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Histological bone structure of Lower Cretaceous dinosaurs from southwest Istria (Croatia)

***Z. Kozarić, †M. Šparica & ‡Z. Bajraktarević**

**Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 41 000 Zagreb, Croatia*
† Institute of Geology, 41 000 Zagreb, Sachsova 2, Croatia
‡ Department of Geology and Palaeontology, Faculty of Science, University of Zagreb, 41 000 Zagreb, Zvonimirova 8, Croatia

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Recently discovered dinosaur bones in submerged near-shore deposits of southwest Istria include one vertebra, one femur, and two rib fragments. They all show well-developed vascularization. The large amounts of primary bone tissue in the vertebral and femoral compacta as well as in one rib fragment suggest that they belong to sub-adult animals which were growing rapidly. The structure of a second rib fragment differs in that it consists of secondary bone. The absence of lines of arrested growth is evident in all investigated bones. This suggests a sustained manner of bone deposition.

KEY WORDS: Upper Hauterivian; ?Lower Barremian; dinosaur bones; palaeofauna; palaeohistology; Croatia.

1. Introduction

Dinosaur footprints on the Istrian peninsula and the associated islands of Brijuni and Fenoliga occur in deposits whose ages range from Barremian to Cenomanian, and very likely also to the Senonian (Figure 1, localities 1-4, 6). The data on these occurrences can be found in Bachofen-Echt (1925), Polăk (1965), Gogala (1975), Gogala & Pavlovec (1978), Tišljar *et al.* (1983), Velić & Tišljar (1987), Leghissa & Leonardi (1990), Dalla Vecchia *et al.* (1993), and Dalla Vecchia (1994). The footprints on the islet of Fenoliga have been considered as early Turonian by Polăk (1965), but Gušić & Jelaska (1990, pp. 69 and 147) convincingly argued for a late Cenomanian age.

Recently, after almost 70 years, dinosaur bones have been found underwater in the near-shore zone of the southwest Istrian coast, possibly in deposits of early Barremian age (Figure 1, locality 5; Boscarolli *et al.*, 1993; Tunis *et al.*, 1994; Kozarić *et al.*, 1994), and in the intertidal zone on the shore (Jurkovšek *et al.*, 1994).

Numerous finds, spanning a rather large stratigraphic range, indicate favourable palaeoecological conditions for terrestrial reptiles (dinosaurs) in this part of the Adriatic carbonate platform. The results of sedimentological and biostratigraphic investigations make possible precise reconstruction of the palaeoenvironmental and palaeozoogeographic circumstances of the region during the Cretaceous.

Changing sea levels reflecting global geodynamic events, caused shallowing and short-lived emersions during the Barremian, Aptian and Albian. Several times, parts of the carbonate platform (Istria) became terrestrial areas of low relief with environments suitable for dinosaurs. The longest emersion phase

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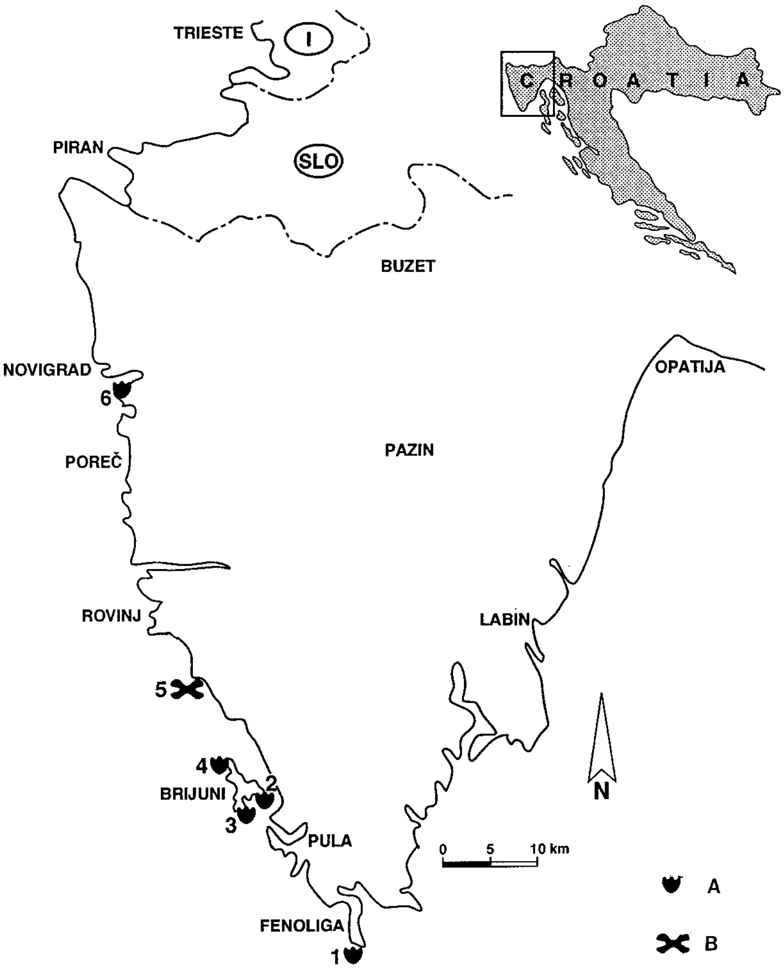

Figure 1. Map showing the location of dinosaur footprints and bones in Istria (Croatia). A = dinosaur footprints; B = dinosaur bones. SLO = Slovenia; I = Italy.

occurred in western Istria. It lasted 10–15 million years and led to largest recorded stratigraphic hiatus, with Upper Albian deposits overlying those of mid Barremian age (Velić *et al.*, 1989).

In this paper we present the first detailed histological analysis of different parts of dinosaur bones from the shallow submerged strata of southwest Istria. Based on the results obtained, we give an interpretation of relative age of the material and number of individuals involved.

2. Materials and methods

The material comprises four specimens of dinosaur bones. These were collected from the sea-bottom, where they spent a long period of time after they were eroded off from the bone-bearing rock (Figure 1, locality 5). They occur in oncolithic rudstones that alternate with laminated algal limestones. These are overlain by a calcareous breccia with a carbonate–siliciclastic and bauxitic matrix. The deposits are lacustrine, and mark an erosional disconformity between the

Upper Hauterivian and ?Lower Barremian (Tunis *et al.*, 1994). According to both the anatomical analysis published earlier (Boscarolli *et al.*, 1993), and our own observations, the investigated bone specimens consist of one vertebra, one femur, and two rib fragments. Recently, Dalla Vecchia (1995) reported the possibility that these bones belong to sauropod, plant-eating, quadrupedal dinosaurs of different sizes.

The bones are now in the collection of Insitute of Geology, Zagreb (Nos IG-1, 2, 3 and 4), and the thin sections (IG-1/BS-1, IG-2/BS-2, IG-3/BS-3 and IG-4/BS-4) are in the collection of the first author. Histological analysis was performed by means of thin sections about 100 μm in thickness, which were made from the cortex of the corpus vertebrae, the compacta of the femur diaphysis, and whole cross-sections of the ribs. The sections were studied and photographed using a Leitz Orthoplan microscope in normal transmitted light and with crossed nicols.

3. Histological description and discussion

According to all observable anatomical characteristics, the vertebra belongs to the caudal part of the vertebral column. The length of the centrum vertebrae is 11.5 cm, the diameter is 8.8 × 9.4 cm, and the height of the vertebra is 15.5 cm (Figure 2). Microscopically, the structure of the superficial part of the bone is not fully preserved; in its deeper part, however, a well pronounced system of vascular canals surrounded by bone tissue can be observed. The superficial part of the bone, in places where it is preserved, has a homogeneous, slightly vascularized appearance, but with numerous, irregularly arranged, large, oval, osteocyte lacunae. In deeper parts of the vertebral compact bone, a well developed network of vascular canals occurs. These are of varying diameter, and surrounded by

Figure 2. Caudal vertebrae of dinosaur IG-1, lateral view.

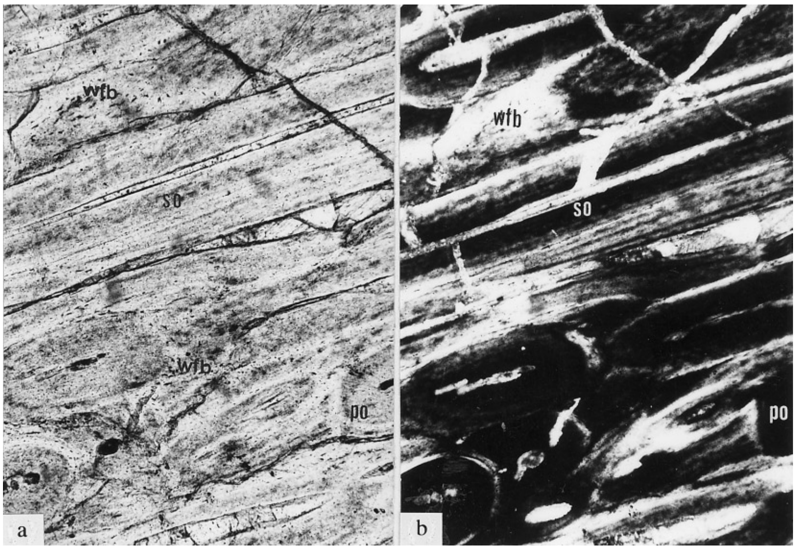

Figure 3a, b. Compact vertebral bone of dinosaur IG-1/BS-1, oblique section. Ordinary light (3a), and cross-polarized light (3b). Primary osteons (po) have a central canal surrounded by lamellar bone tissue with lacunae in regular arrangement. A moderate amount of woven-fibred bone tissue (wfb) surrounds the primary and secondary osteons (so). Magnification ×100.

bone mass with poorly developed lamellar structure and a parallel arrangement of flat osteocyte lacunae, which is characteristic of primary osteons (Enlow, 1963). Secondary osteons are also visible (Figure 3a,b).

In cross-polarized light, a different refraction of light in the bone tissue of the primary and secondary osteons and surrounding woven bone tissue is clearly observable (Figure 3b). Currey (1962) described a similar appearance of bone tissue in prosauropod dinosaurs. Between the primary and secondary osteons there is bone tissue with large, oval osteocyte lacunae which, according to Enlow (1969), is characteristic of woven-fibred bone tissue (Figure 4).

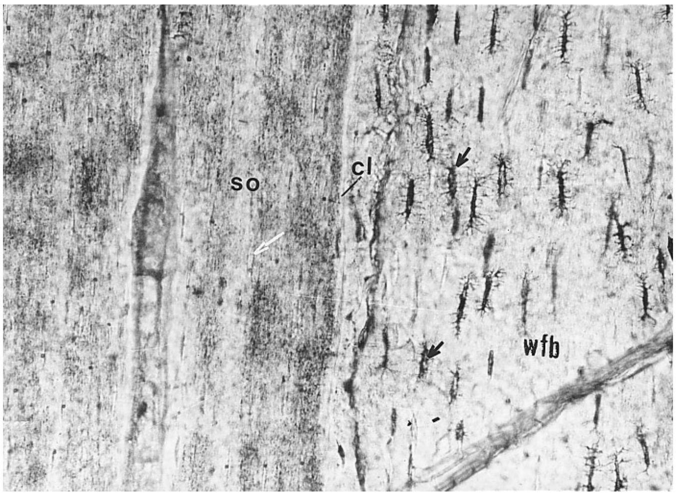

Figure 4. Compact vertebral bone of dinosaur IG-1/BS-1 in ordinary light. Secondary osteons (so) in longitudinal section have very flattened lacunae (white arrow), but in woven-fibred bone tissue (wfb) they are globular and exhibit numerous canaliculi (black arrow). Magnification ×250.

Figure 5. Femur of dinosaur IG-2; epiphysis and part of diaphysis.

The femur consists of the proximal quarter of the bone 37.5 cm long, with a well developed epiphysis and diaphysis 22 cm long (Figure 5). The periosteal part of the diaphysis is not preserved, so that the subperiosteal part of the bone, composed of a well-developed two-dimensional network of vascular canals, occurs on the surface. The canals are arranged in rows parallel with the surface of the diaphysis, some of them being surrounded by woven-fibred bone tissue. In cross-polarized light, this tissue shows a slight birefringence. Such an appearance of the bone compacta was termed fibro-lamellar bone tissue by Ricqlès (1975). This type of tissue is characteristic of some parts of bone compacta in Artiodactyls (Enlow & Brown, 1956, 1957), and some Ornithischian and Saurischian dinosaurs (Currey, 1960; Ricqles, 1983, 1994). According to Enlow (1966), Ricqlès (1983), Reid (1984) and Buffrenil & Mazin (1992), the fibro-lamellar bony tissue is indicative of a fast rate of bone deposition. Chinsamy (1990) found cortical stratification in a *Syntarsus rhodesiensis* femur, but we did not encounter any stratification. Highly vascular, fibro-lamellar bone and primary osteons were found in the region of rapid growth of bone. It is evident that the area occupied by 'blood vessels' correlates with stage of ontogeny. The number of blood vessels in compact bone was higher in juvenile individuals than in adults (Chinsamy, 1993). Recently, Chinsamy (1995) found fibro-lamellar bone without lines of arrested growth in *Dryosaurus lettowvorbecki* bone tissue, which implies that bone was deposited in a rapid and sustained manner.

In deeper parts of the diaphysis compacta, larger amounts of primary osteons occur, whereas the appearance of resorptive cavities represents the onset of Haversian reconstruction and the formation of the secondary osteons (Figure 6).

Histologically, the general characteristic of the investigated vertebral and

Figure 6. Cross-section of dinosaur femur IG-2/BS-2 at diaphysis level. Superficial region (SR) and deeper region (DR) of compact bone in ordinary light. Primary vascular canals (vc) are surrounded by woven-fibred bone tissue (wfb). Magnification ×100.

femoral bone tissue is the presence of a large amount of primary osteonal tissue. The occurrence of fibro-lamellar bone tissue suggests a rapid growth rate which is characteristic of juvenile animals (Reid, 1984; Ricqlès, 1984). The absence of any pause in the deposition of bone tissue suggests a rapid rate of deposition in sustained manner, as noted for *Dryosaurus lettowvorbecki.*

The two rib fragments are 3 cm and 6 cm long respectively, and differ significantly from each other, which means that they belong to different ribs, or different individuals, or different parts of the rib/ribs of an individual (Figure 7). Rib A is circular in cross section; rib B has a more elongated, elliptical shape. In A, the subperiosteal part is built of several longitudinally oriented vascular canals of approximately the same diameter. These are surrounded by concentrically arranged bone tissue with osteocyte lacunae which are embedded in a woven-fibred bone tissue. In deeper parts of the rib, the diameter of the vascular canals increases. Around the canals, layers of bone tissue with concentrically arranged

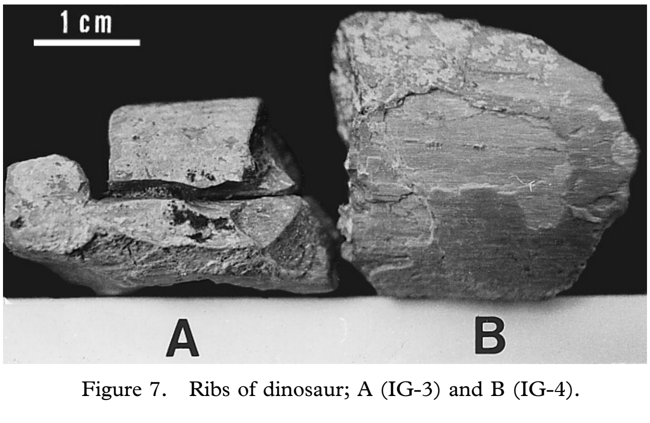

Figure 7. Ribs of dinosaur; A (IG-3) and B (IG-4).

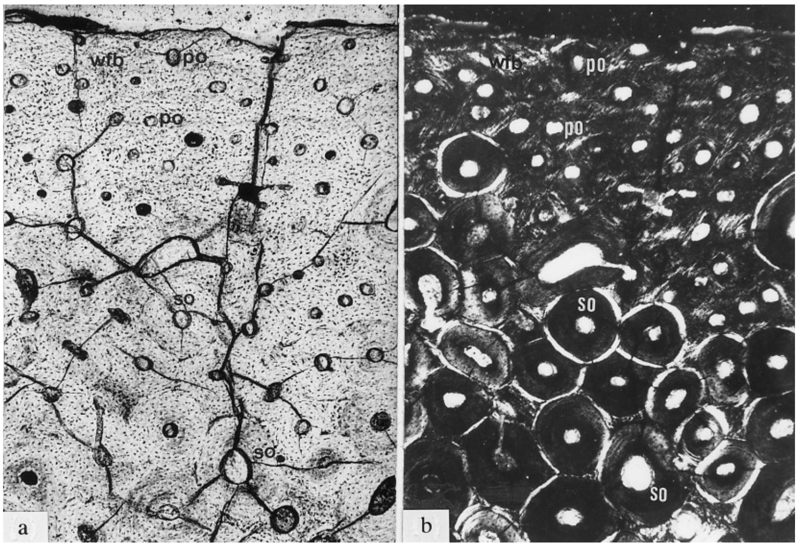

Figure 8a, b. Cross-section of dinosaur rib A (IG-3/BS-3) in ordinary (a) and cross-polarized (b) light. Primary osteons (po) in the superficial region of the rib are surrounded by woven-fibred bone tissue (wfb). The deeper region of rib is made up of numerous secondary osteons (so) oriented longitudinally. Magnification ×100.

osteocyte lacunae appear; these represent the beginning of the formation of secondary, Haversian osteons (Figure 8a). In cross-polarized light, the peripheral woven-fibred bone tissue is slightly birefringent, in contrast to larger, more deeply situated osteons in which the birefringence of both peripheral and central parts of the osteons is clearly pronounced. The amount of woven-fibred bone tissue around the primary osteons is small and the tissue is slightly birefringent in cross-polarized light (Figure 8b).

Rib B shows a similar structure. The superficial part is well vascularized by longitudinally situated vascular canals of approximately constant diameter. In the superficial part, a few primary osteons appear between numerous secondary osteons. In deeper parts of the rib, the number of secondary osteons increases, and these are surrounded by interstitial bone tissue (Figure 9a,b).

The common characteristic of both rib samples is that they lack the central spongy (cancellous) part of the bone. This has been described also in recent crocodiles and in fossil remains of Thecodontia, Saurischia and Ornithischia (Enlow, 1969; Ricqlès, 1976). Reid (1984) described the presence of primary and secondary osteons in *Tyrannosaurus* ribs.

In both of the ribs investigated here growth rings or lines of arrested growth are absent. Rib A is composed of a large amount of primary osteonal tissue. This kind of bone tissue is characteristic of juvenile individuals which are growing rapidly (Ricqlès, 1983; Reid, 1984). In rib B, the dominant structures are secondary osteons which are surrounded by interstitial bone tissue.

4. Conclusions

The vertebral and femoral compact bone studied is built of structurally similar, primary osteonal tissue. The presence of fibro-lamellar bone suggests that in these bones the osteonal tissue was being deposited rapidly. It is impossible to

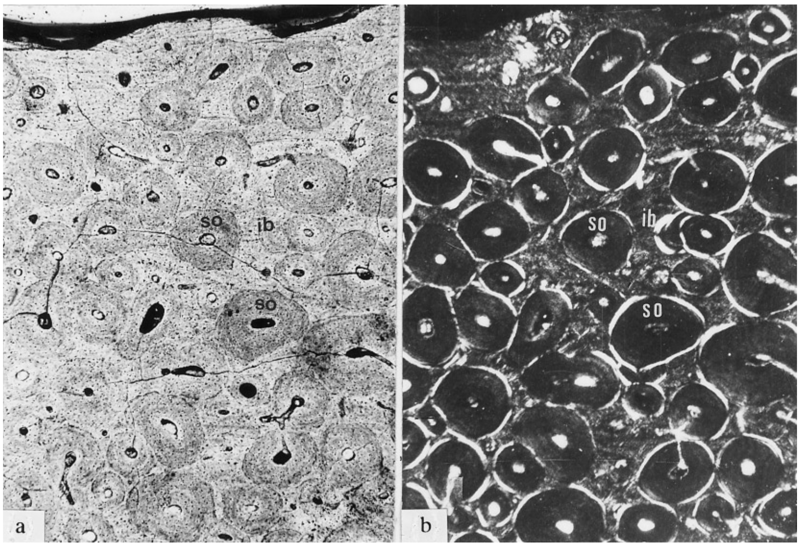

Figure 9a, b. Cross-section of dinosaur rib B (IG-4/BS-4) in ordinary (a), and cross-polarized (9) light. Secondary osteons (so) are abundant and surrounded by interstitial bone tissue (ib). Magnification ×100.

say whether these bones belong to the same animal. The histological structure of the rib fragments is so different that that they might well not be from the same animal. Rib A is built of a primary osteonal bone with a lot of primary osteons and woven bone tissue. In rib B, the primary osteonal bone is replaced by secondary bone (Haversian reconstruction). The general characteristic of all investigated bones is absence of lines of arrested growth, which suggests bone deposition in sustained manner.

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