

Palaeobiological Implications of the Bone Histology of *Pterodaustro guinazui*

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ABSTRACT

This study provides a comprehensive investigation of the bone microstructure of multiple bones of the Early Cretaceous filter-feeder, *Pterodaustro guinazui*, from the Largacito Formation of Central Argentina. We provide information regarding the bone histology of multiple elements from single skeletons, as well as a variety of bones from different individuals. In addition, we analysed changes in bone microstructure through ontogeny in growth series of several long bones of the taxon. Our investigation of skeletal and ontogenetic variation in *Pterodaustro* gives insights into the developmental growth dynamics of this unusual ctenochasmatid pterodactyloid from early ontogeny through to adulthood and also provides information pertaining to histological variability within and between bones of individuals. This study also documents the presence of what appears to be medullary bone tissue within the medullary cavity of a large femur of *Pterodaustro*. This suggests that, like birds, reproductively active female pterosaurs may have deposited a special bone tissue (medullary bone) to cope with the demand of calcium during eggshelling. Our study supports the hypothesis that small Jurassic pterodactyloids took several years to reach adult body size. More specifically, we provide data that suggests that *Pterodaustro* attained sexual maturity at about 2 years of age, and continued to grow for a further 3–4 years doubling in size before attaining skeletal maturity. *Anat Rec*, 292:1462–1477, 2009. © 2009 Wiley-Liss, Inc.

Key words: pterosaur; bone histology; bone microstructure; *Pterodaustro* growth

INTRODUCTION

New pterosaur discoveries and rigorous phylogenetic assessments are refining our understanding of pterosaur evolutionary history and palaeoecology (e.g., Hone and Benton, 2007; Witton and Naish, 2008). However, many questions still remain regarding various aspects of pterosaur biology. Several aspects of the life history of extinct vertebrates are deducible from an analysis of the microstructure of fossilized bone (e.g., Chinsamy-Turan, 2005). For example, histological studies have provided pertinent information regarding the growth dynamics of nonavian dinosaurs (e.g., Erickson, 2005; Chinsamy-Turan, 2005), Mesozoic birds (e.g., Chinsamy et al., 1994; Chinsamy and Elzanowski, 2001), and Mesozoic mammals (Chinsamy and Hurum, 2006).

Grant sponsor: National Research Foundation; Grant number: GUN 441908; Grant sponsor: Universidad Nacional de San Luis; Grant number: N8 340103; Grant sponsor: (SA) Ciencia y Técnica, Universidad Nacional de San Luis; Grant number: Proyect N8 340103; Grant sponsors: National Geographic, Fundacion Antorchas, Consejo Nacional de Investigaciones Científicas y Técnicas.

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Received 9 June 2009; Accepted 9 June 2009

DOI 10.1002/ar.20990

Published online in Wiley InterScience (www.interscience.wiley.com).



Fig. 1. Partial skeleton of *Pterodaustro* showing the thin, upwardly curved lower jaw and the delicate teeth therein.

Histological sections of pterosaur bones have been studied since about the mid-nineteenth century (Bowerbank, 1848; Quekett, 1849; Steel, 2003), and a host of subsequent early studies (e.g., Seitz, 1907; Gross, 1934; Enlow and Brown, 1957; Bramwell, 1972) provided brief accounts of pterosaur osteohistology. Subsequent novel utilization of pterosaur bone microstructure has been in the identification of a new pterosaur taxon (Padian et al., 1995), and in the identification of the pteroid as a true bone (Unwin et al., 1996). The first study that used bone microstructure to determine aspects of ontogenetic growth was conducted on remains of *Pteranodon* (Bennett, 1993). More recently Ricqlés et al. (2000) and Padian et al. (2004) published extensive surveys of pterosaur bone microstructure. Although limited by the availability of different ontogenetic stages within single taxa, these studies provide important insights into the relationship between bone microstructure and development. These researchers deduced that Late Cretaceous pterodactyloids with wingspans of 3–11 m, such as *Montanazhdarcho*, *Pteranodon*, *Quetzalcoatlus*, and related forms, show “typical dinosaurian and even typical bird-like bone growth” (Padian et al., 2004). They further postulated that some smaller basal Jurassic and Triassic

pterosaurs (with wingspans up to about 1.5 m), including *Rhamphorhynchus*, appear to have growth patterns more like that of small birds (Padian et al., 2004). Another independent study that used bone microstructure to understand ontogeny and growth is that by Sayão (2003) who examined multiple elements from two pterodactyloid taxa from the Santana Formation (Crato and Romualdo members) of Brazil. Based on the preserved bone histology, she deduced that one individual was a subadult and the other an adult, and she documented variation in the bone histology of the different skeletal elements (Sayão, 2003). Two recent publications on pterosaur osteohistology are an overview of pterosaur bone microstructure (Steel, 2008), and a brief account of *Pterodaustro*'s bone histology and developmental patterns (Chinsamy et al., 2008).

The study reported on here differs from previous studies by other researchers in that it represents a comprehensive assessment of the bone microstructure of a single pterosaur species. The study is multifaceted in that it assesses the histological variation within and between individuals of the same species, and also documents histological changes associated with growth in various long bones, as well as life-style adaptations evident in the microscopic structure of the bones of *Pterodaustro*.

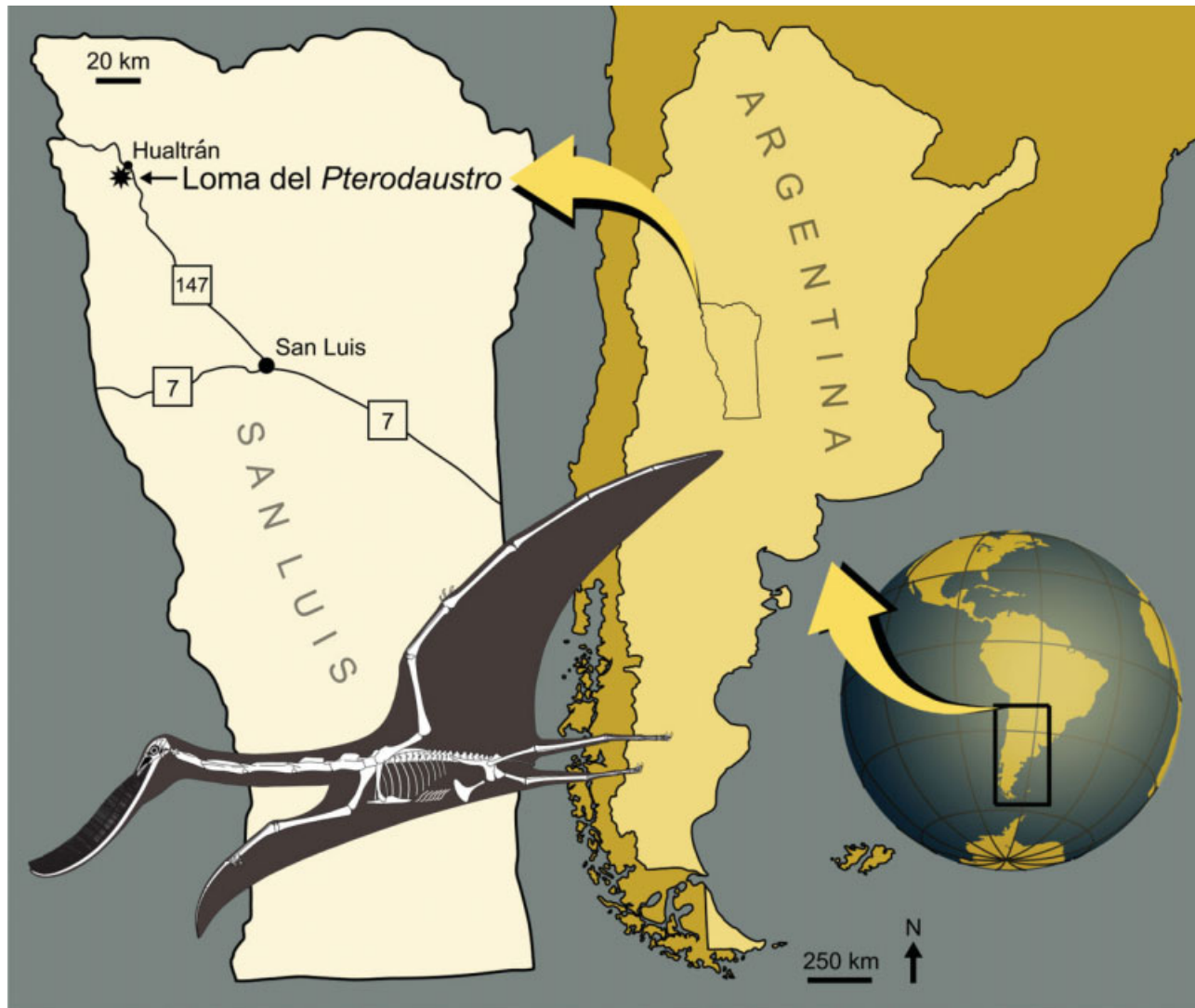


Fig. 2. Map showing the location of the *Pterodaustro* locality in the Lower Cretaceous, Lagarcito Formation of Central Argentina (Sierra de Las Quijadas, San Luis Province).

Pterodaustro is an unusual, medium-sized, filter-feeding ctenochasmatid pterodactyloid (Fig. 1). It has a long, upwardly curved lower jaw with hundreds of delicate teeth, measuring less than a millimeter in transverse dimensions and about 40 mm in length (Chiappe and Chinsamy, 1996; Chiappe et al., 2000). These slender, filament-like teeth would have been efficient for filter-feeding since they would have been capable of large deflections without causing damaging stresses and strains on the dentine and enamel (Currey, 1999). The teeth in the upper jaws were more rounded and conical in shape, and were probably used for crushing filtered material trapped by the specialized dentition of the lower jaws (Chiappe and Chinsamy, 1996).

Pterodaustro guinazui was recovered from Lower Cretaceous (Albian) laminated shales of the Lagarcito Formation (at the Loma del *Pterodaustro* locality, San Luis Province) of Central Argentina (Chiappe et al., 1998; Fig. 2). Analysis of the palaeoenvironment suggests that the deposits formed in an extensive, shallow perennial lake,

and that the nearest marine shoreline was several hundred kilometers away. Since the 1970s, hundreds of specimens—most represented by differently-sized isolated bones—have been recovered from the Lagarcito Formation, making *Pterodaustro* one of the best represented pterosaurs. Multiple individuals are known for several pterosaur taxa, but *Pterodaustro* is unique in that it is represented by a wide range of different-sized individuals – embryonic, hatchling, subadult and adult sizes— with wing spans ranging from 0.3 to 2.5 m (Codorniu and Chiappe, 2004). The broad spectrum of sizes of individuals suggests that *Pterodaustro* had similar growth patterns to that of small Jurassic pterosaurs, such as *Pterodactylus* and *Rhamphorhynchus* (Bennett, 1995), and differed from that of larger pterodactyloids such as *Pteranodon* and *Nyctosaurus*, where adults and immature individuals have fairly similar body sizes (Bennett, 1993, 1996). Although most specimens of *Pterodaustro* are known from isolated bones, the preservation of nearly complete and articulated skeletons corresponding to different stages of

development has permitted the identification of allometric changes in the limb bones as well as in the mid-series cervical vertebrae (Codorniu and Chiappe, 2004). Such studies have made a significant contribution to our understanding of allometric changes in the ontogeny of this ctenochasmatid pterodactyloid, and it is likely that similar allometric changes might have occurred in other pterodactyloid pterosaurs (Codorniu, 2007).

MATERIALS AND METHODS

Since bone microstructure provides information pertaining to various aspects of the biology of extinct animals, our sample was carefully selected to facilitate deductions regarding histological variation of different skeletal elements (both within single skeletons and between different individuals), life style adaptations, and ontogenetic changes during growth.

The remains of *Pterodaustro* are represented by well preserved 3D specimens, as well as by a larger number of crushed (collapsed) bones in finely laminated shale. Since the former are important for morphological analyses, we tended to sample the more crushed material for the destructive process of thin sectioning for histological analyses.

In order to assess variation within single individuals, two partial skeletons of *Pterodaustro* (MMP 1089 and MMP 1207) were extensively sampled (see Table 1), and 15 different skeletal elements were sectioned (including femora, humeri, tibiae, coracoids, vertebrae, ribs, etc.) to assess variation in different skeletal elements (Table 1). To obtain information regarding ontogenetic changes in particular skeletal elements, several different sized long bones (femora, humeri, tibiae, ulnae, and radii) were studied. The relative size of each of the bones was determined relative to the largest specific skeletal element known for *Pterodaustro* (Table 1).

The selected sample of bones was photographed and measured. Prior to removing a sample of bone for histological sectioning, a silicon mold and a cast were made of each of the specimens. Thereafter, using a hand-drill and a fine circular rotating disc, the bones were sampled in appropriate regions (e.g., mid-shaft region for the long bones, since this would be the area least affected by remodeling changes, and also provides a good record of growth through ontogeny or proximal and distal ends of long bones to document histological changes associated with growth in length) (e.g., Chinsamy-Turan, 2005). Once sectioned, the "cut-off" piece was carefully labeled, embedded in resin, and then processed for thin sectioning using the methodology outlined in Chinsamy and Raath (1992) and Chinsamy-Turan (2005). Thin sections were examined using a Nikon petrographic microscope and measurements, and photomicrographs were prepared using a digital Nikon camera and NIS Elements version 2.3.

RESULTS

General Description of the Bone Microstructure of *Pterodaustro*

Generally the bone microstructure was well preserved (Figs. 3–7). Different types of primary and secondary bone tissues were readily identifiable in the compacta of the different bones sectioned. Depending on the particular bones, and location of the section, the compacta con-

TABLE 1.

Skeletal Element	Specimen Number
Humeri	MMP1469 V 89 MMP1207 V 237 MMP1089
Ulnae	MMP1207 V 82 V 231
Radiae	MMP1207 V 16
Pteroid	V 196
Metacarpal IV	MMP1105 V 233 V 1477
Femora	V 56 V 58 V 382
Tibiae	V 161 V 28 V 150
Metatarsal	V 59
Coracoids	V 164 V136
Scapulae	V 193 V136
Cervical vertebrae	MMP1111 V 159 MMP 1089
Rib	V 117 MMP1089
Wing Phalanx 2/3	
Ulna	
Rdius	
Ulna	
Cervical vertebra	
Mandible	
Humerus	
Metatarsal	MMP 1207
Humerus	
Ulna	
Radius	
Wing phalanx 1	
Pteroid	

sisted of a mixture of fibro-lamellar bone, parallel-fibred bone, cancellous bone, compacted coarse cancellous bone, and lamellar and/or lamellated bone tissues. Primary osteons were fairly abundant in the bone walls. Predictably, the bone walls were extremely thin: in the long bones the wall thickness, i.e., from the peripheral edge of the cortical bone to the medullary margin, measured about 1 mm. The thin compact bone walls of

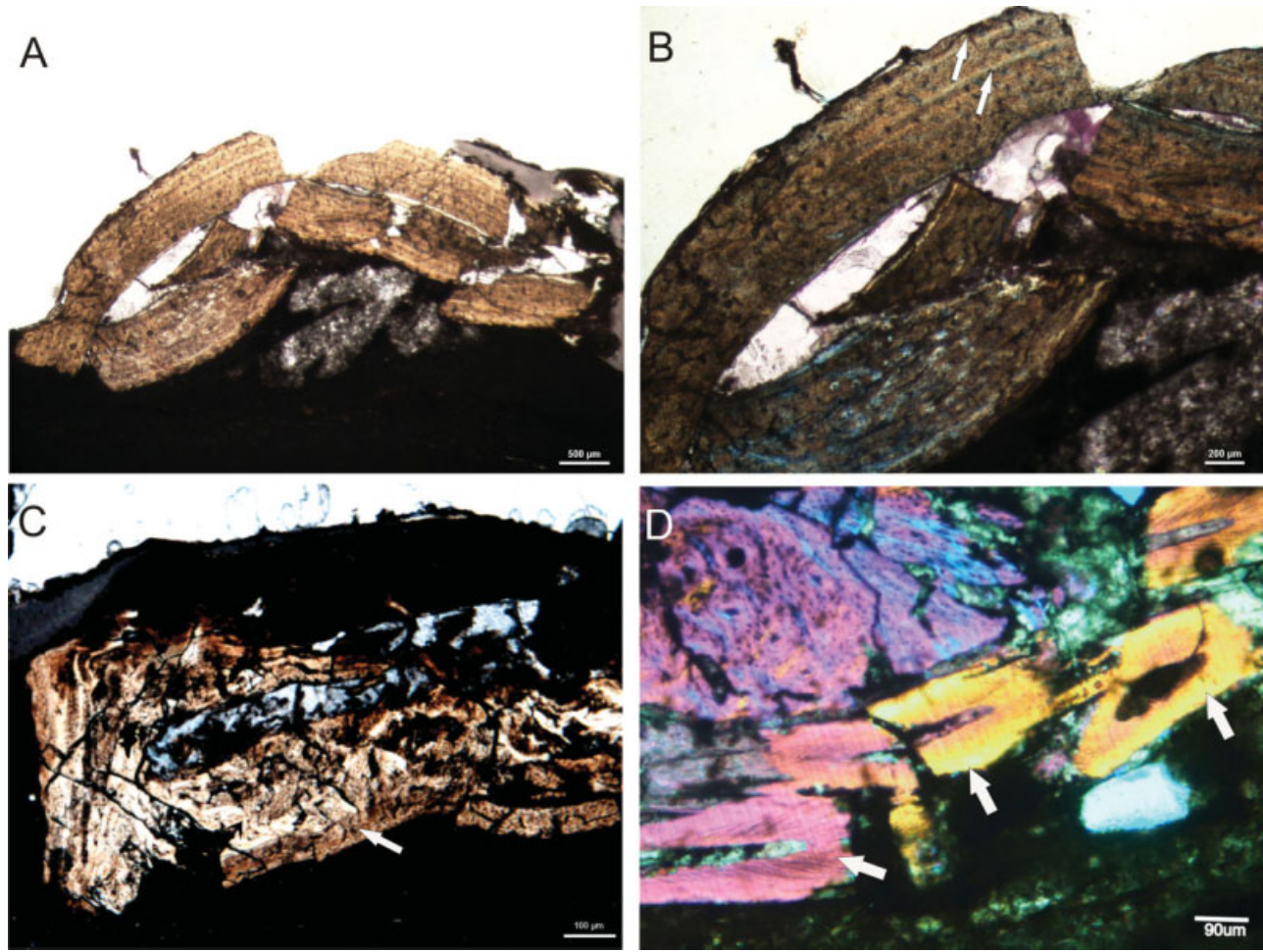


Fig. 3. **A–C:** Humerus V 89, transverse sections. **A:** low magnification showing the general collapsed condition of the bones of *Pterodaustro*. **B:** Higher magnification showing the overall bone histology. **C:** A section through the metaphysis showing a thin rim of periosteal bone (arrow) peripherally followed by a large amount of compacted

coarse cancellous bone. **D:** Humerus, MMP 1089, transverse section, showing the histology of part of the bone wall (upper left of micrograph), and oblique sections through some of the fine filter-feeding teeth (arrows). Dentinal fibres are clearly visible in the teeth.

Pterodaustro's bones were richly supplied with blood vessels, as indicated by the large number of channels in the bone (Starck and Chinsamy, 2002). The orientation of these channels was predominantly longitudinal, with some reticular arrangements. This type of spatial arrangement of bone was found in both wing bones (humeri, radii and ulnae) and leg bones (femora and tibiae). There was a distinct lack of erosion cavities in *Pterodaustro*'s bones and only some isolated occurrences of secondary osteons were noted.

Many of the long bones preserved lines of arrested growth (LAGs) (e.g., Figs. 3B, 4F, 5B, 6A, 7B). Most of the long bones were badly crushed, so the actual size of the medullary cavity was not visible, though it appeared to have been large. In the central regions of the long bones, the shaft consists of a simple cylinder of compact bone and the medullary cavity was free of any cancellous bone tissue (e.g., Figs. 3A,B, 4B,F, 6A). However, towards the proximal and distal ends of elements endochondral bone occurs, and longitudinal sections in this region revealed well preserved calcified cartilage, as well

as a network of bony struts (Fig. 4C,E). A predominance of compacted coarse cancellous bone occurred in these metaphyseal sections (Figs. 3C, 6B). Fortunately, in the sediment underlying a crushed humerus (MMP 1089), thin sections revealed oblique sections of several of the delicate filament-like teeth of the lower jaw (Fig. 3D).

None of the *Pterodaustro* bones sampled showed the "plywood-like" structure that Ricqlès et al. (2000) described as a unique bone tissue among pterosaurs. It seems that this orthogonal "plywood" tissue is not commonly distributed among pterosaurs, and it appears to be restricted to smaller bones of fairly large pterosaurs (Steel, 2008). Recently, Sayão (2003) found this plywood bone tissue in an oblique section of the second phalanx of the fourth digit of a pterodactyloid from the Romualdo Member of the Santana Formation, and Steel (2008) documented this tissue in transverse sections of pteroids of an ornithoherid.

For a more detailed description of the bone microstructure of *Pterodaustro*, we present the results in three categories: (1) ontogenetic changes in bone microstructure; (2)

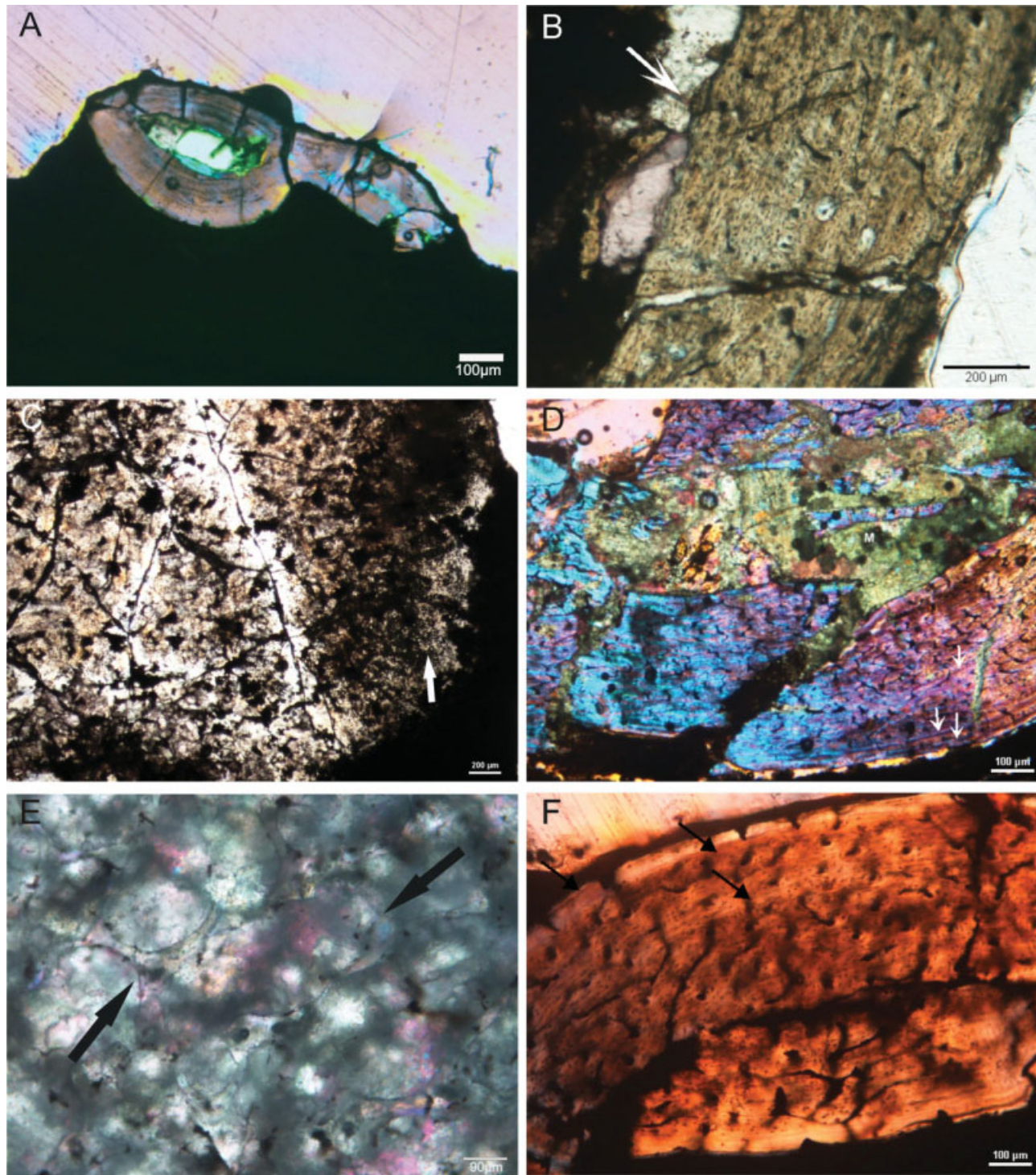


Fig. 4. **A:** Humerus, V 237, transverse section. Note the poorly vascularised compacta. **B:** Humerus MMP 1207, transverse section. The bone wall has a large number of predominantly longitudinally arranged primary osteons. Notice the resorptive surface of the medullary margin of the bone wall (arrow). **C:** Humerus, V 89, Longitudinal section. Arrow points to the large amount of calcified cartilage present in the region of the articular end of the bone. **D:** Humerus, MMP 1469, trans-

verse section showing the general histology of the bone. Arrows indicate three growth rings in the compacta. **E:** Humerus, MMP 1469, longitudinal section. Arrows indicate the web-like nature of the bony struts. **F:** Femur, V 58, transverse section showing primary compact bone tissue with a relatively high density of longitudinal to reticular type of "vascular" channels present. Arrows indicate three growth rings.

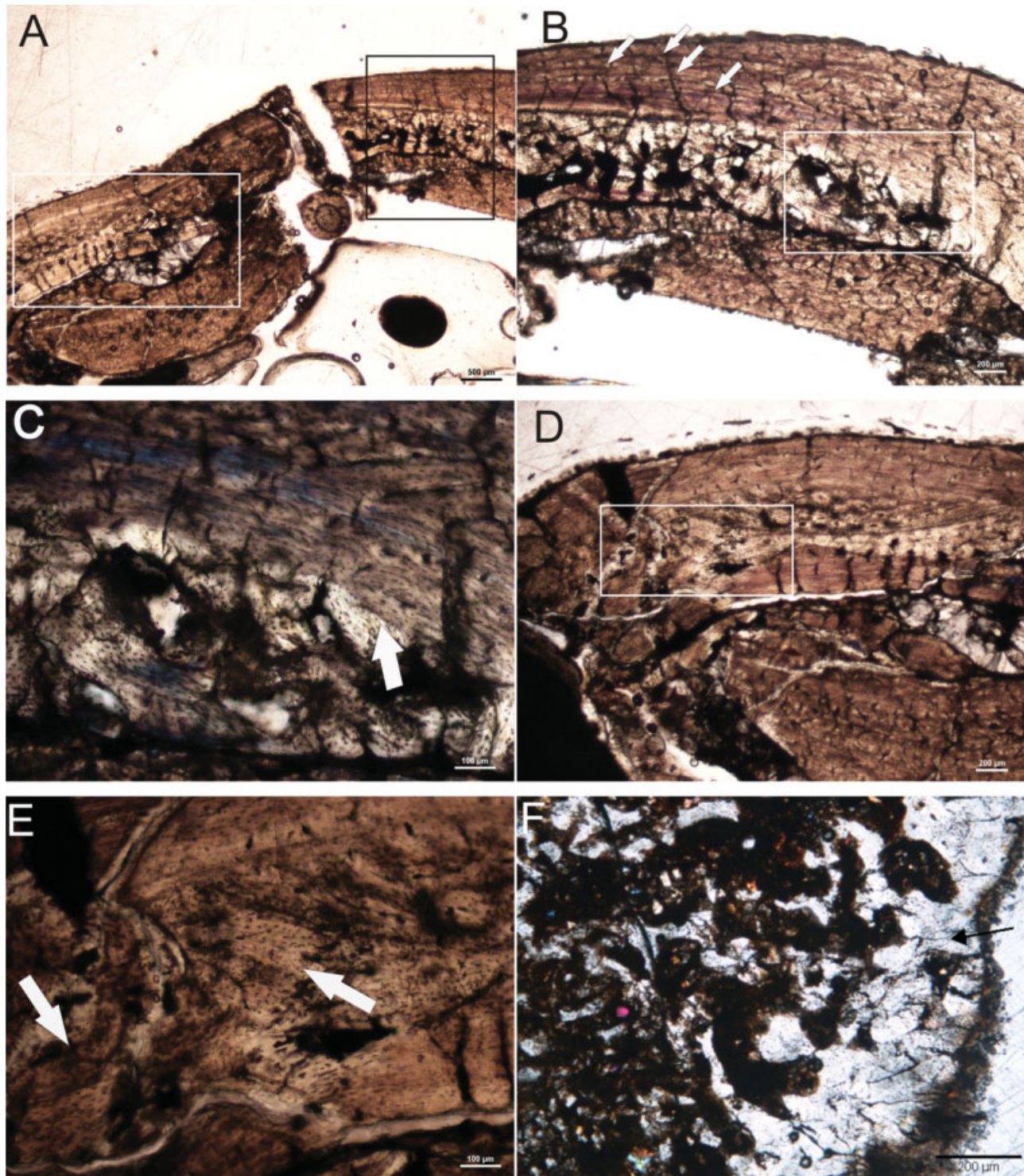


Fig. 5. Femur, V 382, (A–E) transverse sections. A: Low magnification showing an overall view of a section from the midshaft region of the bone. Higher magnification of the black framed area is shown in (B), and that of the white framed area is shown in (D). B: Higher magnification of the frame on the right in (A) shows four growth rings that interrupt bone deposition, and a central region consisting of what is probably medullary bone. C: Framed region in (B), showing a close up of the medullary bone tissue. Arrow indicates the tide line. D: Higher

magnification of the left framed region in (A) showing detail of the bone wall. E: Framed region in (D), showing periosteal bone (top of picture) followed by a region that has a rather unusual compacted bone tissue (arrows), which may be compacted medullary bone from an earlier reproductive cycle. F: Longitudinal section showing a rim of bony tissue (arrow) at the end of the bone indicating that growth in length had ceased at this stage.

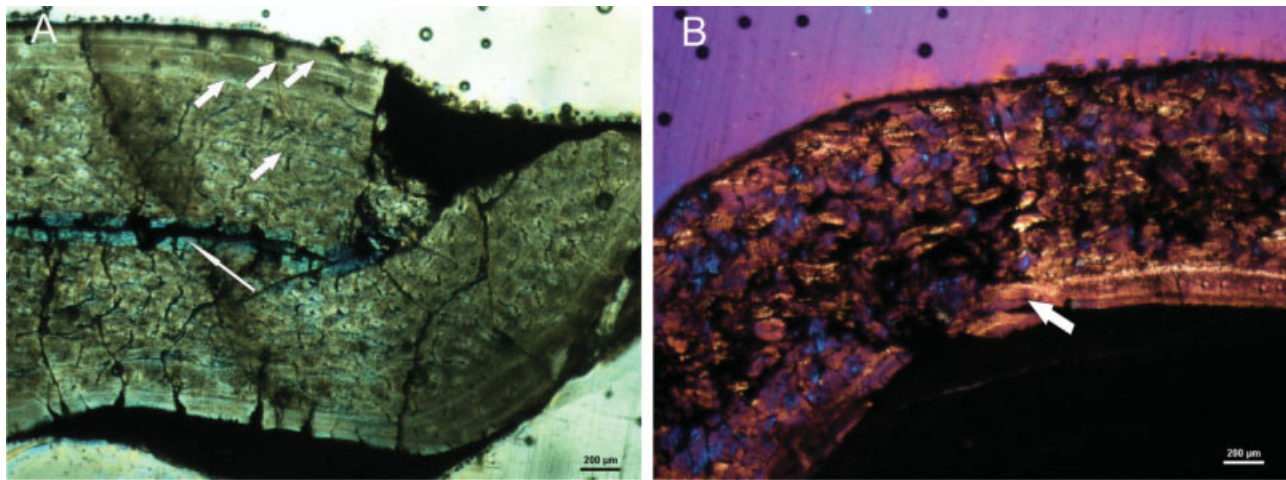


Fig. 6. Tibia, V 28, transverse section. **A**: Midshaft region. Four growth rings (arrows) are clearly visible in the compacta. The thin white arrow points to well developed inner circumferential lamellated bone tissue lining the medullary cavity. **B**: Metaphyseal section showing the predominance of compacted coarse cancellous bone tissue in the bone wall.

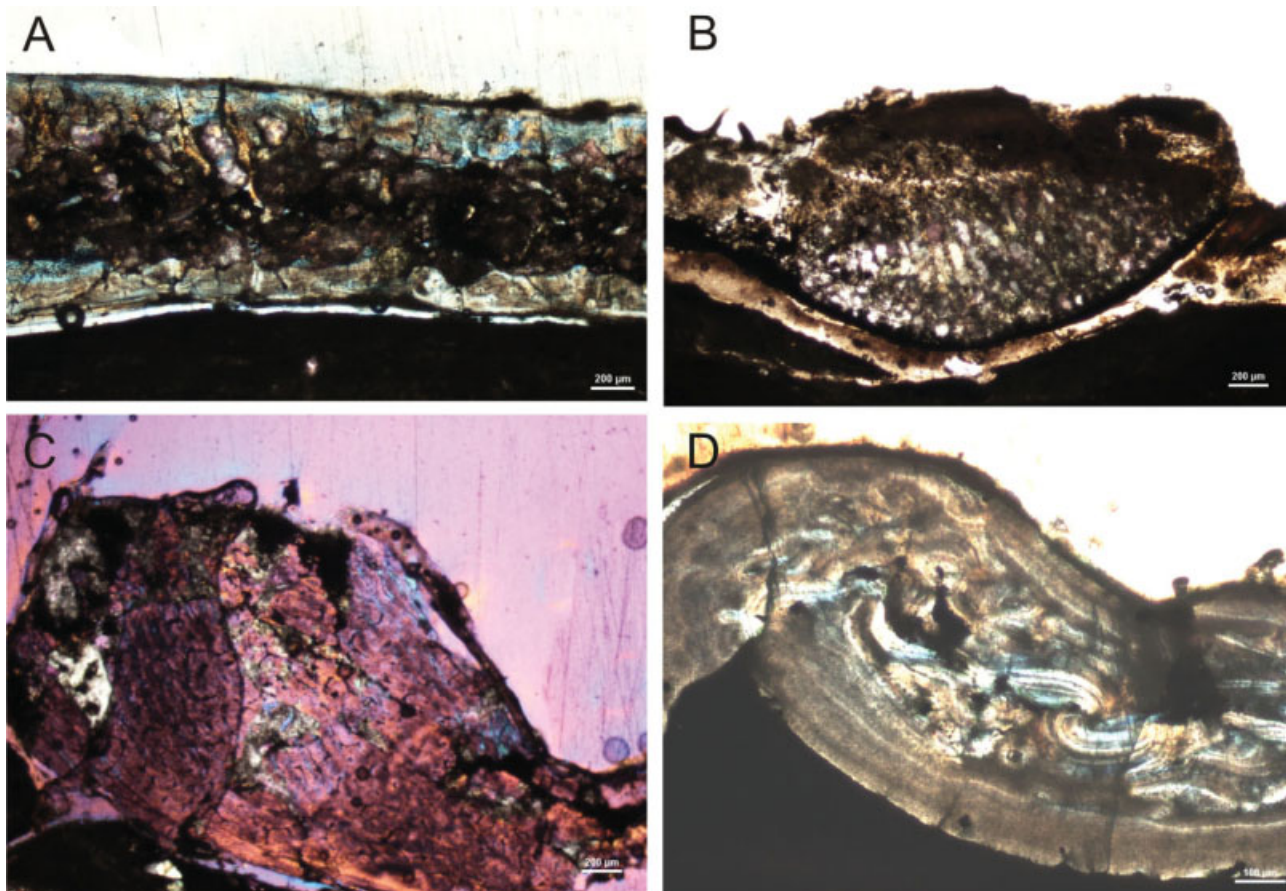


Fig. 7. **A**: Coracoid, V 164, showing a thin rim of periosteal compacted bone, with 1–2 growth lines. This is followed by reconstructed bone tissue (compacted coarse cancellous bone and cancellous bone). **B**: Cervical vertebra, MMP 1089, sectioned across the centrum which shows a thin edge of compact bone enclosing a central region

consisting of a network of cancellous spaces. **C**: Cervical vertebra, sectioned longitudinally in the cranial-caudal direction exhibits well vascularised bone tissue. **D**: Pteroid, V 196. Here, 1–2 growth rings are visible in the outer part of the bone wall. This region is followed by an extensive region of compacted coarse cancellous bone.

TABLE 2.

Specimen	Element length (mm)	% of largest	Number of growth rings					
			Humeri	Femora	Tibiae	Radii	Ulnae	Fibulae
V 237	16.93	13%	X					
MMP1207	59.62	45%	–					
MMP1207	85.58	49%				2	X	
MMP1207	84.93	53%						
V 58	42.85	43%		3				
MMP1089	64.02	49%	X					
V 89	66.92	51%	3					
V 161	106.73	58%			4			
V 231	117.42	67%					2	
V 150	129.52	70%			4			
V 150	–	–						4?
V 56	71.35	71%		4				
V 16	122.28	77%				2		
V 82	136.97	78%					X	
MMP1469	104.39	79%	4					
V 28	168.6	91%			5			
V 382	fragment	100%		5				

–, none observed; X, poor histological preservation; ?, possibly.

histological variation between different skeletal elements; and (3) histological variation within single skeletons.

Ontogenetic changes in bone microstructure.

The analysis of the bone microstructure of the sampled elements provides a direct indication of the type of bone tissues, and hence the relative rate at which bone was deposited during the life history of those particular individuals (Amprino, 1947; Chinsamy-Turan, 2005; Erickson, 2005).

Humerus growth series. This sample comprised five specimens ranging from 16.93 mm in length to 104 mm, which represents a size range from 13% to 79% of the largest humerus known for *Pterodaustro* (Table 1). The bone microstructure of the youngest individual was not very well preserved. However, it appears that a rather unusual bone microstructure is preserved in the youngest individual (V 237). Instead of rapidly formed fibro-lamellar bone tissue, it seems to consist of a band of avascular, possibly parallel-fibred bone tissue with several LAGs (Table 2; Fig. 4A). A humerus 45% the size of the largest known individual clearly showed a fibro-lamellar bone microstructure with a mixture of mainly longitudinal and some reticular “vascular” channels (Fig. 4B). Isolated small secondary osteons were evident in the compacta. Both the perimedullary and peripheral margins of the bone are uneven and have a resorptive appearance. No inner circumferential layer of endosteal bone tissue is evident at this stage of growth (Fig. 4B). A third humerus (MMP 1089) 49% of the size of the largest known humerus was not very well preserved, but clearly showed a well vascularised compacta. Specimen V 89 (51% of the largest humerus) has at least two growth rings visible in the compacta of the bone, and it seems likely that the innermost growth ring had been resorbed (Fig. 3B). The growth rings are visible as distinct layers of lamellar bone (i.e., the annuli) and not as simply lines of arrested growth (LAGs). The osteocytes in this thin layer are clearly flattened and more parallel in their arrangement. At the time of death, it appears that this individual was in a relative fast phase of the

growth cycle. Thus, it seems that there were two episodes of slow rates of bone deposition. Another interesting feature is that in this specimen a narrow layer of inner circumferential lamellae (ICL) is present in the compacta (Fig. 3B). Large amounts of calcified cartilage are evident in the longitudinal sections of the bone (Fig. 4C). Specimen MMP 1469 is 79% the size of the largest humerus known. Cross sections of this specimen reveal a richly vascularised reticular network of vascular channels. Approximately in the middle of the bone wall there appears to be a growth ring, which can be traced in some other parts of the compacta. Near the peripheral margin of the bone wall, two distinct growth lines occur (Fig. 4D). These appear to be LAGs rather than annuli. It is likely that an innermost growth ring has been resorbed. Around the medullary margin a distinct ICL occurs. Longitudinal sections show hardly any amorphous calcified cartilage, and instead bony struts occur right up to the articular end of the bone (Fig. 4E). The bony struts at the ends of the bone tend to form a web-like network (Fig. 4E).

Femur growth series. Three different sized femora were studied. The largest specimen is V 382 and it is considered the largest femur known for the taxon. The two smaller femora, V 58 and V 56 are about 45% and 74%, respectively, of the size of V 382 (Table 1). The bone microstructure of the smallest femur clearly shows three growth rings that interrupt bone deposition (Table 2). The first one appears to be approximately in the middle of the compact bone wall and the other two occur towards the periphery of the compacta and are more closely associated (Fig. 4F). No ICL is evident in the cross section, and the medullary margin appears to be resorptive. Longitudinal sections (LS) of the bone indicate the presence of a large amount of calcified cartilage, but there does not appear to be any definite orientation of the bony struts. The somewhat larger femur, V 56, represents a more distal portion of the femur, and as such the bone growth record is not well documented. A narrow region of periosteal bone occurs in the compacta, and about four rather closely spaced growth rings are evident in the compacta. Because only the distal part of

the bone was available for thin sectioning, the bone wall contains a large amount of compacted coarse cancellous bone. Adjacent to the medullary margin, the channels appear to be predominantly longitudinally oriented. In one part of the section, two growth rings are visible. The medullary margin shows marked evidence of resorption, and it is evident that bone resorption and medullary expansion were still underway in this individual. In the largest of the femora studied, V 382 (Fig. 5), four growth rings are evident in its compacta, and it is likely that an innermost one has been resorbed. Three of these growth rings interrupt the deposition of fibro-lamellar bone tissue, while the fourth seems to mark a change in bone deposition to a more lamellar type (Fig. 5B). The medullary cavity has a large amount of endosteally formed bone (Fig. 5A,B). The presence of this tissue is rather unexpected since the section is taken from the mid-shaft region and is not at all close to the metaphysis (where it is more usual to find bone tissue occupying the medullary cavity to varying degrees). Could this tissue be medullary bone formed during egg-laying? This is an intriguing possibility. In the same section there seems to be a secondarily compacted bone tissue enclosed by lamellar bone (Fig. 5D,E). The longitudinal section of this femur shows a well developed layer of bone at the articular margin of the bone (Fig. 5F).

Tibia growth series. This sample consists of three tibiae: V 161, V 150, and V 28 that are, respectively, 58%, 70%, and 91% the size of the largest known tibia (Table 1, 2). In the smallest of the three tibiae, four to five growth rings are clearly visible in the compacta (Table 2). The bone between consecutive growth rings, the zonal regions, becomes progressively less "vascularised" after subsequent growth rings. The initial zone is wide and there are a large number of channels located here (indicating rich "vascularisation" during life). The bone external to the peripheral growth rings is mainly lamellar and indicates a much slower rate of bone deposition. In this individual an ICL lines the medullary cavity. Specimen V 150 consists of a proximal section of both tibia and fibula. The tibia bone wall consists essentially of compacted coarse cancellous bone with a thin rim of periosteal bone. The fibula consists of periosteal bone tissue and four poorly defined growth rings are evident in the compacta. Longitudinal sections of this tibia show a narrow region of calcified cartilage at the articular end of the bone. In V 28 the bone wall preserves a well-vascularised compacta with at least four growth rings (Fig. 6A). The innermost growth ring is preceded and followed by a richly vascularised fibro-lamellar type of bone suggesting that this is bone formed during early ontogeny, and it is possible that an inner growth ring has been resorbed. The third zone, following the second growth ring, is visibly less well-vascularised (Fig. 6A). This is followed by a third and fourth ring bounding zonal regions that are rather poorly vascularised with a lamellar type of tissue. Longitudinal sections of this element exhibit compact bone tissue that extends right to the end of the bone, without any traces of calcified cartilage. A more proximal section shows a large amount of compacted coarse cancellous bone in the medullary cavity (Fig. 6B).

Radius growth series. The two radii studied, MMP 1207 and V 16, are respectively 53% and 77% of the largest known radius (Tables 1 and 2). Two growth rings

are evident in the compacta of MMP 1207. The first is close to the medullary cavity, while the second is nearer the peripheral edge (Table 2). Specimen V 16 consists of a more distal part of the radius and the compacta is almost entirely composed of compacted coarse cancellous bone. A narrow peripheral region of periosteal bone was also present, and contained two growth rings (Table 2).

Ulna growth series. Three specimens were studied, MMP 1207, V 231, and V 82, that represent 49%, 67%, and 78% respectively of the largest known ulna (Tables 1 and 2). In V 231, a narrow region of periosteal bone tissue and a central region of compacted fine cancellous bone are visible. Toward the medullary region, two growth rings of lamellar tissue are clearly visible (Table 2). The histology of MMP1207 and V 82 was not well preserved.

Histological variation within different skeletal elements. Sections at the proximal and distal ends often reveal calcified bone tissues, though the extent of this varied depending on the "maturity" of the individual (Figs. 4C,E, 5F). In the metaphyseal regions of the shafts of long bones, there is a predominance of compacted coarse cancellous bone tissue and usually only a narrow outermost peripheral band of primary bone (Figs. 3C, 7A,B). In these sections, the medullary cavity often has an extensive development of cancellous bone tissues. Sections taken in the mid-shaft regions of long bones often preserved most of the primary bone, and in many cases provided a good assessment of the appositional growth that occurred throughout the life of the individual (Figs. 3A,B, 4A,B,D,F, 5B, 6A).

Several other bones such as pteroids, vertebrae, metacarpals, coracoids, etc. (Fig. 7) were also studied, but it seems that they do not preserve a good record of growth processes. For example, a pteroid (V 196) consisted of a narrow region of periosteal bone with one, and possibly a second, LAG visible, while the medullary cavity was packed with compacted coarse cancellous bone (Fig. 7D). A cervical, MMP 1089, sectioned longitudinally from the cranial to caudal region along the edge of the vertebra (Fig. 7C) shows a compacted bony tissue, while a section through the centrum shows a central region filled with thin bony cancelli (Fig. 7B). This central region of bony spaces (Fig. 7B) may have been a biomechanical adaptation for lightening the bone, or may have been associated with the development of diverticulae originating from a system of pulmonary air sacs. A coracoid, V164 exhibited a rim of compact bone wall and several bony struts that projected toward the centre of the bone (Fig. 7A).

Histological variation within single skeletons. A partial skeleton, MMP 1207, yielded a humerus, radius, and an ulna that were sectioned for this study. No growth rings were observed in the humerus, yet the radius showed two growth rings (Table 2). Unfortunately, the histology of the ulna was not well preserved. The proximal part of both a tibia and fibula of specimen V 150 were sectioned. The tibia consisted, essentially, of compacted coarse cancellous bone with a thin rim of periosteal bone, while the fibula consisted of periosteal bone tissue containing four poorly defined growth rings (Table 2).

As expected, the tissues observed in the various bones of MMP 1089 differed according to their location in the skeleton. Unfortunately, because of poor preservation, most of these bones were unsuitable for making any deductions regarding bone depositional patterns.

DISCUSSION

General Observations of the Bone Microstructure of *Pterodaustro*

The thin-walled bones of *Pterodaustro* were generally crushed (collapsed), but histological detail was usually discernible. Although the nature of the bone tissue varied according to the “maturity” of the individual, location of the section within the skeleton and skeletal element, generally the walls of adult and juvenile long bones were thin (about 1 mm in thickness). Midshaft sections of long bones generally consisted of a ring of compacted bone, while bony struts tended to be located more proximally and distally along the shafts, with extensive web-like development towards the metaphysis. These features possibly reflect biomechanical adaptations.

The observation that *Pterodaustro* has a predominantly longitudinal arrangement of “vascular” channels is noteworthy. In a recent analysis of macro and microstructural analysis of long bones in birds, de Margerie et al. (2002) found a strong correlation between torsion-resisting features and histologic characteristics (thin bone walls, circular shaft cross sections, oblique collagen fibres and laminar arrangement of tissue). de Margerie et al. (2002) found that the ulna had the highest concentration of circumferentially oriented vascular channels in response to the flight loads exerted on the ulna. However, among the Procellariiformes studied (an albatross and 2 petrels), they found a different arrangement, i.e., low amounts of laminar tissue and their bones were relatively thick walled (as compared to the other flying birds sampled). Since these birds are fairly large (e.g., Albatross has a wing span of about 2.5 m) and soar on stiff wings often in strong winds, their long narrow wings are not well suited for constant flapping flight, and it is quite likely that they have different loading forces on their bones. Interestingly, a Lesser Black-Backed Gull and an unrelated charadriiform seabird with similar flight modes also showed histological similarity to the Procellariiformes studied. Given these findings in modern taxa and *Pterodaustro*'s similarity to them, it is reasonable to assume that the wing bones of *Pterodaustro* did not experience large torsional loads while flying and that perhaps it had a flight mode similar to the Procellariiformes. It seems that the longitudinal organization of the bone tissues in *Pterodaustro* and Procellariiformes may have been an adaptation to longitudinal stresses caused by compression, tension, or bending loads.

In the preserved compacta, the lack of erosion cavities in *Pterodaustro*'s bones and only isolated occurrences of secondary osteons concur with previous reports on pterosaur cortical bone tissues (e.g., Ricqlès et al., 2000; Sayão, 2003; Padian et al., 2004; Steel, 2008). The current study provides evidence that this is the case throughout ontogeny in *Pterodaustro*. In fact, only in large azhdarchids has dense haversian bone been observed, and this was in the region where endosteal struts met the deep cortex (Ricqlès et al., 2000).

One femur (V 382) is interesting because the medullary cavity has a rather unusual type of bone tissue that seems to be endosteally derived. This intriguing tissue and its presence in the medullary cavity, raises the possibility that it might be homologous to avian medullary bone, i.e., a bone tissue that female birds deposit during reproduction which is used as a calcium reservoir during egg shell formation). Interestingly, there is some debate about whether pterosaurs laid soft or hard-shelled eggs (Unwin and Deeming, 2008). Only three pterosaur eggs are known: two eggs are from the Yixian Formation in Liaoning in China (Wang and Zhou, 2004; Ji et al., 2004), while the third is an egg of *Pterodaustro* (Chiappe et al., 2004). Of the two eggs from China, only one preserves microstructural details and is reported to have been a soft-shelled egg because of the lack of evidence of calcite (Ji et al., 2004). However, the eggshell of the *Pterodaustro* egg described by Chiappe et al. (2004) has distinctive calcite crystals therein, and the egg was described as having a hard-shell. In the detailed analysis of the eggshell structure of pterosaurs, Unwin and Deeming (2008) suggest that pterosaurs may have had parchment-shelled eggs similar to those of extant squamates, and that like the latter, they may have exhibited variation in the degree of calcification of the eggshells, which may have been as a consequence of differing nesting or climatic conditions (Unwin and Deeming, 2008). The presence of the calcite in the eggshell of the *Pterodaustro* egg (Chiappe et al., 2004), suggest that there must have been some demand for calcium during eggshelling in *Pterodaustro*. Given that pterosaurs have relatively thin-walled bones, it seems conceivable that they might not have obtained calcium directly from their bones as is the case among crocodiles (Wink and Elsey, 1986; Wink et al., 1987), and that like birds, they may have deposited medullary bone to protect their structural skeleton from bone resorption (Miller and Bowman, 1981). This deduction is also supported by the observation that there are hardly any secondary osteons, or erosion cavities, observed in the bones of pterosaurs (e.g., Ricqlès et al., 2000; Padian et al., 2004; Steel, 2008). It seems reasonable to assume therefore that, like modern birds, *Pterodaustro* may have formed medullary bone, which would have acted as a labile store of calcium during egg-shelling (Simkiss, 1967; Whitehead, 2004). Another feature of this bone is that, in a localized region of the same section (Fig. 5A) an unusual compacted bone tissue appears to be sandwiched by layers of lamellar bone (Fig. 5D,E). It is possible that this tissue represents remains of medullary bone from a previous reproductive cycle that had become compacted and incorporated into the bone wall. These histology features have been observed in modern bird bones (Whitehead, 2004).

Recently, endosteal bone tissues homologous to avian medullary bone have been reported in three nonavian dinosaurs: a femur of *Tyrannosaurus rex* (Schweitzer et al., 2005), a tibia of *Allosaurus* and a femur and tibia of *Tenontosaurus* (Lee and Werning, 2008). If these endosteal tissues in the nonavian dinosaurs and the pterosaur are indeed medullary bone, it would imply that medullary bone was possibly plesiomorphic for Ornithodira. However, an alternative hypothesis has been proposed for these endosteally derived bone tissues in the nonavian dinosaurs (Chinsamy and Turmarkin-Deratzian, 2009). It is equally probable that medullary

bone was independently acquired among birds and pterosaurs as a consequence of the biomechanical constraints that precluded their thin bone walls acting as a calcium reserve for eggshelling.

Growth Dynamics of Pterosaurs

Our understanding of the growth and life history patterns of pterosaurs has been limited by the fact that multiple individuals of pterosaurs representing different ontogenetic aged individuals of the same taxon are relatively rare (e.g., Unwin, 2006). The sample of *Pterodaustro* material analysed in this study is unique since it represents the most complete ontogenetic series known so far for a pterosaur taxon. Previous studies that have tried to determine life history patterns and growth trajectories of pterosaurs include morphological studies by Bennett on *Pteranodon* (1993) and *Rhamphorhynchus* (1995) and histological studies (Bennett, 1993; Sayão, 2003; Padian et al., 2004).

In his assessment of 707 specimens of *Pteranodon*, Bennett (1993) found that only 15% of the sample represented immature individuals and, interestingly, these young individuals were similar in size when compared to the adults. He found that the smallest individual in the sample was only about 70% the size of the largest individual (Bennett, 1993). Bennett reported that body size was unreliable for differentiating between ontogenetic stages and that the extent of fusion evident in the skeleton was a better way of distinguishing between the similar sized subadults and adults. He also found that *Nyctosaurus*, *Ornithocheirus*, and *Anhanguera/Santadactylus* did not show a marked size difference between adults and immature individuals. Considering these findings, Bennett (1993) proposed that juveniles of *Pteranodon*, *Nyctosaurus*, *Ornithocheirus* and *Anhanguera/Santadactylus* grew rapidly to adult size and then practically stopped growing.

Following on from this earlier study, Bennett (1995) undertook a statistical and morphological study of size and morphological variation of the Late Jurassic basal pterosaur *Rhamphorhynchus muensteri* from the Solnhofen Limestone. *Rhamphorhynchus muensteri* is represented by over 100 individuals with wingspans ranging from between 290 and 295 mm to about 1.81 m. Unlike *Pteranodon* and the other relatively smaller pterosaurs studied previously, Bennett (1995) found that specimens could easily be separated into distinct size classes that he interpreted as representing different ontogenetic stages. He found that the skeletons of the smallest individuals lacked fusion and many of the bones were still incompletely ossified, while those of the middle age class showed some fusions and better ossification. Skeletons of the largest age class were well ossified, and showed fusion of the various skeletal elements, such as bones of skull and limb girdles. Interpreting the age classes as annual, he further suggested that *Rhamphorhynchus* grew over a period of 3 years and that young individuals could fly and feed themselves before they reached adult size (Bennett, 1995; Unwin, 2006). In attempting to assess growth dynamics of *Rhamphorhynchus*, Bennett (1995) deduced the modal and average body lengths of *Rhamphorhynchus* year-classes, and compared this to growth rate data for alligators. He found that the growth rates of *Rhamphorhynchus* and the alligators

were comparable, therefore suggesting slower growth rates for *Rhamphorhynchus* relative to that postulated for large pterodactyloids such as *Pteranodon*. Bennett (1995) further suggested that the growth rate data do not provide evidence of high metabolic rates, and suggested the possibility that *Rhamphorhynchus* and other “rhamphorhynchoid” pterosaurs may have been ectothermic, but perhaps with a greater capacity for aerobic metabolism than modern reptiles.

In further attempts to understand the growth and biology of pterosaurs, Ricqlès et al. (2000), and Padian et al. (2004) conducted histological analyses of several pterosaur taxa. Padian et al. (2004) analysed two specimens of *Rhamphorhynchus* representing the first and second age classes proposed by Bennett (1995). On the basis of histological features, Padian et al. (2004) suggested that during early ontogeny this pterosaur had rapid growth rates—indeed, as rapid as juvenile dinosaurs—but that this later declined to much slower rates. Padian et al. (2004) further proposed that Late Cretaceous pterodactyloids with wingspans of 3–11 m such as *Montanazhdarcho*, *Pteranodon*, and *Quetzalcoatlus* had “typical dinosaurian and even typical bird-like bone growth”, whereas some smaller basal pterosaurs of the Jurassic and Triassic (with wingspans of up to 1.5 m) grew more like smaller birds (Padian et al., 2004).

In her analysis of two pterodactyloid taxa from the Santana Formation of Brazil, Sayão (2008) found differing bone tissue patterns that suggested different ontogenetic status for the individuals. She suggested that as juveniles, the pterosaurs experienced rapid bone depositional rates (as reflected by the presence of uninterrupted fibro-lamellar bone) and that as they matured their bone growth was interrupted with the formation of LAGs. A similar deduction was made by Steel (2008) who suggested that pterosaurs had rapid bone depositional rates, but that they occasionally experienced cyclical interruption. However, Steel (2008) did not specify how this changed during ontogeny. In her analysis Steel (2008) mentions that there is a quantitative change in bone “vascularization” in younger and older bone tissues. She also documents the presence of thin avascular lamellae peripherally which mark the attainment of determinate body size (Steel, 2008).

Growth Patterns of *Pterodaustro* as Reconstructed From its Bone Microstructure

That different growth stages are quite easily discernible makes *Pterodaustro* different from *Pteranodon* and *Nyctosaurus*, and more like small Jurassic pterosaurs, e.g., *Pterodactylus* and *Rhamphorhynchus*. The histological characteristics of *Pterodaustro*'s bones provide direct evidence regarding the growth processes that the individuals experienced. Since our sample contained different sized individuals, we were able to deduce growth patterns during ontogeny.

As described earlier, the bone microstructure of the smallest individual (V 237) was rather not very well preserved, and perplexing. Usually, very young individuals show evidence of rapid bone deposition (Chinsamy-Turan, 2005), yet this individual seems to have lamellar bone in the compacta that suggests it was slowly formed. Why should this have been the case? It is possible that this simple ring of uniformly textured bone is

biomechanically stronger (Carter and Spengler, 1978) than other bone tissues and may have been important for an animal that might have been precocial (e.g., Unwin, 2006). It is possible too that perhaps this tissue reflects the type of bone formed before hatching, i.e., embryonic bone. Castanet et al. (1988) have reported a similar type of embryonic bone in *Sphenodon punctatus*, although the embryonic bone of enantiornithines, a basal group of Mesozoic birds, shows a very different kind of bone tissue that is much more “vascularised” and woven-textured (Chinsamy-Turan, 2005). This unusual bone tissue has not been observed in any of the other bones of *Pterodaustro*, but perhaps this is because most of our sample contains individuals that are considerably larger than the young hatchling studied here.

In the next smallest individual (MMP 1207), the occurrence of fibro-lamellar bone tissue suggests that bone deposition (and hence growth) was rapid. Thereafter, the deposition of a thin layer of lamellar bone tissue, the annulus, indicates a comparatively slower rate of bone formation. Steel (2008) also illustrates circumferential lamella interrupting the fibro-lamellar bone deposition in a diaphysis of an ulna of an ornithocheirid. Such alternating fast (zones) and slow growth (annulus) cycles are known to form annually in lepidosaurs (e.g., Castanet et al., 1988), and crocodylians (Peabody, 1961), and are also present and assumed to be annual in avian (Chinsamy et al., 1994) and nonavian dinosaurs (Reid, 1981; Chinsamy, 1990; Erickson et al., 2004; Chinsamy-Turan, 2005; Erickson, 2005; Erickson et al., 2007). Thus, given that such annual growth patterns are present among many diapsids, it is reasonable to assume that they are also annual features in *Pterodaustro*. Two cycles of zones and annuli are observed in V 89, although the second cycle of rapid growth appears to be reduced. With increasing age, bone deposition periodically stopped leading to the formation of LAGs instead of annuli, and the bone deposited in the zones changed to a more parallel-fibred bone tissue with fewer channels for vascularisation, suggesting a slower rate of bone formation. Later in ontogeny the spacing of the LAGs tend to become visibly closer as a result of less bone being appositionally deposited, another indication of slower growth rates. LAGs have been described in several pterosaurs (Ricqlès et al., 2000; Sayão, 2003; Padian et al., 2004; Steel, 2008). For example, in a pterodactyloid metatarsal from the Romualdo Member of the Santana Formation of Brazil about seven to eight LAGs are distinctly evident in the compacta (Steel, 2008, Fig. 5).

Two phases of posthatching growth are observed in *Pterodaustro*:

1. An early phase of relatively rapid growth cycles composed of zonal fibro-lamellar bone with annuli.
2. A later phase of relatively slower growth with cycles of parallel-fibred to lamellar bone tissue interrupted by LAGs.

On the basis of these histological findings, we suggest that *Pterodaustro* hatchlings grew rapidly for about 2 years, until they were about 53% adult size (using the largest sampled individual as a proxy of adult size). Thereafter, the change from fibro-lamellar bone tissue to parallel-fibred bone tissue may be in response to energy

being redirected from growth to reproduction (Chinsamy-Turan, 2005; Erickson, 2005; Erickson et al., 2007). This trend has been observed in a variety of extant lepidosaurs (e.g., Castanet et al., 1988), and in a variety of nonavian dinosaurs (e.g., Sander, 2000; Erickson et al., 2007). Thus, we suggest that the change in growth rate may mark the onset of sexual maturity at about 53% adult size and, thereafter, growth in length and diameter occurred at slower rates for another 3–4 years until larger body sizes were attained (Fig. 8).

By about half adult size, an inner circumferential layer (ICL) of bone in the humerus has developed, which suggests that most of the medullary expansion had occurred already by this stage. Contrary to Steele (2008) in *Pterodaustro* the presence of the ICL is not commensurate with the attainment of adult body size.

Our histological findings thus support the inferences made on the basis of morphology, that Late Jurassic taxa such as *Rhamphorhynchus* and *Pterodactylus* are represented by multiple year classes and took several years to attain adulthood (Bennett, 1996). Thus, our findings are contradict Padian et al.’s (2004) suggestion that small basal pterosaurs of the Jurassic and Triassic (with wingspans up to 1.5 m), including *Rhamphorhynchus*, appear to have growth patterns more like small birds, which achieve adult size within a few weeks or months.

We found that the bone microstructure of *Pterodaustro* differs significantly from modern birds: firstly, unlike modern birds, *Pterodaustro* shows periodic growth and likely took several years to reach skeletal maturity; and secondly, unlike modern birds that attain sexual maturity after reaching full adult size (e.g., Erickson et al., 2007), *Pterodaustro* may have continued growing for several years after attaining sexual maturity. If this is correct, the timing of sexual maturity in *Pterodaustro* is more similar to that of extant squamates and crocodylians (Chabreck and Joanen, 1979; Wilkinson and Rhodes, 1997), nonavian dinosaurs (Erickson et al., 2007), and basal Mesozoic birds (Chinsamy et al., 1994; Chinsamy and Elzanowski, 2001; Chinsamy-Turan, 2005).

Although bone microstructure cannot be directly used to infer metabolic physiology (Chinsamy and Hillenius, 2004), the actual nature and texture of the histological patterns observed in *Pterodaustro* certainly suggest faster bone depositional rates than ectotherms such as crocodylians (Chinsamy-Turan, 2005). However, in having periodic cycles of bone deposition, and in taking several years to reach skeletal maturity, *Pterodaustro*’s growth appears not to have been as fast as modern endotherms.

Longitudinal sections of long bones of small individuals in the region of the epiphysis show large amounts of calcified cartilage. Bony trabeculae also extend into the metaphyseal area, and in many instances islands of calcified cartilage are also present. These characteristics suggest that elongation of the bone occurred rapidly at these early stages of growth. Such epiphyseal features have been observed in several pterosaur taxa, e.g., *Pteranodon* and *Montanazhdarcho* (Ricqlès et al., 2000) and several wing phalanges of a pterodactyloid (Steel, 2008). However, in the largest individuals of *Pterodaustro* (V 28, V 382) the bony struts extend right to the articular end of the bone, implying that diaphyseal elongation had ceased and that maximum skeletal size had been attained.

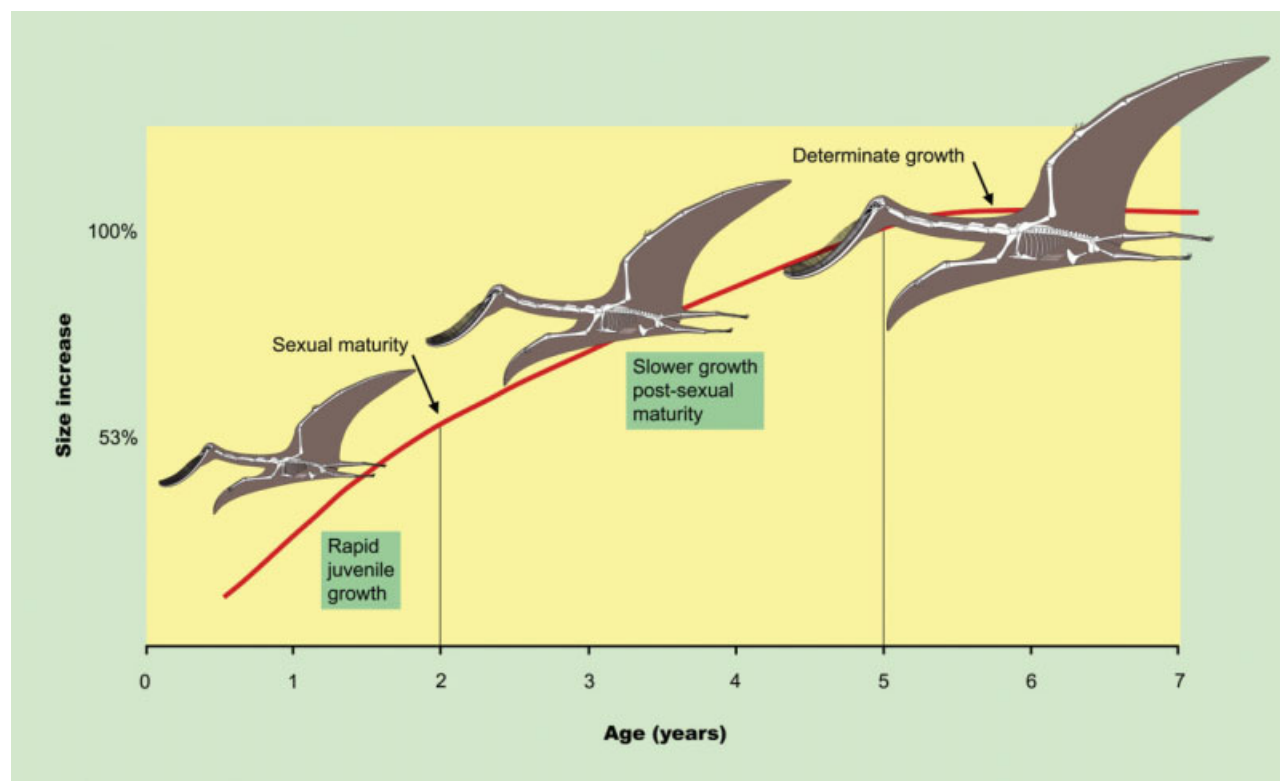


Fig. 8. Schematic growth curve of *Pterodaustro guinazui* as deduced from the analysis of the growth patterns in the bone microstructure of the ontogenetic series of its long bones.

Histological Variation of Bone Tissues Within Single Individuals and Between Different Skeletons

A host of previous studies on a variety of vertebrates have found that the histology of bones in a skeleton varies according to the particular bone and its location in the skeleton (e.g., Enlow, 1969). Indeed, the overall morphology and biomechanical functioning of a particular element within the skeleton also affect the nature and type of bone tissues that are present (e.g., Chinsamy-Turan, 2005).

In the current study, the histological analysis of multiple elements of single individuals showed that certain skeletal elements were better for skeletochronological assessment than others. For example, in MMP 1207 it was clear that the radius rather than the humerus preserved a better histological record of development. Interestingly, when comparing bones from different individuals, we also found that the humeri tended not to be the best skeletal element for skeletochronology. The femur and tibia seemed to be better skeletal elements to use for such analyses. In terms of histological variation between and within different bones we found that mid-shaft sections better preserved the appositional growth record than more proximal or distally located sections, which tended to reflect metaphyseal remodeling and have large amounts of secondary bone, such as compacted coarse cancellous bone. The importance of examining the histology of “articular ends” of the bones was

also apparent in this study since it permitted deductions regarding bone elongation.

In conclusion, this investigation of the bone microstructure of one of the best represented pterosaurs has provided unparalleled information regarding its paleobiology. The possibility of endosteally formed bone tissue in the medullary cavity of the largest femur, suggests that like birds, pterosaurs having thin walled bones may have used medullary bone for calcium required for egg shell formation (instead of mobilizing calcium from their thin walled bones; Simkiss, 1967; Miller and Bowman, 1981; Wilson and Thorp, 1998; Whitehead, 2004). This comprehensive analysis of the bone microstructure of different bones and different sized individuals of the filter-feeder, *Pterodaustro*, provides pertinent information regarding histological variation within a single taxon, and insights into its possible developmental trajectory. These results further suggest that other small to medium sized pterosaurs probably also experienced several cycles of periodic growth before reaching a determinate body size.

ACKNOWLEDGMENTS

The National Geographic Society and the Fundacion Antorchas supported fieldwork and overall research. The National Research Foundation, South Africa, supported the histological analysis. A. Dondas (MMP) is thanked for access to the *Pterodaustro* material under his care.

Stephanie Abramowicz (Dinosaur Institute, Natural History Museum of Los Angeles County) is thanked for having drawn the map and the scaled reconstructions of *Pterodaustro*. Allison Tumarkin-Deratzian is thanked for discussions. Christopher Bennett and David Unwin are acknowledged for reviewing and making constructive comments that have improved this manuscript.

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