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 of early tetrapods has hitherto been based exclusively on the continuous evolution of anatomical features during the ossification, thereby not allowing one to mark precisely the timing of transition from one ontogenetical stage to another (e.g. Steyer 2000; Schoch 2001). Skeletochronology, inferred from hard-tissue analyses, provides detailed information about growth, somatic age and sexual maturity in extant as well as extinct vertebrates (Castanet *et al.* 1993), especially in dinosaurs (e.g. Padian *et al.* 2001). However, there are still no such analyses for Palaeozoic vertebrates, even though the bone histology of several Palaeozoic tetrapods is partly known (e.g. de Ricqlès 1981). Here we demonstrate how fossil bone histology can illuminate evolutionary problems, such as those that surround the origin of amniotes.

2. Material and methods

In order to understand the evolution of growth strategies in Palaeozoic tetrapods, this study focus on the seymouriamorph *Discosauriscus austriacus* (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 & Bartík 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 1995). Histological and growth patterns in *Discosauriscus* are compared with that of extant morphotypes of the same size and general developmental 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-lamellar (even if locally lamellar or parallel fibred; figure 1a), indicating a relatively slow, well-organized bone deposition (de Ricqlès *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).

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The long-bone epiphyseal organization of *Discosauriscus* resembles that of urodeles (e.g. *Pleurodeles waltl*; de Ricqlès 1965; figure 1*e–g*), 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 1*g*). *Discosauriscus'* epiphyses have no secondary centre of ossification, which so far has only been observed in amniotes (figure 1*h*).

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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 1*a*), whereas the oldest one was at least 10 years old (10 LAGs; figure 1*d*). A decrease in the growth-mark width towards the cortical periphery is obvious in the oldest specimens (figure 1*d*), 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 2-[-anchor not formatted-]-*a*). 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 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 2*a*) and then left the lacustrine environment for a more terrestrial life, as already suggested by anatomical data (Klembara *et al*. 2001). These obser-vations 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 Ricqlès *et al*. (1991); and 73–120 μm yr^{-1} in saurians of the same size; Castanet 1985; figure 2*a*).

In conclusion, this new palaeohistological analysis clearly shows that seymouriamorphs retained a sala-mander-like developmental pattern, although their limbs were certainly already largely adapted to terres-trial 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 (epiphyseal structure, figure 2*b*–*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.

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