphotypes of the same size and general development stage among amniotes and urodeles. Frogs and caecilians are not considered in this comparison owing to their distinct morphology and locomotion, which could bias the measurements of bone growth.

3. LIMB-BONE HISTOLOGY OF *DISCOSAURISCUS*

This is the first skeletochronological study of a large fossil growth series of such an ancient early tetrapod. The mid-shaft bone tissue organization of *Discosauriscus* is very similar to that of extant tetrapods of small body size: saurians (e.g. *Lacerta viridis*, Castanet 1985) and urodeles (e.g. *Euproctus asper*, Montori 1990; *Desmognathus monticola*, Castanet et al. 1996). Primary bone tissue is mostly sub-lamelar (even if locally lamellar or parallel fibred; figure 1a), indicating a relatively slow, well-organized bone deposition (de Ricqles et al. 1991). Numerous radial Sharpey's fibres (S.f.), showing muscle attachments, are revealed under polarized light (PL) in many long-bone diaphyses (figure 1b). At the periphery of the medullary cavity, bone remodelling (erosion/reconstruction process) leaves a distinct endosteal margin visible under PL (figure 1a). This endosteal tissue, made of parallel-fibred bone, shows that remodelling, mainly linked to morphogenesis and mechanical constraints (Francillon-Vieillot et al. 1990), had already occurred in late larval specimens. The periosteal cortex is split into numerous growth layers bordered by lines of arrested growth (LAGs; figure 1). The K-index of the cortical thinness (Currey & Alexander 1985) indicates that bone compacta is thinner in the humeri, femora and radii (0.33<K<0.73) than in the ulnae, fibulae and tibiae (0.24<K<0.55). Bone trabeculae (b.t.) are visible in the marrow cavity of the femora and humeri. A sparse vascularization, composed of primary radial and longitudinal vascular canals in young specimens, intensifies in the femur, humerus and tibia towards a mainly radial arrangement among the largest specimens (figure 1d).

The bone cortex (b.c.) in older specimens is relatively compact (up to 93%) and characteristic of mostly terrestrial extant tetrapods (Germain & Laurin 2005). The long-bone epiphyseal organization of *Discosauriscus* resembles that of urodeles (e.g. *Pleurodeles walii*, de Ricqles 1965; figure 1g), but the numerous trabeculae made of parallel-fibred bone and the absence of calcified cartilage in juvenile individuals suggest a relatively faster endochondral ossification and epiphyseal growth. As in living salamanders, the epiphyses of *Discosauriscus* were probably covered by...
a cartilaginous structure that extended to the metaphysis (figure 1g). Discosauriscus’ epiphyses have no secondary centre of ossification, which so far has only been observed in amniotes (figure 1h).

4. BONE GROWTH OF THE SEYMOURIAMORPH DISCOSAURISCUS AND EVOLUTIONARY IMPLICATIONS

Long-bone diaphyses of Discosauriscus show a simple classic LAG pattern as in extant tetrapods (Castanet et al. 1993). LAGs, separating two tissue types of differential bone densities, punctuate quiescent osteogeneses followed by sudden resumptions, expressed in living poikilotherms during annual aestivations or hibernations (Castanet et al. 1993). The LAGs of similar structure in Discosauriscus thus imply a probable annual periodicity. The skeletochronological analysis indicates that Discosauriscus, which lived under a tropical climate (Ziegler 1990), shows a seasonal annual life cycle similar to that of extant tropical Caudata (Castanet et al. 2003). The youngest sampled specimen was at least 4 years old when it died (four LAGs; figure 1a), whereas the oldest one was at least 10 years old (10 LAGs; figure 1d). A decrease in the growth-mark width towards the cortical periphery is obvious in the oldest specimens (figure 1d), suggesting that the diaphyseal growth strongly and definitively slows in thickness from the seventh or eighth year on. In extant tetrapods, such a transition in LAG spacing is linked with the acquisition of sexual maturity (Castanet et al. 2003). By comparison, it can be assumed that sexual maturity was reached late in development and probably not before 9 or 10 years old, allowing extended larval (until 6 years old) and juvenile stages (figure 2a). Palaeohistology therefore elaborates upon anatomical data (Klembara 1997) by showing that specimens up to 10 years old were subadults. Owing to their longevity, at least twice the

Figure 1. Diaphyseal and epiphyseal bone histology of D. austriacus. (a) Radius, (under polarized light, PL) of the youngest larval specimen (RCZ6) showing four LAGs underlined by lamellar bone (in white) and spaced out by sub-lamellar bone. (b) and (c) Tibia and fibula, respectively (under natural light, NL), of the late larval specimen KO88 showing eight LAGs in both cortices and radial Sharpey’s fibres (S.F.). (d) Tibia (NL) of the oldest subadult SNM Z 15568 showing 10 LAGs and a radial vascularization. (e) Distal femoral end (NL) of the metamorphic specimen (K0224) showing bone trabeculae (b.t., built by endochondral ossification) and the metaphyseal bone cortex (b.c.). (f) Detail of the trabeculae (PL) made of parallel-fibred bone (in white). (g) Comparative stained distal humeral end (NL) of a metamorphosed urodele, Pleurodeles waltl, showing a cartilaginous zone of growth (c.z.g.) and a cartilaginous top (c.). (h) Comparative stained distal humeral end (NL) of a juvenile amniote, Anolis sp., showing a secondary centre of ossification (s.c.o.). Scale bars: (sections in (a–d)), 0.1 mm; (long bones in (a–d)), 0.5 cm.
The age of acquisition of sexual maturity in extant urodeles (e.g. *E. asper*; Castanet et al. 2003), it can be reasonably estimated that the overall longevity of *Discosauriscus* may also be at least twice the immature period, i.e. at least 20 years. Finally, the life-history traits revealed by palaeohistology support the idea that young individuals lived for a long time until they reached the subadult stage (figure 2a) and then left the lacustrine environment for a more terrestrial life, as already suggested by anatomical data (Klembara et al. 2001). These observations confirm previous assertions concerning changes in environmental habitat co-occurring with metamorphosis (e.g. Schoch 2002). *Discosauriscus* probably became adult during its terrestrial style of life.

The similarities in bone growth pattern between this seymouriamorph and urodeles are also confirmed by a similar average diaphyseal growth rate (26–82 μm yr⁻¹) on average in juvenile femora of *Discosauriscus*; 48–97 μm yr⁻¹ in lissamphibians of the same size; de Ricqles et al. (1991); and 73–120 μm yr⁻¹ in saurians of the same size; Castanet 1985; figure 2a).

In conclusion, this new palaeohistological analysis clearly shows that seymouriamorphs retained a salamander-like developmental pattern, although their limbs were certainly already largely adapted to terrestrial locomotion. Although the phylogeny of early tetrapods is still debated (e.g. Vallin & Laurin 2004; Anderson 2007; Ruta & Coates 2007), this study concludes that the salamander-like ossification (epiphysial structure, figure 2b–d; diaphyseal deposition) is a shared primitive trait through the evolution of tetrapods until closer to the amniote crown. Next it will be interesting to complete these results by applying skeletochronology on a growth series of lepospondyls (remaining up to now impossible given the scarcity of material) to further test the different phylogenetic hypotheses.
All animals were anaesthetized and killed by deep freezing before dissection and did not belong to the CITES list of protected animals. All procedures were carried out under the ethics guidelines of France.

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