

COMPARATIVE POPULATION HISTOVARIABILITY WITHIN THE
ARCHOSAURIA

by

HollyNoelle Woodward Ballard

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Earth Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2012

©COPYRIGHT

by

HollyNoelle Woodward Ballard

2012

All Rights Reserved

APPROVAL

of a dissertation submitted by

HollyNoelle Woodward Ballard

This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency and is ready for submission to The Graduate School.

Dr. John R. Horner

Approved for the Department of Earth Sciences

Dr. David Mogk

Approved for The Graduate School

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

In presenting this dissertation in partial fulfillment of the requirements for a doctoral degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this dissertation is allowable only for scholarly purposes, consistent with “fair use” as prescribed in the U.S. Copyright Law. Requests for extensive copying or reproduction of this dissertation should be referred to ProQuest Information and Learning, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted “the exclusive right to reproduce and distribute my dissertation in and from microform along with the non-exclusive right to reproduce and distribute my abstract in any format in whole or in part.”

HollyNoelle Woodward Ballard

April 2012

ACKNOWLEDGEMENTS

Research funding was provided by the Jurassic Foundation, the Geologic Society of America, the National Science Foundation (proposal number OISE-1015130), and the Australian Academy of Science. Thanks to Dr. Jack Horner and the Museum of the Rockies, Gerry Ohrstrom, and the Department of Cell Biology & Neuroscience for multiple years of graduate student funding. I am grateful for the extensive histology training and assistance provided by Ellen Lamm, and the Museum of the Rockies provided the necessary facilities for thin section analysis and photo imaging. I appreciate the time, patience, and guidance given by my committee members Dr. Jack Horner, Dr. David Varricchio, Dr. James Farlow, and Susan Gibson. Dr. Todd Kaiser, my graduate representative, also set aside his time to attend committee meetings and defenses. My deepest gratitude goes to my advisor, Dr. Jack Horner, for helping me become a better scientist. He taught me how to think ontogenetically, phylogenetically, and stratigraphically, and in my years at MSU I have learned so much from him. My fellow graduate students, past and present, not only provided the much needed social support to see me through to the end, but were invaluable resources for memorable discussions, ideas, and helpful criticisms. Lastly, I acknowledge and thank my family, Ron and Pam Woodward, Tom and Sandy Ballard, and my husband Tom for their love and support throughout this long process. I am grateful that they always believed in me. Lastly, I am especially appreciative of my husband for keeping me on track with my dissertation, and for also insisting I take some time out every once in a while to enjoy life.

TABLE OF CONTENTS

1. INTRODUCTION TO DISSERTATION	1
A Brief History of Paleohistology	1
Significant Paleohistology Contributions Prior to 1980	1
Important Contributions to Dinosaur Skeletochronology	4
Summary of Dissertation	9
Literature Cited	16
2. QUANTIFICATION OF INTRASKELETAL HISTOVARIAILITY IN <i>ALLIGATOR MISSISSIPPIENSIS</i> WITH IMPLICATIONS FOR NON-AVIAN DINOSAUR PALEOHISTOLOGY	20
Contribution of Authors and Co-Authors	20
Manuscript Information Page	21
Abstract	22
Introduction	23
Materials and Methods	26
Ethics Statement	26
Procedure	26
Results	28
Humerus	30
Radius	31
Ulna	32
Femur	33
Tibia	34
Fibula	35
Coracoid	36
Scapula	37
Osteoderms	38
Discussion	38
General Bone Tissue Observations	38
Growth Marks	40
Statistical Analyses and Growth Curves	43
Conclusions	48
Acknowledgements	50
Literature Cited	93
3. OSTEOHISTOLOGICAL EVIDENCE FOR DETERMINATE GROWTH IN THE AMERICAN ALLIGATOR	98
Contribution of Authors and Co-Authors	98

TABLE OF CONTENTS – CONTINUED

Manuscript Information Page	99
Abstract	100
Introduction	101
Materials and Methods	102
Results	103
Discussion	104
Conclusions	106
Acknowledgements	108
Literature Cited	111
 4. GROWTH DYNAMICS OF AUSTRALIA’S POLAR DINOSAURS	115
Contribution of Authors and Co-Authors	115
Manuscript Information Page	116
Abstract	117
Introduction	118
Results	120
Discussion	121
Materials and Methods	124
Ethics Statement	124
Procedure	124
Acknowledgements	126
Literature Cited	131
 5. REANALYSIS OF “ <i>RAPTOREX KRIEGSTEIN</i> ”: A JUVENILE TYRANNOSAURID DINOSAUR FROM MONGOLIA	135
Contribution of Authors and Co-Authors	135
Manuscript Information Page	136
Abstract	137
Introduction	138
Materials and Methods	140
Results and Discussion	141
Stratigraphy	141
Ontogeny	144
Osteohistological Indications of Maturity	146
Histology	148
Taxonomy	152
Conclusions	154
Acknowledgements	155
Literature Cited	158

TABLE OF CONTENTS - CONTINUED

6. A POPULATION HISTOVARIABILITY ANALYSIS OF THE HADROSAURID DINOSAUR <i>MAIASAURA PEEBLESORUM</i>	166
Contribution of Authors and Co-Authors	166
Manuscript Information Page	167
Abstract	168
Introduction	168
Materials and Methods	171
Background	171
Procedure	173
Results	177
Tibia Lengths 33 cm – 40 cm (n = 7)	178
Tibia Lengths 40 cm – 50 cm (n = 22)	179
Tibia Lengths 50 cm – 60 cm (n = 1)	181
Tibia Lengths 60 cm – 70 cm (n = 3)	181
Tibia Lengths 70 cm – 80 cm (n = 3)	183
Tibia Lengths 80 cm – 90 cm (n = 6)	184
Tibia Lengths 90 cm – 100 cm (n = 5)	185
Discussion	186
Ontogenetic Histology Analysis	186
Regressions and Growth Curves	191
Frequency Graph	195
Conclusions	196
Acknowledgements	197
Literature Cited	222
7. CONCLUSIONS	226
Literature Cited	229
REFERENCES CITED	230

LIST OF TABLES

Table	Page
2.1. Annual average skeletal apposition rates for the three alligator individuals included in this study	51
2.2. Data collected for the three alligator individuals included in this study	56
2.3. Results of Student's <i>t</i> -tests performed on perimeter measurements of paired homologous elements.....	57
4.1. Data for Australian polar dinosaur fossils included in this study	127
6.1. Data collected from forty eight histologically sectioned <i>Maiasaura</i> tibiae	199
6.2. Average daily apposition rates for each year of growth, assuming 370 days in the Campanian year	200
6.3. Estimates of <i>Maiasaura</i> femur length, body length, and body mass based on regression formulae and mass equations	201

LIST OF FIGURES

Figure	Page
2.1. A plot comparing bone growth mark and surface perimeters of left and right homologous elements.....	60
2.2. Intraskkeletal annual average cortical thicknesses	61
2.3. Intraskkeletal annual average cortical area	62
2.4. Osteohistology of the mid-diaphyseal humerus of alligator MOR 2007-05RC3	63
2.5. Osteohistology of the mid-diaphyseal humerus of alligator MOR 2007-05RC4	64
2.6. Osteohistology of the mid-diaphyseal humerus of alligator MOR 2007-05RC5	65
2.7. Osteohistology of the mid-diaphyseal radius of alligator MOR 2007-05RC3	66
2.8. Osteohistology of the mid-diaphyseal radius of alligator MOR 2007-05RC4	67
2.9. Osteohistology of the mid-diaphyseal radius of alligator MOR 2007-05RC5	68
2.10. Osteohistology of the mid-diaphyseal ulna of alligator MOR 2007-05RC3	69
2.11. Osteohistology of the mid-diaphyseal ulna of alligator MOR 2007-05RC4	70
2.12. Osteohistology of the mid-diaphyseal ulna of alligator MOR 2007-05RC5	71
2.13. Osteohistology of the mid-diaphyseal femur of alligator MOR 2007-05RC3	72
2.14. Osteohistology of the mid-diaphyseal femur of alligator MOR 2007-05RC4	73

LIST OF FIGURES – CONTINUED

Figure	Page
2.15. Osteohistology of the mid-diaphyseal femur of alligator MOR 2007-05RC5	74
2.16. Osteohistology of the mid-diaphyseal tibia of alligator MOR 2007-05RC3	75
2.17. Osteohistology of the mid-diaphyseal tibia of alligator MOR 2007-05RC4	76
2.18. Osteohistology of the mid-diaphyseal tibia of alligator MOR 2007-05RC5	77
2.19. Osteohistology of the mid-diaphyseal fibula of alligator MOR 2007-05RC3	78
2.20. Osteohistology of the mid-diaphyseal fibula of alligator MOR 2007-05RC4	79
2.21. Osteohistology of the mid-diaphyseal fibula of alligator MOR 2007-05RC5	80
2.22. Osteohistology of the coracoid blade of alligator MOR 2007-05RC3	81
2.23. Osteohistology of the coracoid blade of alligator MOR 2007-05RC4	82
2.24. Osteohistology of the coracoid blade of alligator MOR 2007-05RC5	83
2.25. Osteohistology of the scapula blade of alligator MOR 2007-05RC3	84
2.26. Osteohistology of the scapula blade of alligator MOR 2007-05RC4	85
2.27. Osteohistology of the scapula blade of alligator MOR 2007-05RC5	86

LIST OF FIGURES – CONTINUED

Figure	Page
2.28. Osteohistology of an osteoderm from alligator MOR 2007-05RC3	87
2.29. Osteohistology of an osteoderm from alligator MOR 2007-05RC4	88
2.30. Osteohistology of an osteoderm from alligator MOR 2007-05RC5	89
2.31. Examples of woven tissue and primary osteons within diaphyseal sections of various elements	90
2.32. Third and fourth growth marks in every sampled element of MOR 2007-05RC5.....	91
2.33. Detail of the outer cortex from elements of MOR 2007-05RC4 and MOR 2007-05RC5 showing a fifth growth mark.....	92
3.1. Bone histology of a captive <i>Alligator</i> , a <i>Tyrannosaurus</i> , and a <i>Desmotosuchus</i>	109
3.2. Generalized cladogram of Sauropsida (after Ricqlès et al., 2003) diagramming the presence of an EFS in various groups	110
4.1. Bone microstructure of a mature polar hypsilophodontid	128
4.2. Hypsilophodontid ontogenetic bone microstructure	129
4.3. Theropod osteohistology.....	130
5.1 Comparison of the LH PV18 fish centrum D) to an ellimmichthyiform centrum (A-C) (Horseshoeichthyes) from the Dinosaur Park Formation (Campanian) of Alberta.....	156
5.2. Osteohistological features of LH PV18 and a near adult <i>Tyrannosaurus rex</i> for comparison	157

LIST OF FIGURES – CONTINUED

Figure	Page
6.1. Tibia length plotted against A) diaphyseal perimeter, B) bone wall thickness, and C) transverse section area	203
6.2. Tibia length plotted against A) medullary cavity perimeter and B) medullary cavity area	204
6.3. Ontogenetic growth curves for nine <i>Maiasaura</i> individuals	205
6.4. Transverse sections from the seven <i>Maiasaura</i> tibiae between 33 cm and 40 cm in length.....	205
6.5. Possible nestling bone preserved within tibia lengths between 33 and 40 cm	206
6.6. Reticular to laminar vascular organization observed in two tibiae (A, T-1; B, T-8) between 33 cm and 40 cm in length.....	207
6.7. An unusual banding pattern is observed in many tibiae less than 50 cm in length.....	208
6.8. Radially oriented vascularity at the periosteal surface suggests possible osteopetrosis in three tibiae of <i>Maiasaura</i>	209
6.9. Transverse sections from the twenty-two <i>Maiasaura</i> tibiae between 40 cm and 50 cm in length.....	210
6.10. Bone tissue features present in tibiae between 40 cm and 50 cm in length.....	211
6.11. Several tibiae within the 40 cm – 50 cm size range possess resorption cavities arranged in one or more circular rings within the mid cortex.....	212

LIST OF FIGURES – CONTINUED

Figure	Page
6.12. Transverse sections from the four <i>Maiasaura</i> tibiae between 50 cm and 70 cm in length.....	213
6.13. Bone tissue features present in tibiae between 50 cm and 70 cm in length.....	214
6.14. Transverse sections from the three <i>Maiasaura</i> tibiae between 70 cm and 80 cm in length.....	215
6.15. Bone tissue features present in tibiae between 70 cm and 80 cm in length.....	216
6.16. Transverse sections from the six <i>Maiasaura</i> tibiae between 80 cm and 90 cm in length.....	217
6.17. Bone tissue features present in tibiae between 80 cm and 90 cm in length.....	218
6.18. Transverse sections from the five <i>Maiasaura</i> tibiae between 90 cm and 100 cm in length.....	219
6.19. Bone tissue features present in tibiae between 80 cm and 90 cm in length.....	220
6.20. An age frequency chart showing the distribution of the <i>Maiasaura</i> sample set based on LAG counts	221

GLOSSARY

Bone Formation

Endochondral ossification- the method by which long bones form. A preformed cartilaginous element is slowly eroded and replaced by bone mineral.

Perichondrium- fibrous, connective tissue sheath covering the cartilaginous long bone model. Once bone mineral replaces the cartilage model, this connective tissue sheath is found only on the epiphyses of growing animals, excluding the articular surfaces.

Periosteum- fibrous, connective tissue sheath covering the shaft, or diaphysis, of long bones (Fig. 1).

Endosteum- fibrous, connective tissue sheath lining bone bordering the medullary cavity.

Osteoblast- cell arising from the periosteum and endosteum and synthesizes bone matrix.

Osteocyte- cell derived from osteoblasts that have become surrounded by newly formed bone matrix.

Lacuna- the space occupied by an osteocyte. In fossilized bone, this is often the only evidence of osteocyte presence.

Osteoclast- a multinucleated cell responsible for bone resorption. Uneven, scalloped resorption cavities provide evidence of its presence in fossil bone (Fig. 2).

Long Bone Anatomy

Epiphysis- a cartilage mass capping either end of a long bone, and is responsible for bone elongation. Surrounded by a perichondrium except at the articular surface (Fig. 1).

Metaphysis- region at the base of an epiphysis, separating the zone of calcified cartilage from the zone of ossification (Fig. 1).

Diaphysis- the shaft of a long bone, responsible for increase in bone diameter.

Surrounded by the periosteum (Fig. 1).

General Bone Architecture

Cortex- peripheral portion of the diaphysis (Fig. 1)

Medulla- inner portion of the diaphysis, containing the marrow cavity (Fig. 1).

Compacta- bone consisting of a higher volume of tissue than pore space. Usually, but not always, characteristic of the cortex. Is often converted to cancellous spongiosa during growth.

Spongiosa- bone consisting of a higher volume of pore space than tissue. Usually, but not always, characteristic of the medulla. Can be divided further into “fine cancellous” (the least porous), “coarse cancellous”, and “trabecular” (the most porous).

Compact Bone Vascularity

Haversian canal- a vascular canal running the length of the long bone shaft (Fig. 1).

Volkman's canal- a radial vascular canal connecting Haversian canals (Fig. 1).

Simple primary vascular canal- formed when a blood vessel becomes encased within the bone matrix as it is deposited.

Primary Haversian cavities- large spaces containing many blood vessels, left within the bone matrix after the mineralization front moves away (Fig. 2).

Primary osteon- forms when a blood vessel within a primary Haversian cavity is surrounded by concentric layers of slowly deposited tissue (Fig. 2).

Avascular bone- bone lacking a vascular network (Fig. 3).

Secondary osteons- term given to the resulting structure after the bone around a vascular canal is resorbed by osteoclasts and later filled in by concentric layers of bone (Fig. 2).

Cementing line- the outline of the resorbed cavity before infilling, differentiating secondary osteons from primary osteons (Fig. 2).

Dense Haversian tissue- when multiple generations of secondary osteons overlap each other (Fig. 2).

Lamina- the distance from the center of one vascular layer to the center of the next vascular layer (Fig. 2).

Bone Matrix Organization

Acellular tissue- lacks obvious osteocytes, which are either very small or have withered and died.

Woven-fibered bone matrix- consists of randomly arranged coarse collagen fibers, which look isotropic in polarized light. It is often formed in fast growing juveniles (Fig. 3).

Lamellar matrix- contains thin layers, or “lamellae”, of bone matrix resulting in a high degree of spatial organization and anisotropy (Fig. 3). It is deposited in a plywood-like pattern, where the collagenous fibers alternate direction of deposition for each concentric layer. It is the tissue type filling spaces left from resorption to form secondary osteons, as well as around vascular canals within primary Haversian cavities to form primary osteons.

Parallel-fibered tissue- contains parallel collagenous fibers but is not organized to the degree that lamellar tissue is, although it too is anisotropic in polarized light (Fig. 3).

Fibro-lamellar tissue- primary osteons within a woven-fibered matrix (Fig. 4). Different sub-types of this tissue are defined based on vascularity, and together make up the “fibro-lamellar complex.”

Vascular Organizations of the Fibro-Lamellar Complex

Laminar- vascular canals are longitudinally arranged (Fig. 4).

Radiating- vascular canals are arranged radially, as spokes on a wheel (Fig. 4).

Associated with very fast bone deposition.

Circular- vascular canals are parallel to the transverse section and arranged in circumferential layers (Fig. 4).

Plexiform- circular vascular canals connected by radiating vascular canals (Fig. 4).

Reticular- vascular canals obliquely oriented to the transverse section and anastomose irregularly (Fig. 4).

Growth Marks

Annulus- made of either parallel-fibered or lamellar bone, representing a period of slow growth (Fig. 5).

Lines of arrested growth (LAGs)- a thin line of hypermineralized matrix representing a temporary, but complete cessation of growth (Fig. 5).

Zone- a region of bone mineral bounded by growth marks (Fig. 5).

Lamellar-zonal- a zone consisting of parallel-fibered or lamellar tissue bounded by growth marks (Fig. 5).

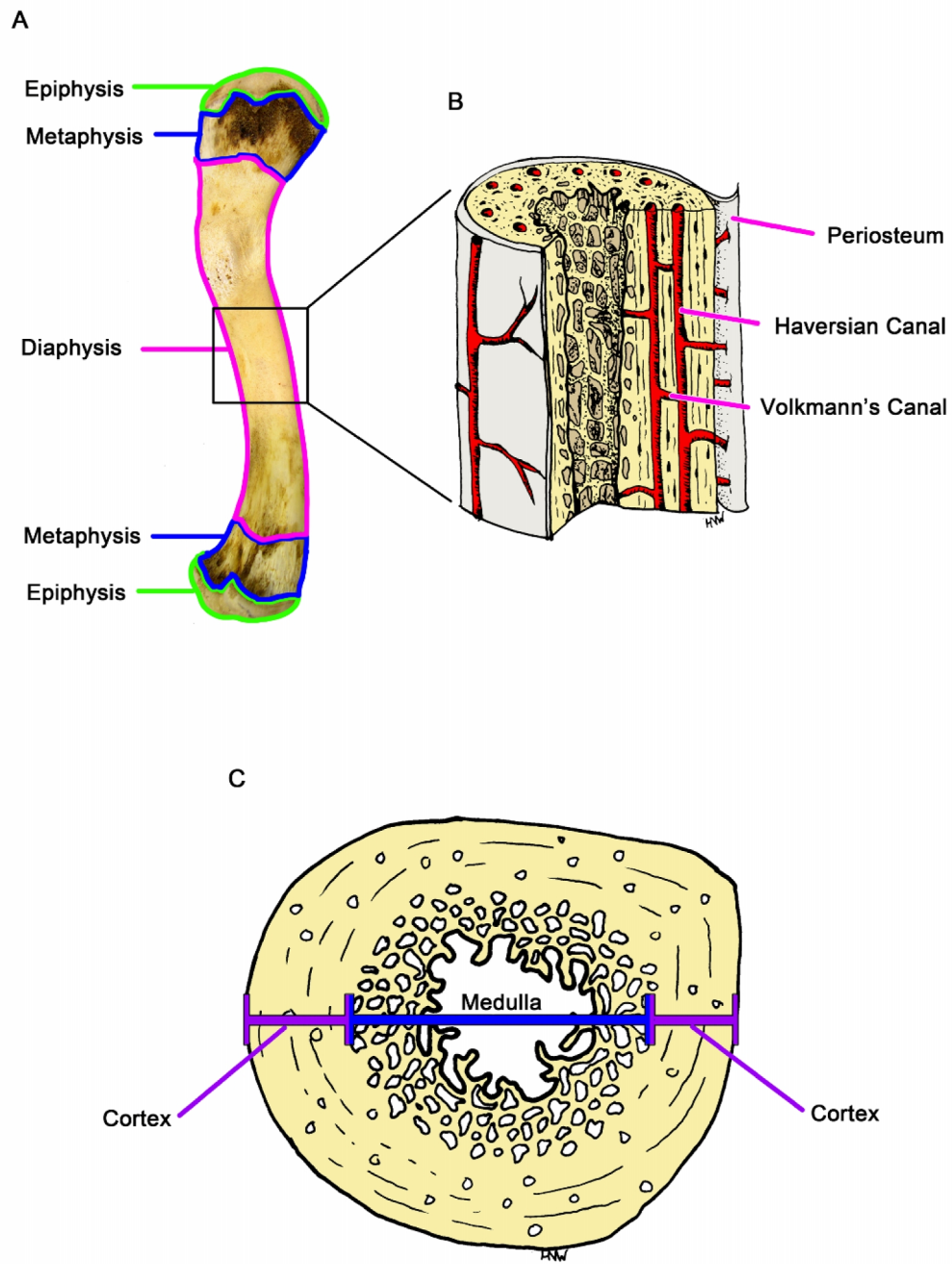


Figure 1. Anatomy and general architecture of an endochondral long bone. A) Alligator femur with the regions of the epiphyses, metaphyses, and diaphysis highlighted; B) a schematic enlargement of the diaphysis labeling the periosteum, a Haversian canal, and a Volkmann's canal; C) a schematic of a diaphysis observed in transverse section, with the cortex region shown in purple and the medulla shown in blue.

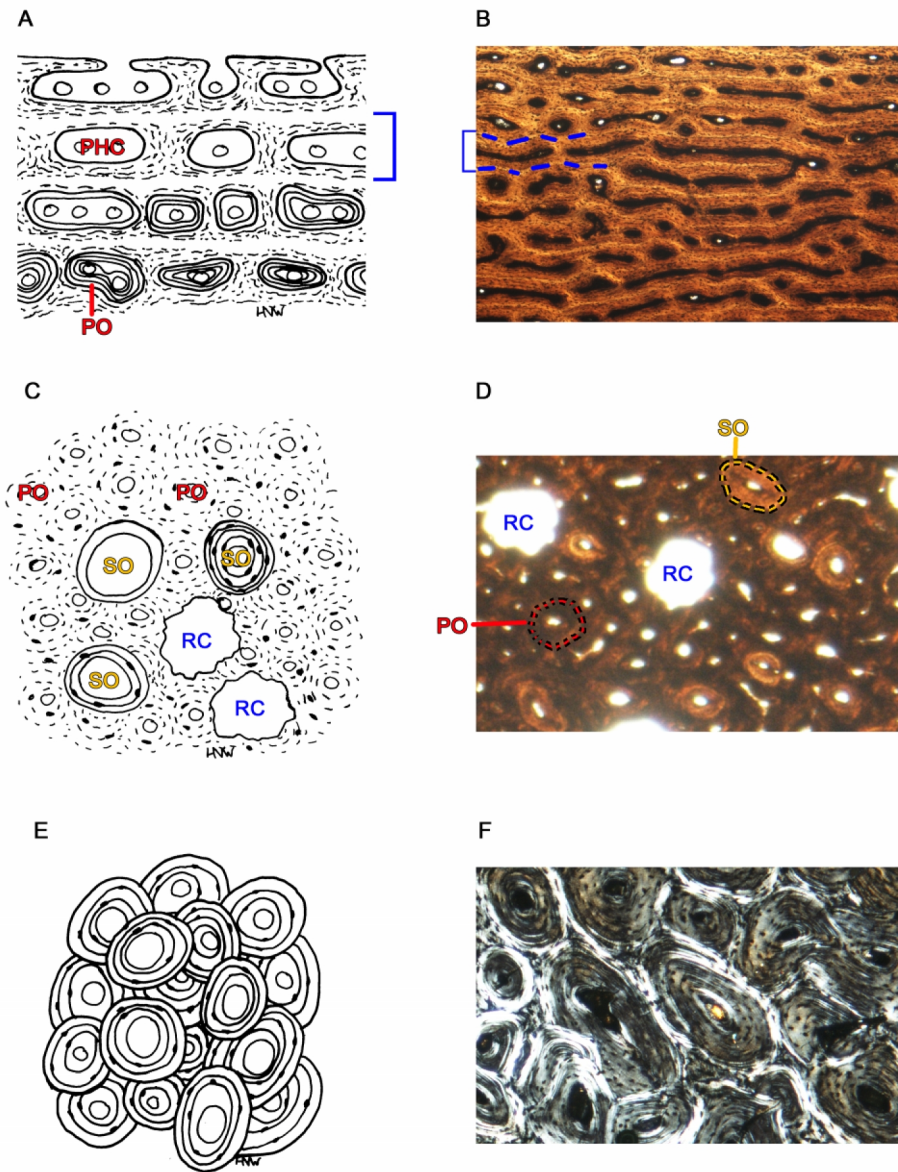


Figure 2. Bone cellular processes and resulting structures. A) a schematic of woven tissue. Vessels are surrounded by woven tissue forming primary Haversian canals (PHC). Older primary Haversian canals are later enclosed by more slowly formed lamellar tissue, forming a primary osteon (PO). A lamina (blue bracket) is the distance from the center of one vascular layer to the center of another vascular layer; B) Woven bone from dinosaur *Troodon* with primary osteons and evident laminae (dashed blue lines); C) a schematic of osteoclast resorption cavities (RC). Secondary osteons (SO) result when resorption cavities are filled in with lamellar tissue and cut across the original bone fabric; D) primary osteons, secondary osteons, and resorption cavities within the tibia of the dinosaur *Maiasaura*; E) a schematic of dense Haversian tissue, made of multiple generations of secondary osteons; F) a cow tibia showing dense Haversian tissue.

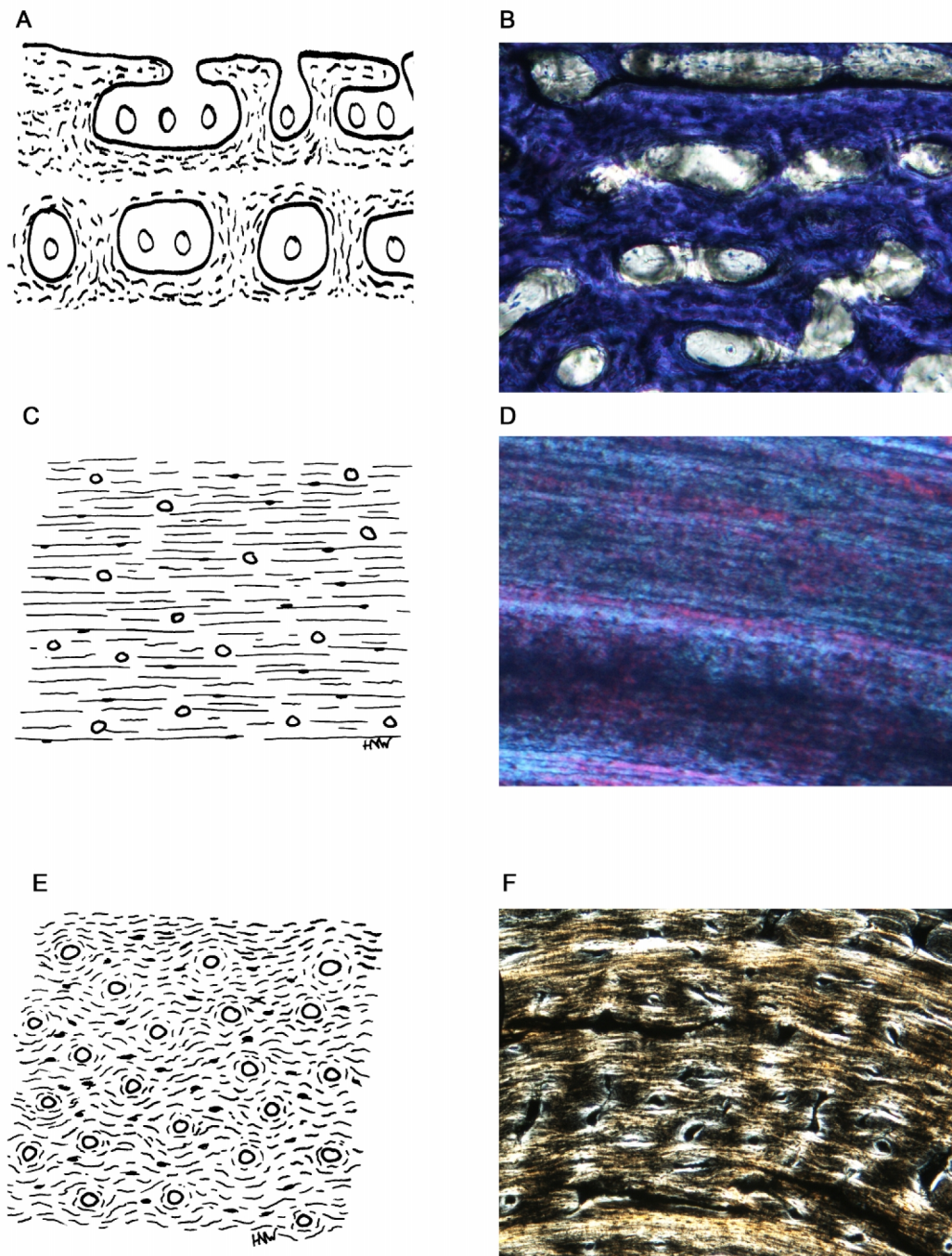


Figure 3. Bone matrix organization. A) a schematic of woven fibered tissue with primary Haversian cavities, which is illustrated by B), an emu femur; C) a schematic of lamellar bone tissue, made of highly organized collagen fibers; D) lamellar tissue is anisotropic, as seen in this alligator scapula; E) the schematic of parallel-fibered tissue shows that collagen fibers are organized in parallel but not to the degree found in lamellar tissue; F) the anisotropy and parallel collagen fiber arrangement is seen in this alligator humerus.

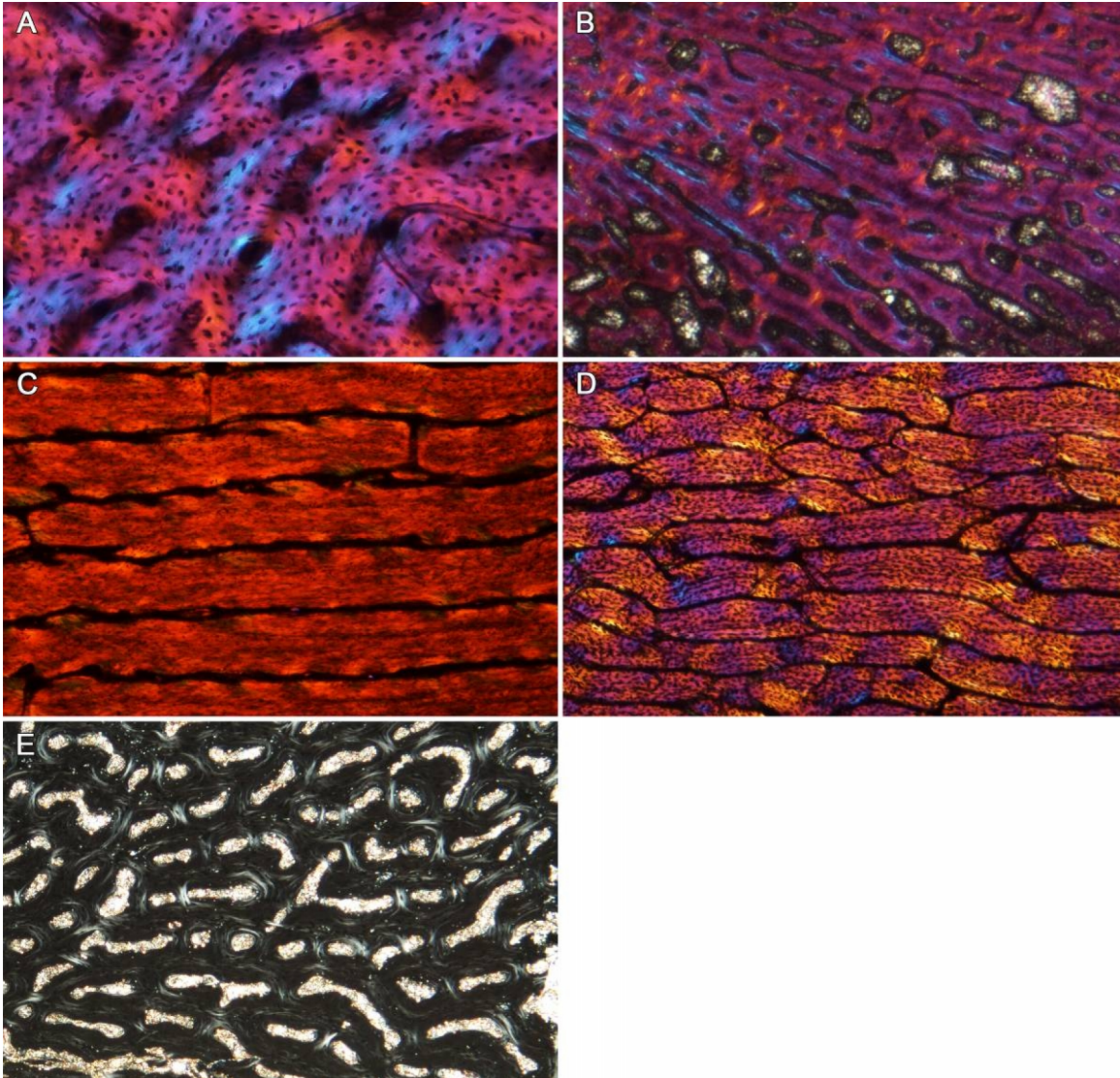


Figure 4. Vascular orientations within the “fibro-lamellar complex” as seen in the transverse plane. A) laminar vascularity results from longitudinally oriented vascular canals; B) radial vascular canals radiate from within the cortex to the periosteal surface; C) circular vascular canals are arranged parallel to the plane of section and in circumferential layers; D) when circular vascular canals are connected by radial vascular canals the vascularity is said to be plexiform; E) if the vascular canals are oriented obliquely to the plane of section, as if anastomosing, they are reticular.

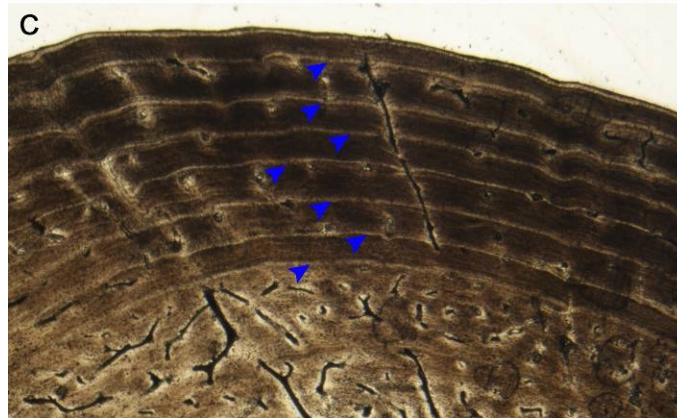
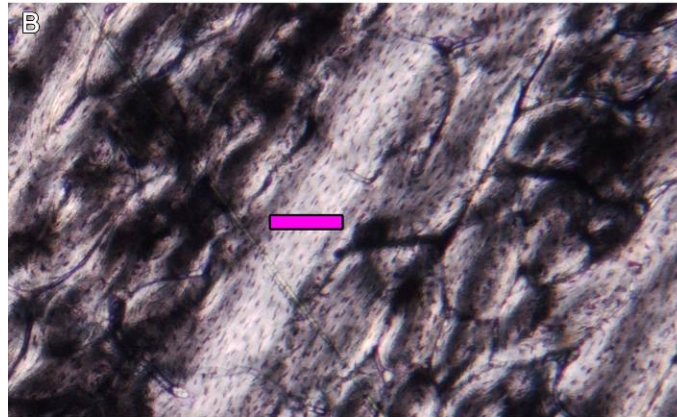
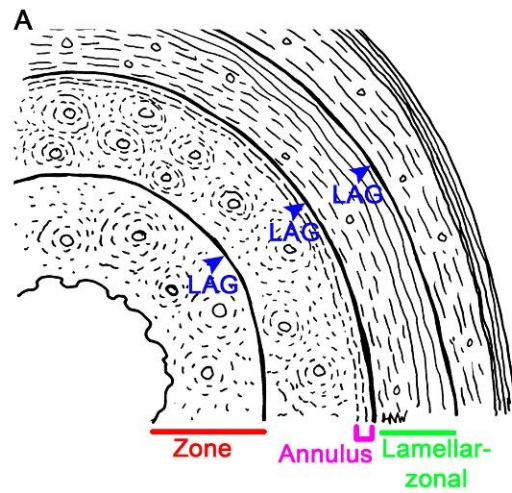


Figure 5. Growth marks. A) a generalized schematic showing various growth marks. An annulus indicates dramatically slowed growth, whereas a line of arrested growth (LAG) indicates a cessation of growth. A zone is a region of bone tissue bounded by growth marks. Lamellar-zonal bone occurs when a zone is comprised of either parallel-fibered or lamellar tissue; B) the purple bar in this elk femur spans the width of an annulus; C) blue arrows indicate multiple LAGs in this alligator femur.

ABSTRACT

The microstructure of bone can be used to infer growth rate, physiology, and age of a taxon. Because of this, bone histology is frequently employed in studies of non-avian dinosaurs to better understand how they grew. Unfortunately, the potential for individual skeletal variability is rarely accounted for in extant studies, making interpreting the growth histories of dinosaurs somewhat ambiguous. This dissertation addresses the question of individual variability in the bone microstructure of an extant dinosaur relative, the American alligator, thus aiding in establishing a baseline from which to draw conclusions about the growth of dinosaurs. Appendicular elements of thirty three alligators demonstrate growth rate variability within elements of the same individual, and also across individuals. Regardless, no element skipped yearly growth mark deposition. Additionally, some alligators possessed an external fundamental system (EFS), revealing that skeletal maturity was achieved. This suggests that dinosaurs likely also had determinate growth. Next, the results of the alligator studies were used to reassess the interpretations of previous dinosaur paleohistology studies. Reanalysis of polar dinosaur histology using seventeen samples falsifies the hypothesis that growth marks are related to hibernation, demonstrating the danger of drawing conclusions about behavior from only two samples. The histology of *Raptorex* was similarly reassessed, with the microstructure here interpreted as that of an immature individual rather than a subadult or early adult. Finally, a new histological study on the dinosaur *Maiasaura* demonstrates the growth rate variability within a taxon that often goes unaccounted for in smaller sample sets. With forty eight tibia specimens, this represents the largest single-element dinosaur histology study to date. In general, the first growth mark, indicating yearlings, appears in animals that would have been between 2.5 m and 3.5 m in body length. The largest individuals have not only LAGs but also EFS. Growth appears to plateau between nine and ten years, supporting the findings of the original *Maiasaura* growth study. However, this study also shows there is a considerable degree of individual variation in growth rates, and cautions against constructing generalized growth curves using only a handful of specimens.

CHAPTER ONE

INTRODUCTION TO DISSERTATION

The study of fossil bone microstructure (“paleohistology”) provides paleontologists with details about non-avian dinosaur growth and physiology that could not otherwise be obtained from simply examining bone morphology. Bone microstructure records information about growth rate, skeletal maturity, physiology, and even the age at death. Paleohistology was established in the 1850s but has only within the last thirty years begun to play a major role in the understanding of dinosaur biology. This increased attention was likely fueled by the discovery of growth marks within dinosaur long bones—used in extant animals for skeletochronology. Together with skeletochronology, bone tissue organization is utilized in assessing dinosaur ontogenetic status and skeletal maturity. This has, in recent years, led to growth curve models for numerous dinosaur taxa. Below is a brief account of the origins of paleohistology and significant contributions enabling the advancement of the field to its present state.

A Brief History of PaleohistologySignificant Paleohistology
Contributions Prior to 1980

The earliest publications concerning dinosaur bone microstructure were little more than casual descriptions (e.g., Mantell, 1850). One of the first systematic studies was performed by Seitz in 1907, objectively describing and illustrating the histology of fossils spanning the Permian to the Tertiary, including dinosaurs, marine reptiles,

amphibians, and pterosaurs. Paleohistology was further advanced by Gross (1934), who accurately identified the presence of primary osteons in a matrix of fibrous bone within dinosaur fossils. This fibro-lamellar arrangement is significant because it indicates rapid growth rates, suggesting that at least some dinosaurs did not fit the overgrown reptilian image popular at that time.

The first report to utilize osteohistology to infer aspects of dinosaur growth was published by Nopsca and Heidsieck (1933). By comparing differences in tissue structure, the authors concluded that two separate crested hadrosaur dinosaur genera were simply immature morphs of the same genus, and proposed that hadrosaur cranial outgrowths occurred late in ontogeny. Although their study demonstrated the usefulness of histology beyond pure description, their hypothesis regarding hadrosaur cranial growth would not again be seriously reviewed for forty years.

During the 1950's, Enlow and Brown (1956, 1957) published the first major comparative review of animal osteohistology, describing bone microstructure patterns found within vertebrates including dinosaurs. They recognized that dinosaur bone tissue was much different from amphibians, snakes, and lizards, describing it as "similar to, if not identical with, the bone tissues of many living mammals, including man" (Enlow and Brown, 1957, p. 200).

Enlow (1969) later extensively discussed the similarities between turtle and crocodilian bone tissue, and contrasted 'typical' reptilian bone microstructure with that of dinosaurs. He observed that dinosaurs were the only group of reptiles to have frequently and extensively deposited Haversian systems, as well as the only reptilian group to

possess plexiform bone tissue also found in artiodactyl mammals. In addition, Enlow made important observations regarding the function of growth marks, then documented only in fish, amphibians, and reptiles, suggesting that the cyclic layering of ‘growth rings’ in reptiles was related to seasonal factors.

The field of paleohistology (as well as paleobiology) was also being advanced at this time by Ricqlès, beginning in 1968 with a paper describing plexiform tissue found within the long bones of prosauropod dinosaurs. This was the first paper in a series describing the osteohistology of various Permian and Mesozoic groups (Ricqlès, 1969, 1972, 1974a, b). During this time Ricqlès was also expanding his histological descriptions in an additional three part series (Ricqlès, 1975, 1976, 1977b, a, 1978b, a). The first of this series named and diagrammed bone tissue structures as well as vascular canal orientations (Ricqlès, 1975). The second part defined and described general histological features found within a bone and detailed bone fracture histology (Ricqlès, 1976, 1977b, 1978b). The papers also discussed how tissue structure changed with age in mammals, the mechanical constraints of Haversian bone, the organization and function of spongiosa during ontogeny (Ricqlès, 1976), and bone tissue types commonly found in specialized ecological adaptations such as flying and swimming (Ricqlès, 1977b). Part three of the series described the bone histology of archosaurs and therapsids and discussed the evolution of endothermy, osteons, mineralization, cartilage, and finally, speculated on the genetic role in bone tissue development (Ricqlès, 1978b, a).

Important Contributions to Dinosaur Skeletochronology

Increased interest in dinosaur osteohistology since the 1980s is likely attributable to the discovery that dinosaur bone, like the bone of many extant vertebrates, contains Lines of Arrested Growth (“LAGs”). The first report of dinosaur bone with LAGs was published by Reid in 1981. He examined a portion from the pubis and ischium of an unidentified sauropod and recognized zonal bone structure. The presence of LAGs in sauropods was confirmed by Ricqlès in 1983 when he found numerous rest lines in the rapidly deposited tissue of a humerus from an immature *Bothriospondylus*. Growth marks were soon being found in many different dinosaur groups. In a paper published in 1990, Reid surveyed a variety of dinosaur fossils from both ornithischian and saurischian groups to demonstrate the common presence of zonal bone growth in many dinosaur lineages, including *Massospondylus*, *Euskelosaurus*, *Camarasaurus*, *Megalosaurus*, *Allosaurus*, *Baryonyx*, *Albertosaurus*, *Tyrannosaurus*, *Iguanodon*, *Orthomerus*, *Rhabdodon*, *Stegosaurus*, and a ceratopsian. He also examined more than one bone from each species, finding that some elements produced non-zonal bone, while other elements had zonal bone (Reid, 1990).

Annual growth marks are often associated with ectothermic vertebrates (Peabody, 1961; Hemelaar and van Gelder, 1980; Hutton, 1986; Buffrénil and Castanet, 2000). They are however quite common in many extant vertebrate groups including fish, amphibians, reptiles, and mammals (e.g., Peabody, 1961; Ricqlès, 1980; Ricqlès et al., 2001; Castanet et al., 2004; Turvey et al., 2005; Bourdon et al., 2009; Köhler, 2011). Recently, scientists reported the presence of LAGs in the kiwi bird as well (Bourdon et

al., 2009), making this the only extant bird known to take more than a year to reach skeletal maturity and therefore form growth marks. Because growth marks are found in so many extant vertebrate groups including, it is likely they are a plesiomorphic trait expressed regardless of metabolic physiology (Horner et al., 1999; Ricqlès et al., 2001).

Although the presence of growth marks in dinosaur bone was first reported in the early 1980s, it was not until a decade later that growth marks were used as skeletochronological indicators. Various studies have demonstrated that growth mark deposition occurs annually in many extant reptile groups (Peabody, 1961; Hemelaar and van Gelder, 1980; Hutton, 1986; Buffrénil and Castanet, 2000). If it is assumed that growth mark deposition was annual in dinosaurs as well, this would provide a way to obtain age at death estimates for individuals, as well as determine yearly growth rates. In addition, body mass growth curves can be constructed using various models, such as logistic and Gompertz.

The first dinosaur skeletochronology study was done by Chinsamy in 1993. She sectioned sixteen femora of various sizes from the prosauropod *Massospondylus* just below the fourth trochanter. Innermost growth marks were obliterated in the larger femora due to secondary remodeling. Chinsamy estimated the original number of missing growth lines by using the width of the initial zones in smaller and therefore presumably younger femora, thereby restoring the majority of growth lines through ‘retro-calculating’. The number of growth marks estimated to have been destroyed by reconstruction was then added to the number of marks present in the larger specimens to obtain an age estimate. Chinsamy stressed the importance of sectioning a standardized

region of bone across specimens, as bone microstructure can change in different regions of the same bone. This was reemphasized by Horner et al. (1999, p. 299), “...because of local variations in bone tissue, any other bone or even a different section of the same bone could easily yield a different estimate”. They went on to suggest that because remodeling occurs at different rates in different bones (and therefore the number of LAGs preserved differs), examining an ontogenetic sequence should be used whenever possible.

The next major ontogenetic study examined the hadrosaurid *Maiasaura* *peeblesorum* (Horner et al., 2000). Horner et al. divided *Maiasaura* bones into six growth stages ranging from small nestlings to adults based on relative bone size and histological patterns. Over 50 bones were sectioned including vertebrae, ribs, limb girdles, and long bones. Growth rates were estimated using LAGs from the femora, and the first femur to record a LAG was from an individual that would have been over three meters long. If LAGs were deposited yearly, the authors inferred that *Maiasaura* would have grown three meters in its first year. The onset of adulthood was hypothesized to occur at age seven by first determining the rate at which the cortex was deposited, based on conservative growth measurements on extant animals. This study is important because it is the first to use not only a large number of individuals for a histological analysis, but also a large number of different elements so that skeletal variation could be observed.

Another multiple-element ontogenetic analysis was performed by Erickson and Tumanova (2000) on *Psittacosaurus*. Elements studied included humeri, tibiae, and femora from 11 individuals. The authors devised a method for determining body mass from the femoral diaphyseal circumference of each individual in the ontogenetic series.

Using the estimated masses along with estimated ages, a sigmoidal growth curve for this genus was produced. However, the authors admit that the largest specimen sampled may not actually have attained adult size (Erickson and Tumanova, 2000). This was the first time mass estimates were determined and used to produce a dinosaur growth curve from an ontogenetic series.

Several years later, Horner and Padian (2004) used known growth rates in bones of modern birds to estimate growth rates in dinosaurs using LAG counts as an independent test. The authors hoped to assess: 1) whether the largest specimens of *Tyrannosaurus* showed a cessation in growth, 2) if smaller specimens showed a slowing of growth, and 3) how old these specimens were at the time of death. The innermost growth lines of the tibiae sampled were lost due to marrow cavity enlargement and secondary reconstruction. Therefore, the bone deposition rates early in the animal's life had to be estimated. It was assumed that appositional growth rates were higher in young dinosaurs, since this is the case for all other living amniotes. The number of missing LAGs was retro-calculated by three different methods. The distance from the center of the tibia to the first LAG was divided by 1) the largest width between two successive LAGs; 2) the next to last LAG interval; 3) the mean width of all preserved LAG intervals. The results suggested that *Tyrannosaurus rex* growth was slightly higher than that of an African elephant and that growth ceased between 25 and 35 years of age (Horner and Padian, 2004).

The timing of somatic and sexual maturity can also be examined histologically. Erickson et al. (2007) sampled ribs, gastralia, phalanges, fibulae, femora, and tibiae from

four oviraptoroids and one deinonychosaur from Asia as well as two North American deinonychosaurs, all either captured in brooding posture or closely associated with eggs. By deriving mass estimates from femoral circumferences, the authors were able to determine the developmental stage at which the animal perished. The authors also examined the spacing of LAGs within the bone to determine whether adult size had been reached (closely spaced LAGs) or if the brooding dinosaur was still growing (widely spaced LAGs). Although five of the seven taxa examined exhibited external fundamental systems, two possessed widely spaced LAGs. This implies the individuals, and by extension non-avian dinosaurs in general, reached sexual maturity prior to skeletal maturity (Erickson et al., 2007).

In a similar study, Lee and Werning (2008) report the presence of medullary bone in *Allosaurus*, *Tenontosaurus*, and *Tyrannosaurus*. Among extant animals, medullary bone is only found in female birds in the process of shelling their eggs. Because medullary bone was found within long bones that had not stopped growing, the authors concluded that these taxa attained reproductive maturity before reaching adult size. Growth curves were produced by fitting LAG retro-calculations to growth rate equations. The point at which reproductive maturity was attained for *Tenontosaurus*, *Allosaurus*, and *Tyrannosaurus* was calculated to be 8, 10, and 18 years respectively. The authors demonstrated that these ages correspond with the inflection point in the growth curve for each taxon, well before approaching skeletal maturity (Lee and Werning, 2008).

Summary of Dissertation

To further advance the field of paleohistology, more studies examining the variation in bone tissue microstructure of both extinct and extant taxa are necessary for establishing a baseline from which to draw conclusions about the growth of extinct animals. Unfortunately, very few extant studies sample multiple skeletal elements or multiple individuals, thus casting doubt on the reliability of assumptions made in paleohistology. Some problems include: assuming that annual growth mark deposition is variable across skeletal elements; there is no quantified consensus on which skeletal elements are best for reconstructing growth curves; most growth curves are made based on observations from one or a few individuals at each size class. Essentially, the field of dinosaur paleohistology has forged on for years without thoroughly addressing central, key questions: how much skeletal variation is there in a particular taxon, and is this necessary to take into consideration before attempting to reconstruct or interpret the growth history of an animal?

Therefore, the purpose of this dissertation is to address how individual variability may affect interpretations of paleohistology, and will contribute to the foundation of information from which dinosaur paleohistology studies should be based upon. This will be done by assessing the potential for skeletal variability within a living relative of dinosaurs, the alligator. Then, the conclusions of the alligator studies will be applied to a reanalysis of several recent histological studies to demonstrate the importance of large sample sizes. Finally, information obtained from these studies will be applied to interpret

a large fossil sample set, producing a robust interpretation of the growth history for a single dinosaur taxon.

To understand non-avian dinosaur growth, the histology of their living relatives- birds and crocodylians- must be examined. Of the two groups, birds are more closely related to dinosaurs. In fact, birds and dinosaurs share similarities in woven fibered bone deposition. When bone fibers are woven and disorganized, this is an indication of rapid growth. In contrast, crocodylian bone fibers are well organized, indicating slower apposition rates than for either dinosaurs or birds. However, both crocodylians and dinosaurs deposit growth marks called “lines of arrested growth” (LAGs), which resemble tree rings within a bone. They represent annual cessations of bone deposition in extant vertebrates. Because of this, LAGs are very important in dinosaur paleohistology, allowing inferences about age at death and yearly growth rates. Almost every extant bird taxon achieves skeletal maturity in less than a year, so that their bones lack growth marks. Because of this, to understand growth mark and annual growth rate variability, crocodylian- rather than bird- histology must be examined.

Chapter Two details the first of two alligator studies included in this dissertation. This study examines multiple skeletal elements from three individuals to determine if growth mark counts are consistent across elements. In addition, histologically examining multiple individuals reveals whether or not variability is an important factor to consider when reconstructing dinosaur growth curves. Eight pairs of appendicular elements were histologically sampled from one wild caught male alligator and two captive bred males. Student’s *t*-tests revealed no significant difference in the perimeters of growth marks

between homologous left and right elements for each individual. This is supported by the close relationship of perimeter measurements ($R^2 = 0.98$) when left and right growth mark perimeters of homologous elements were plotted against one another. In addition, apparent inconsistencies in growth mark counts across elements seems to be the result of variable marrow cavity expansion rates, as the innermost growth marks were nearly destroyed in some specimens but not in others. When annual growth rates for each skeletal element are plotted, it becomes obvious that there is considerable variability in the growth of skeletal elements across individuals. The high degree of growth rate variability, even between the two captive individuals, cautions against drawing sweeping conclusions about the growth of extinct taxa based solely on a handful of samples. Although there is considerable growth rate variability for each element when compared across individuals, the femur, humerus, and tibia consistently had the highest growth rates within each animal. Thus, for determining growth rates, weight-bearing bones (femur, tibia, humerus in quadrupeds; femur and tibia in bipeds) may provide maximum growth rate estimates for dinosaurs.

Chapter Three discusses a histological analysis performed on thirty known-age alligators from a single population. These alligators were examined for evidence of skeletal maturity, so that inferences could be made regarding dinosaur growth trajectories and senescence. It is often assumed that alligators and other reptiles continue to grow throughout their lives. Because of this, dinosaurs are frequently assumed to have continually grown as well.

In mammals, determination of skeletal maturity is fairly straightforward. As long bones develop, they form secondary centers of ossification within the epiphyses. When the epiphyses are unfused, growth in bone length is possible. But once the ossified epiphyses fuse to the bone shaft, bone can no longer expand in length. However, birds and most other reptiles lack secondary centers of ossification but this does not necessarily mean that growth in reptiles is ongoing. Aside from epiphyseal fusion, the only way to know if an animal is skeletally mature is to find an external fundamental system (EFS) within the long bone shaft. A EFS is a region of often avascular compact parallel tissue within the outer cortex of a long bone, signaling a virtual cessation in growth (Cormack, 1987). This structure is well documented in non-avian dinosaurs and in extant animals including birds, mammals, and some reptiles (e.g., Cormack, 1987; Castanet et al., 1988; Horner and Padian, 2004; Ponton et al., 2004) .

Out of the thirty captive alligators examined for this study, all but three possessed a EFS, suggesting that most had achieved skeletal maturity before their third decade of life. Because the alligators were skeletally mature at a relatively young age, this suggests that dinosaurs and other extinct animals could have lived for many years more after achieving skeletal maturity, and thus EFS should not equate with senescence when interpreting the life histories of extinct taxa. In addition, because EFS has been found in various animal groups, the absence of a EFS in extinct animals should not lead one to the conclusion that their growth was indeterminate but rather that the animal had not reached skeletal maturity before death. Results of this study suggest that more histological

analyses are needed to establish whether indeterminate growth is indeed a real phenomenon or simply an illusion caused by incomplete sampling of very old animals.

The conclusions of these alligator studies are used to reassess the results of three different dinosaur paleohistology studies in chapters 4-6. The first of these demonstrates what can and cannot be interpreted about dinosaur behavior based on small sample sets. In 1998, Chinsamy et al. histologically examined a femur from a small bodied plant eating ornithopod and a femur from a theropod, both from high latitude sediments. The authors found no LAGs in the ornithopod, but many LAGs in the theropod. To account for the differences in histology, the authors proposed that the ornithopod was active throughout the long polar winter, but that the theropod hibernated to avoid the cold.

The original material used in that study, as well as additional samples, was used to reassess the initial findings. One additional theropod femur was provided for analysis. This femur is smaller than the original, and no LAGs are present. The tissue organization is woven, and strongly resembles tissue from the lower latitude theropod *Troodon*. Because the smaller femur lacks LAGs and the larger femur has them, this is evidence that LAGs form annually and are not indicators of hibernation- especially since dinosaurs at lower, warmer latitudes have LAGs. Sixteen samples of small bodied ornithopod femora and tibiae, in addition to the original ornithopod femur, were also examined. In all but the smallest two ornithopods growth marks were present, further falsifying the hibernation hypothesis. As with the theropod, bone tissue microstructure is similar to related dinosaurs from lower latitudes. This reassessment suggests there is no bone microstructural evidence for behavioral differences between high latitude and low

latitude ornithopods or theropods, and that behavioral adaptations should not be proposed using such a restricted sample set (i.e., two femora).

The Asian theropod *Raptorex* was recently described by Sereno et al. on the basis of a single individual. In part because the specimen was fairly small and thought to be an adult, the authors concluded that the general tyrannosaur body plan- namely greatly reduced forearms- evolved first in a small bodied ancestor. As part of their analysis, Sereno et al. histologically examined the right femur and found that it contained at least three LAGS. In addition, bone tissue organization was reported as woven, indicating that growth was rapid. Despite this, the authors suggested that the LAG spacing implied growth was slowing down and that this animal was either a subadult or young adult. Chapter Five details the reexamination of the histological description and images from the *Raptorex* manuscript, revealing that at the resolution available in the paper, it is impossible to say for certain how many LAGs are present- if any. If the animal was a subadult or young adult, there should be evidence of a EFS or at least some bone remodeling in the form of secondary osteons, which is a feature found in more mature animals. Taken together, the woven tissue, absence of a EFS, and absence of secondary osteons strongly suggests *Raptorex* is an immature animal and not an ancestral adult. This reassessment highlights the danger of misinterpreting bone tissue microstructure, and demonstrates the risk of naming a new taxon based on the remains of a single immature individual.

The final study in this dissertation, detailed in Chapter Six, examines a large histological sample set from a dinosaur taxon and incorporates the findings of the previous chapters when interpreting the results. Forty eight tibiae of the hadrosaurid

Maiasaura, representing a minimum of thirty individuals, were collected for histological examination from a single Campanian-aged bone bed. Chemical analyses and very little weathering of the bones suggest they were not exposed at the surface for many years (Trueman, 1999; Horner et al., 2000). Although some time averaging is likely, this bone bed nevertheless represents a population of animals that were experiencing similar seasonal conditions. This study assesses the variation in growth rates in a single element across multiple individuals of varying ontogenetic status. A previous study by Horner et al. examined only six femora and concluded that *Maiasaura* achieved its adult body size of seven meters in as little as seven years. Samples in the present study correspond to individuals between 2.5 and 7.2 m in body length, and represent the largest single-element, monospecific dinosaur microanalysis to date. In general, the smallest tibiae consist of woven tissue with reticular or laminar vascularity. The first growth mark, indicating yearlings, appears in animals that would have been between 2.5 m and 3.5 m in body length. Older, still growing individuals have woven tissue but vascularity changes to predominately plexiform. The largest individuals have not only LAGs but also EFS. Growth appears to plateau between six and nine years, supporting the findings of the original *Maiasaura* growth study. However, this study also shows there is a considerable degree of individual variation in growth rates, and cautions against constructing generalized growth curves using only a few specimens for data.

Literature Cited

- Bourdon, E., J. Castanet, A. d. Ricqlès, P. Scofield, A. Tennyson, H. Lamrous, and J. Cubo. 2009. Bone growth marks reveal protracted growth in New Zealand kiwi (Aves, Apterygidae). *Biology Letters* 5:639-642.
- Buffrénil, V. d., and J. Castanet. 2000. Age estimation by skeletochronology in the Nile monitor (*Varanus niloticus*), a highly exploited species. *Journal of Herpetology* 34:414-424.
- Castanet, J., D. G. Newman, and H. S. Giron. 1988. Skeletochronological data on the growth, age, and population structure of the tuatara, *Sphenodon punctatus*, on Stephens and Lady Alice Islands, New Zealand. *Herpetologica* 44:25-37.
- Castanet, J., S. Croci, F. Aujard, M. Perret, J. Cubo, and E. d. Margerie. 2004. Lines of arrested growth in bone and age estimation in a small primate: *Microcebus murinus*. *Journal of Zoology, London* 263:31-39.
- Chinsamy, A. 1993. Bone histology and growth trajectory of the prosauropod dinosaur *Massospondylus carinatus* Owen. *Modern Geology* 18:319-329.
- Chinsamy, A., T. Rich, and P. Vickers-Rich. 1998. Polar dinosaur bone histology. *Journal of Vertebrate Paleontology* 18:385-390.
- Cormack, D. 1987. *Ham's Histology*. Lippincott Williams & Wilkins, New York, 732 pp.
- Enlow, D. H. 1969. The bone of reptiles; pp. 45-80 in A. d. A. Bellairs and T. S. Parsons (eds.), *Biology of the Reptilia*. Academic Press, New York.
- Enlow, D. H., and S. O. Brown. 1956. A comparative histological study of fossil and recent bone tissues, Part I. *The Texas Journal of Science* 8:405-443.
- Enlow, D. H., and S. O. Brown. 1957. A comparative histological study of fossil and recent bone tissues, Part II. *The Texas Journal of Science* 8:405-443.
- Erickson, G. M., and T. A. Tumanova. 2000. Growth curve of *Psittacosaurus mongoliensis* Osborn (Ceratopsia: Psittacosauridae) inferred from long bone histology. *Zoological Journal of the Linnean Society* 130:551-566.
- Erickson, G. M., K. Curry Rogers, D. J. Varricchio, M. A. Norell, and X. Xu. 2007. Growth patterns in brooding dinosaurs reveals the timing of sexual maturity in non-avian dinosaurs and genesis of the avian condition. *Biology Letters: Paleontology*.

- Gross, W. 1934. Die Typen des mikroskopischen Knochenbaues bei fossilen Stegocephalen und Reptilien. *Zeitschrift für Anatomie und Entwicklungsgeschichte*:731-764.
- Hemelaar, A. S. M., and J. J. van Gelder. 1980. Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. *Netherlands Journal of Zoology* 30:129-135.
- Horner, J. R., and K. Padian. 2004. Age and growth dynamics of *Tyrannosaurus rex*. *Proceedings of the Royal Society of London Series B* 271:1875-1880.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 1999. Variation in dinosaur skeletochronology indicators: implications for age assessment and physiology. *Paleobiology* 25:295-304.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 2000. Long bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. *Journal of Vertebrate Paleontology* 20:115-129.
- Hutton, J. M. 1986. Age determination of living Nile crocodiles from the cortical stratification of bone. *Copeia* 2:332-341.
- Köhler, M. 2011. On the significance of fibrolamellar zonal bone in bovids; pp. 54 in *First International Symposium on Paleohistology*, Vol. 1, I. C. d. P. M. Crusafont ed Gràfica Masana, Sabadell, Spain.
- Lee, A. H., and S. Werning. 2008. Sexual maturity in growing dinosaurs does not fit reptilian growth models. *Proceedings of the National Academy of Sciences, USA* 105:582-587.
- Mantell, G. A. 1850. On the Pelorosaurus; an undescribed gigantic terrestrial reptile whose remains are associated with those of the iguanodon and other saurians in the strata of Tilgate Forest, in Sussex. *Philosophical Transactions of the Royal Society of London* 140:379-390.
- Nopcsa, F. B., and E. Heidsieck. 1933. On the histology of the ribs in immature and half-grown trachodont dinosaurs. *Proceedings of the Zoological Society of London*:221-226.
- Peabody, F. E. 1961. Annual growth zones in living and fossil vertebrates. *Journal of Morphology* 108:11-62.

- Ponton, F., A. Elzanowski, J. Castanet, A. Chinsamy, E. d. Margerie, A. d. Ricqlès, and J. Cubo. 2004. Variation of the outer circumferential layer in the limb bones of birds. *Acta Ornithologica* 39:21-24.
- Reid, R. H. 1981. Lamellar-zonal bone with zones and annuli in the pelvis of a sauropod dinosaur. *Nature* 292:49-51.
- Reid, R. H. 1990. Zonal "growth rings" in dinosaurs. *Modern Geology* 15:19-48.
- Ricqlès, A. d. 1968. Recherches paléohistologiques sur les os longs des tétrapodes I. - origine du tissu osseux plexiforme des dinosauriens sauropodes. *Annales de Paléontologie (Vertébrés)* 54:133-145 with one plate.
- Ricqlès, A. d. 1969. Recherches paléohistologiques sur les os longs des tétrapodes II. - quelques observations sur la structure des os longs des Thériodontes. *Annales de Paléontologie (Vertébrés)* 55:3-52 with eight plates.
- Ricqlès, A. d. 1972. Recherches paléohistologiques sur les os longs des tétrapodes III. - Titanosuchiens, Dinocéphales et Dicynodontes. *Annales de Paléontologie (Vertébrés)* 58:17-60 with eight plates.
- Ricqlès, A. d. 1974a. Recherches paléohistologiques sur les os longs des tétrapodes IV. - Éothériodontes et Pélycosaures. *Annales de Paléontologie (Vertébrés)* 60:3-39 with eight plates.
- Ricqlès, A. d. 1974b. Recherches paléohistologiques sur les os longs des tétrapodes V. - Cotylosaures et Mésosaures. *Annales de Paléontologie (Vertébrés)* 60:171-216 with seven plates.
- Ricqlès, A. d. 1975. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (première partie.). *Annales de Paléontologie (Vertébrés)* 61:51-129 with nine plates.
- Ricqlès, A. d. 1976. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux de tétrapodes (deuxième partie.). *Annales de Paléontologie (Vertébrés)* 62:71-119 with four plates.
- Ricqlès, A. d. 1977a. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (deuxième partie, fin.). *Annales de Paléontologie (Vertébrés)* 63:133-159.

- Ricqlès, A. d. 1977b. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (deuxième partie, suite.). *Annales de Paléontologie (Vertébrés)* 63:33-55.
- Ricqlès, A. d. 1978a. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (troisième partie, fin.). *Annales de Paléontologie (Vertébrés)* 64:153-184 with six plates.
- Ricqlès, A. d. 1978b. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (troisième partie.). *Annales de Paléontologie (Vertébrés)* 64:85-111.
- Ricqlès, A. d. 1980. Tissue structures of dinosaur bone: functional significance and possible relation to dinosaur physiology; pp. 103-138 in R. D. K. Thomas and E. C. Olson (eds.), *A Cold Look at the Warm Blooded Dinosaurs*. Westview Press, Boulder.
- Ricqlès, A. d. 1983. Cyclical growth in the long limb bones of a sauropod dinosaur. *Acta Palaeontologica Polonica* 28:225-232.
- Ricqlès, A. d., K. Padian, and J. R. Horner. 2001: The bone histology of basal birds in phylogenetic and ontogenetic perspectives. *New Perspectives on the Origin and Early Evolution of Birds: Proceedings of the International Symposium in Honor of John H. Ostrom*.
- Seitz, A. L. 1907. Vergleichende Studien über den mikroskopischen Knochenbau fossiler und rezenter Reptilien, *Nova Acta*, Volume 87. *Abhandlungen der Kaiserlichen Leopold-Carolignischen deutschen Akademie der Naturforscher*, 230-370 pp.
- Sereno, P. C., L. Tan, S. L. Brusatte, H. J. Kriegstein, X. Zhao, and K. Cloward. 2009. Tyrannosaurid skeletal design first evolved at small body size. *Science* 326:418-422.
- Trueman, C. L. 1999. Rare earth element geochemistry and taphonomy of terrestrial vertebrate assemblages. *Palaios* 14:555-568.
- Turvey, S. T., O. R. Green, and R. Holdaway. 2005. Cortical growth marks reveal extended juvenile development in New Zealand moa. *Nature* 435:940-943.

CHAPTER TWO

QUANTIFICATION OF INTRASKELETAL HISTOVARIABILITY IN *ALLIGATOR MISSISSIPPIENSIS* WITH IMPLICATIONS FOR NON-AVIAN DINOSAUR PALEOHISTOLOGY

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: HollyNoelle W. Ballard

Contributions: Conceived the study, prepared, examined, and photographed specimens, analyzed data, interpreted results, and wrote the manuscript.

Co-author: John R. Horner

Contributions: Provided funding and microphotograph equipment. Assisted with study design, discussed implications of results, and edited earlier drafts of the manuscript.

Co-author: James O. Farlow

Contributions: Assisted with statistical analyses and interpretations, discussed results, and edited earlier drafts of the manuscript.

Manuscript Information Page

Holly N. Woodward, John R. Horner, James O. Farlow

Status of Manuscript:

☒ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☐ Published in a peer-reviewed journal

Abstract

Bone microanalyses of extant archosaurs provide a necessary framework from which to form hypotheses regarding the growth and aging of non-avian dinosaurs. Here, the bone microstructure of paired appendicular elements and osteoderms from three juvenile American alligators (*Alligator mississippiensis*) are described and histologic variability quantified to assess growth mark and tissue organization consistency both within and amongst individuals. Results confirm previous observations that tissue organization and growth mark count varies across the skeletal elements of an archosaur, but several trends emerged as well. The femur, tibia, and humerus had the highest annual apposition rates in each individual, and although growth mark count varied intraskeletally, data suggest the inconsistencies are simply due to differing medullary cavity expansion rates. There was also no appreciable variation ($R^2 = 0.984$) in growth mark perimeters between homologous elements of an individual if sampled from roughly the same location. Although considerable intraskeletal data and consistency was obtained, enough variability in apposition and cortical area existed when comparing homologous elements across individuals to suggest that more than three specimens must be examined before generalizing about alligator element growth rates. Thus, these quantified results support previous suggestions that enough skeletal variability exists amongst individual extant archosaurs to caution against generalized interpretations of non-avian dinosaur growth based on small sample sizes. Additionally, comparing graphs of annual cortical thickness and annual cortical area illustrate the potential for large errors in dinosaur mass estimates and for misinterpreting growth curves. For greater transparency, linear measurement

curves used to obtain mass should be reported alongside curves of mass estimates.

Introduction

In recent years the study of fossil bone microstructure has become increasingly important for determining aspects of non-avian dinosaur physiology by providing information about ontogenetic growth, age at sexual maturity, skeletal maturity, and identifying ontogenetic morphs of the same taxon (e.g., Bybee et al., 2006; Lee and Werning, 2008; Horner and Goodwin, 2009; Scannella and Horner, 2010; Fowler et al., 2011). Although numerous extant animal studies provide the basis for paleohistological interpretations, little is known about bone tissue variability within a skeleton or between homologous elements of different individuals. These are important factors to consider, as interpretations about growth and development of different dinosaur taxa are often based on very small sample sizes that do not take individual variability into account. To understand the potential for intraskeletal as well as individual variation within the bone microstructure of dinosaurs, skeletal variation must be assessed in their extant relatives (i.e., birds and crocodylians). Results can then be used as a framework for comparison. To accomplish this, here the bone microstructure of paired appendicular elements and osteoderms from three American alligators (*Alligator mississippiensis*) is described and skeletal variation quantified.

Although extant birds and crocodylians share a common ancestor with non-avian dinosaurs (Witmer, 1995), bone tissue organization suggests dinosaurs grew more like their avian descendants than like crocodylians (Horner et al., 2000). Both bird and non-

avian dinosaur bone often consists of highly vascularized, rapidly deposited woven tissue (Ricqlès et al., 2001) while crocodylian bone is primarily of parallel-fibered or lamellar tissue forming at much slower rates (Ricqlès, 1974; Castanet et al., 1996; Montes et al., 2010). But unlike extant birds, non-avian dinosaur bone tissue is often separated by cyclical marks representing periodically slowed or arrested growth (Peabody, 1961; Frylestam and von Schantz, 1977; Hemelaar and van Gelder, 1980; Ricqlès, 1980; Hutton, 1986; Tucker, 1997).

Growth marks are frequently associated with ectothermic vertebrates, but their occurrence is actually widespread in taxa that take more than a single year to reach skeletal maturity, including mammals (e.g., Peabody, 1961; Ricqlès, 1980; Ricqlès et al., 2001; Castanet et al., 2004; Turvey et al., 2005; Bourdon et al., 2009). Because growth marks have an annual periodicity in extant vertebrates for which data exists (Castanet et al., 1993), the presence of growth marks is heavily relied upon for determination of dinosaur growth rates and age estimates. However, dinosaur studies reveal that growth mark count varies based on the skeletal element sampled and even by sampling locality within the bone (Chinsamy, 1993; Horner et al., 1999, 2000), casting doubt on the validity of paleohistological interpretations involving growth marks (Horner et al., 2000).

Despite being necessary for verifying their utility in dinosaur paleohistology, the extent to which growth marks vary intraskeletally within extant archosaur taxa has not been extensively assessed. With the exception of the endangered kiwi (Bourdon et al., 2009), extant birds achieve skeletal maturity within a single year (Padian and Horner, 2002) and thus do not form growth marks, making them inappropriate for growth mark

studies. Therefore, despite their dissimilar bone tissue arrangement, crocodylians were chosen as the most appropriate archosaur analogue for assessing the variability of growth mark deposition and intraskeletal growth rates in dinosaur bone.

In addition to analyzing growth mark variability, intraskeletal examination of American alligators will assess changes in bone tissue organization both within and between individuals. Many previous studies have examined the growth rates of different elements within an individual (e.g., Ricqlès et al., 1991; Castanet et al., 1996; Castanet et al., 2000; Starck and Chinsamy, 2002), but with respect to extant archosaurs such extensive intraskeletal studies are largely concerned with various avian taxa. The general consensus from examining taxa such as ratites, ducks, and quail is that tissue organization and growth rates vary within the same element, between elements in the same individual, and between homologous elements in different individuals (Castanet et al., 1996; Castanet et al., 2000; Starck and Chinsamy, 2002). After coming to this conclusion from examining ratite histology, Castanet et al. (2000) questioned the validity of paleobiological conclusions made from single-element studies, and the results of the aforementioned studies together emphasize the need for more microanalyses of extant archosaurs so that assumptions regarding aspects of dinosaur growth are based on robust histological observations. To this end, this alligator study will also address as many sources of variability as possible including medullary cavity expansion, growth mark count, and tissue organization, contributing data necessary for more informed interpretations of non-avian dinosaur bone microstructure.

Materials and Methods

Ethics Statement

Appendicular elements (excluding manus and pes) and the largest two nuchal (i.e., postoccipital) osteoderms were obtained from three immature male American alligators (*Alligator mississippiensis*) that were humanely sacrificed by biological staff in February 2007 at the Rockefeller Wildlife Refuge in Grand Chenier, Louisiana, USA.

Procedure

The skeletal elements were prepared for osteohistology and examined at the Museum of the Rockies (MOR) in Bozeman, Montana, USA. Two individuals, MOR 2007-05RC4 (30.8 cm total length) and MOR 2007-05RC5 (141 cm total length), were raised in captivity while MOR 2007-05RC3 (95.3 cm total length) was wild-caught. Unfortunately, no age or mass data was available. Transverse sections were removed from two nuchal osteoderms (*sensu* Tucker, 1997) as well as from the minimum diaphyseal circumference of long bones where cortex is thickest (Sander and Andr  ssy, 2006), and from the thickest region of scapula and coracoid blades. Both left and right elements were sampled for all bones, except the scapula and coracoid of MOR 2007-05RC5 and the radius of MOR 2007-05RC4. Only a single element was available for examination in these cases. Thin sections were prepared using published techniques for extant bone (Schweitzer et al., 2007). At least two thin section slides were made from each specimen, and one of each pair was stained with Toluidine chloride (“Toluidine blue”) in case this made any tissue structures more apparent.

Every thin-sectioned element was analyzed with either 4 X or 10 X objectives using a Nikon Optiphot-Pol polarizing microscope using a circular polarizer, a $\frac{1}{4}$ lambda plate, or a full lambda (530 nm) plate. Images were obtained with a Nikon DS-Fi1 digital sight camera, and compiled using NIS-Elements BR 3.0 software. The field of histology is a highly visual one, but in the past, most if not all histological reports have been limited in some way with regard to the number of figures, or access to publishing figures in color. These restrictions often prevent authors from successfully illustrating accompanying descriptions which has contributed to a considerable amount of ambiguity when attempting to interpret or independently identify previously described structures. Fortunately, with the advent of digital publishing, the aforementioned limitations have essentially been lifted. This publishing format allows the descriptions in this paper to be extensively figured.

For each element, the perimeter of the periosteal surface as well as the perimeter of each growth mark was traced in Adobe Photoshop CS3. Perimeter tracings, cortical area and thickness at each growth mark, geometric centroid, and principal axes were quantified using the BoneJ plugin (Doubé et al., 2010) for NIH ImageJ. Measurements can be found in Table 2.1. Paired Student's *t*-tests (sample for means, $\alpha = 0.05$) were performed to compare growth mark as well as periosteal surface perimeters between homologous elements within an individual (Table 2.2). To visually represent the results of the *t*-test as well as to obtain an R^2 value and a regression equation, a plot of left versus right homologous element growth marks and surface perimeters for all three individuals was also constructed.

Finally, daily apposition rates were determined and graphs of both annual increase in cortical thickness as well as annual increase in cortical area were constructed.

Apposition rates were obtained by measuring the distance from the geometric centroid of the thin section to each successive growth mark along principal axes. The four measurements were then averaged to estimate cumulative cortical thickness. Annual average cortical thickness was obtained by subtracting cumulative cortical thickness at growth mark n from the cumulative cortical thickness at growth mark $n+1$. Annual average cortical area was similarly determined, by subtracting the cumulative cortical area at growth mark n from the cumulative cortical area at growth mark $n+1$. Average daily bone apposition rates were obtained by dividing annual average cortical thicknesses by an estimated 214 growing days in a year (Joanen and McNease, 1987). If growth marks or their principal axes could not be accurately traced, corresponding measurements were omitted from the relevant data tables.

Estimates of initial hatchling element perimeters and areas were obtained by measuring previously prepared diaphyseal thin sections from a hatchling alligator (MOR 1998-04R). Because the extreme medullary drift in scapular and coracoid elements prevented measurements of annual cortical thickness from a geometric centroid, apposition rates and cortical thicknesses were not determined for these elements.

Results

Quantitative analyses reveal important trends in skeletal growth rates and variability. A Student's t -test was performed on a total of twenty paired elements, and

demonstrated that with only two exceptions (the tibia and ulna of MOR 2007-05RC5), there was no significant difference in the perimeters of growth marks or in the periosteal surfaces between the paired skeletal elements of each individual. The results of the *t*-test are found in Table 2.2. A plot of right versus left homologous element growth mark and surface perimeters (Fig. 2.1) support the results of the *t*-test, with the fitted linear curve ($y = 1x - 0.047$) producing an R^2 value of 0.98. Additionally, average daily bone apposition rates (Table 2.3), annual average cortical thickness (Fig. 2.2), and annual average cortical area (Fig. 2.3) demonstrate both trends and variability in intraskeletal bone growth across individuals.

Several observations generalize the bone microstructure patterns observed in thin section samples. Medullary cavities are enclosed by an endosteal layer made of flattened osteocytes embedded within highly organized lamellar tissue. Thick fiber bundles are randomly scattered throughout the cortex and most are arranged radially, but some are also circumferential. Radial fibers are often especially concentrated along the perimeter of cortical growth marks. These fibers are commonly referred to as Sharpey's fibers, but because differentiation of Sharpey's fibers from other fibers inserting into bone is difficult (Hall, 2005), they will be referred to here as "periosteal attachment fibers". Vascularity consists of either simple canals incorporated directly into the bone matrix, but also of poorly developed primary osteons (see Discussion). In many elements, the perimeter of the medullary cavity is less than or nearly equal to the periosteal surface perimeter of homologous hatchling elements, indicating in these instances that no growth marks were completely lost to medullary expansion.

Growth marks are present in all specimens in the form of either LAGs or annuli. A LAG appears as either a white or birefringent line and indicates an area of hypermineralized tissue formed due to a temporary cessation of growth (Castanet et al., 1993). An abrupt decrease rather than a pause in apposition is reflected by a band of well-organized fibers arranged in parallel, containing flattened osteocytes and little to no vascularization. This band of tissue is termed an annulus (Francillon-Vieillot et al., 1990).

Below are qualitative descriptions of the bone microstructures for each alligator. Unless noted, no great differences in tissue organization were observed between left and right elements.

Humerus

In the left humerus of MOR 2007-05RC3 (Fig. 2.4), three growth marks are visible but only the outermost is fully traceable. The innermost annulus in the left humerus has been partially eroded by medullary expansion and the second annulus is too faint to accurately trace. Medullary expansion in the right humerus may have already obliterated the presence of the innermost growth mark. However the third growth mark on both humerii consists of an annulus terminating in a LAG and is easily traced. The innermost cortex is vascularized by a combination of longitudinal and anastomosing vessels. These are found within a mineralized matrix of woven fibers. Vascularity sharply decreases approaching mid-cortex and the second annulus, and the fibers become parallel. The outer cortex remains parallel-fibered but resembles the inner cortex in vascular density.

Small resorption cavities are scattered within the inner cortex of MOR 2007-05RC4 (Fig. 2.5) indicating that medullary expansion was active. The entire cortex consists of mineralized fibers arranged in parallel. Longitudinal vascular canals dominate the sample, but anastomosing canals are also common beyond the inner cortex, especially approaching the periosteal surface. Within the cortex five annuli are present, but the innermost cannot be fully traced. The outermost annulus is very close to the periosteal surface and is easily overlooked but can be fully traced in the left humerus.

The cortex of MOR 2007-05RC5 (Fig. 2.6) is entirely parallel-fibered, although approaching the periosteal surface the fibers are more loosely parallel. Resorption cavities are scattered within the innermost cortex. Five growth marks are present. The third and fourth annuli are unusually close together and the fifth mark is a LAG so close to the periosteal surface that it is difficult to fully trace. Vascular density is uniform throughout, consisting primarily of longitudinal canals prior to the first annulus, then becoming anastomosing to radial.

Radius

The cortical matrix in MOR 2007-05RC3 (Fig. 2.7) is lamellar. There is a concentration of longitudinal simple vascular canals in the inner cortex. Otherwise there are sparsely scattered simple longitudinal vascular canals within the cortex, but the outermost cortex is nearly avascular. Three LAGs are visible and distinct from the lamellae.

Most of the cortex of MOR 2007-05RC4 (Fig. 2.8) is avascular save for a few scattered longitudinal simple vascular canals and the entire cortex is lamellar. The lamellar endosteal layer is well developed. Four LAGs are visible.

The cortex of MOR 2007-05RC5 (Fig. 2.9) is also of highly organized lamellar tissue. The lamellar endosteal layer is very thick and portions of the innermost cortex are composed of secondarily remodeled tissue indicating some medullary drift. Vascularity is sparse but when present is longitudinal and simple. There are five LAGs within the cortex, and LAGs 3 and 4 are unusually closely spaced.

Ulna

A partial LAG is visible in the inner cortex of MOR 2007-05RC3 (Fig. 2.10) but almost destroyed. Two more LAGs within the mid-cortex are easily traced. The cortex is made of highly organized, lamellar fibers except in a region of the inner to mid cortex prior to the second LAG. This area is disorganized and woven. Longitudinal vascular canals are sparsely scattered throughout the cortex except within the disorganized woven matrix where there is a higher concentration.

There is evidence of extreme medullary drift in the ulna of MOR 2007-05RC4 (Fig. 2.11). The medullary cavity is surrounded by an endosteal layer, but this layer cuts across a considerable amount of primary as well as secondary tissue in the inner cortex. Vascularity is sparse but consists of longitudinal canals. Primary tissue is lamellar throughout. There are three LAGs, including one very close to the periosteal surface. Because of the extent of medullary drift and secondary remodeling it is likely several LAGs are missing.

Similar to MOR 2007-05RC4, the medullary cavity of MOR 2007-05RC5 (Fig. 2.12) is surrounded by an endosteal layer that cuts across a considerable amount of secondary tissue indicating a shift in the position of the medullary cavity with respect to the cortical bone. There is a fairly even scattering of longitudinal vascular canals from inner to outer cortex. Four LAGs present. Two of the LAGs are very close together in the mid-cortex, and another is very close to the periosteal surface. Due to the large amount of medullary drift and reconstruction, it is likely at least one LAG is missing within the innermost cortex.

Femur

The inner to mid cortex of MOR 2007-05RC3 (Fig. 2.13) is well vascularized by radial anastomosing canals. Vascularity decreases mid cortex but increases again near the periosteal surface. A partial LAG is present near the medullary cavity. A second growth mark in the form of an annulus and a third growth mark consisting of an annulus followed by a LAG are within the mid-cortex. The tissue between the innermost partial LAG and the medullary cavity is parallel-fibered but after this LAG, tissue becomes more vascularized and woven. After the second growth mark, fibers again become parallel and the tissue is less vascularized. After the third growth mark vascularity increases but fibers remain parallel.

The endosteal layer bounding the medullary cavity of MOR 2007-05RC4 (Fig. 2.14) is being resorbed and the entire cortex is of loosely parallel fibers. Vascularity is fairly consistent throughout the section, predominately simple longitudinal but with some simple radial anastomoses. Three annuli are observed within the cortex. There is also a

LAG very close to the periosteal surface on the left femur but cannot be traced completely. Due to the size of the medullary cavity with respect to the perimeter of a hatchling femur, it is likely that at least one growth mark was obliterated by medullary expansion.

As with MOR 2007-05RC4, the endosteal layer surrounding the medullary cavity of MOR 2007-05RC5 (Fig. 2.15) was in the process of being resorbed. There is also considerable medullary drift as a large amount of secondary tissue is present in the innermost cortex. A partial annulus is still visible near the medullary cavity. There are three more annuli within the cortex. Tissue is organized loosely in parallel and is vascularized largely by longitudinal vascular canals but there are also some radiating vascular canals as well. As with other elements of MOR 2007-05RC5, the third and fourth growth marks are very close together.

Tibia

There are three growth marks in MOR 2007-05RC3 (Fig. 2.16), although the innermost LAG is all but destroyed. The second growth mark is an annulus, and the third growth mark is an annulus followed by a LAG. The innermost cortex is woven and vascularized by both longitudinal and anastomosing vascular canals. After the second growth mark the mid-cortex becomes less vascularized and the fiber orientation becomes parallel. Although vascularity again increases in the outer cortex, fiber arrangement remains parallel and vascularity is largely simple.

Large resorption cavities with only a thin endosteal lining indicate recent resorption and medullary enlargement in MOR 2007-05RC4 (Fig. 2.17). Fibers are

organized in parallel throughout the cortex with evenly distributed longitudinal to somewhat radial simple vascular canals. Five annuli are present. The innermost annulus is almost fully destroyed and cannot be traced. The second annulus is also partially destroyed by medullary expansion, but enough is present that the perimeter can be reasonably estimated. The fifth annulus is very close to the periosteal surface and sometimes appears to merge with it.

Four annuli are visible in MOR 2007-05RC5 (Fig. 2.18), the innermost being very close to the medullary cavity. Tissue is woven from the inner cortex to the second annulus in some areas. Growth marks 3 and 4 are closer to each other than either is to another annulus. Tissue arrangement changes to loosely parallel after the second annulus. Vascular density is consistent throughout the cortex and is a combination of longitudinal and anastomosing vascular canals.

Fibula

In MOR 2007-05RC3 (Fig. 2.19), three LAGs are observed within the cortex. The left fibula also has a fourth LAG very close to the periosteal surface, but no fourth external growth mark is visible in the right fibula. The innermost cortex prior to the first LAG is avascular, consisting of lamellar tissue. Between the first and second LAG tissue becomes woven in areas with longitudinal vascular canals but becomes lamellar again from the mid-cortex to the surface. Periosteal attachment fibers are densely scattered throughout the section.

The cortex of MOR 2007-05RC4 (Fig. 2.20) is sparsely vascularized by longitudinal simple vascular canals. Tissue consists of highly organized, lamellar fibers

throughout. Five LAGs are visible, but the innermost is not fully traceable. The outermost LAG is very close to the periosteal surface and often merges with it. Periosteal attachment fibers are frequent.

The cortex of MOR 2007-05RC5 (Fig. 2.21) consists mostly of highly organized lamellar fibers and some parallel-fibered tissue, and contains sparse simple longitudinal vascular canals. Four LAGs are visible, including a closely spaced third and fourth LAG.

Coracoid

In MOR 2007-05RC3 (Fig. 2.22), there are struts of secondary bone within the medullary cavity, which is enclosed by an endosteal layer. The entire cortex is lamellar with scattered longitudinal vascular canals. Two annuli are visible and it is very likely an inner growth mark was destroyed by medullary expansion due to the size of the medullary cavity.

As with MOR 2007-05RC3, the medullary cavity of MOR 2007-05RC4 (Fig. 2.23) is lined by endosteal tissue and there are struts of remodeled bone within it. The lamellar cortex contains simple longitudinal and radial vascular canals. Four annuli are visible, although the innermost is partially destroyed by medullary expansion. The fourth annulus is faint in areas.

The medullary cavity of MOR 2007-05RC5 (Fig. 2.24) is also lined by a thin endosteal layer and there are struts of remodeled bone within the cavity. There are scattered resorption cavities in the innermost cortex. Tissue ranges from lamellar to loosely parallel-fibered. Vascularity is longitudinal. Four LAGs are clearly visible, but

the innermost is nearly destroyed. Two LAGs in the mid-cortex are unusually close to each other, as in other elements of MOR 2007-05RC5.

Scapula

The left and right scapulae of MOR 2007-05RC3 were not sectioned at approximately the same location, so the overall shape of the cross sections are different. Despite this, the same number of growth marks is found in both scapulae. A partial LAG remains near the medullary cavity and two annuli are observed within the mid-cortex. The entire cortex is made of predominately avascular lamellar tissue, although nearer the medullary cavity in places vascularity consists of longitudinal canals (Fig. 2.25).

Considerable medullary drift is evident in MOR 2007-05RC4 (Fig. 2.26) due to the large amount of secondary tissue and resorption cavities in the innermost cortex. The cortex ranges from lamellar to loosely parallel-fibered. As with MOR 2007-05RC3, the right and left scapulae were not sectioned at approximately the same region, resulting in different shapes for cross sections. There are five annuli followed by LAGs in both scapulae, but the innermost growth mark is almost destroyed by medullary expansion. The fifth LAG is so close to the periosteal surface that it often merges with it. Vascularity is by simple longitudinal and radial canals.

There is substantial secondary remodeling and medullary drift within the cortex of MOR 2007-05RC5 (Fig. 2.27). The cortex is lamellar to loosely parallel-fibered with simple longitudinal vascular canals. Four annuli followed by LAGs are present. The inner two growth marks are partially destroyed by medullary drift. The third and fourth LAGs are fairly closely spaced.

Osteoderms

For each alligator it was very difficult to count growth marks within the osteoderms, and impossible to fully trace their perimeters (Figs. 28-30). As Tucker (1997) observed, the best location for counting growth marks was on the ventral surface, where remodeling was reduced. The smallest osteoderms are from MOR 2007-05RC3 (Fig. 2.28), and they are the most symmetrical in transverse section. In general, tissue within osteoderms is woven, but more parallel-fibered near the exterior. They are richly vascularized, and vascularity is highest in the center and in patches near the surface. Approaching the keel, bone density decreases and the osteoderm becomes more porous. Periosteal attachment fibers are frequent and often dense throughout the base of the osteoderm and may obscure some growth marks, accounting for the discrepancies.

Discussion

General Bone Tissue Observations

As reported in other studies of both fossil and recent bone, tissue organization and growth mark number vary between skeletal elements and between alligator individuals (Chinsamy, 1993; Horner et al., 1999; Castanet et al., 2000; Horner et al., 2000; Margerie, 2002). However, tissue organization between homologous elements within a single alligator does not vary greatly. Interestingly, regions of woven tissue were found within the humerus, ulna, femur, tibia, and fibula of wild-captured MOR 2007-05RC3, but only during the second year of life. Elements of MOR 2007-05RC4 were completely lacking in woven tissue, and only the tibia of MOR 2007-05RC5 had a region of woven

tissue- also formed during the second year of its life. It is unusual that a wild alligator, living in sub-optimal conditions, would produce woven tissue in more elements than either MOR 2007-05RC4 or MOR 2007-05RC5, which were raised in captivity. These differences in tissue organization spanning both captive and wild animals suggest individual variation in growth unrelated to seasonal stresses and should further dissuade researchers from making generalizations about the growth of an extinct taxon based on only a few samples.

The woven-fibered regions within elements of MOR 2007-05RC3 and MOR 2007-05RC5 not only contain simple vascular canals, but also vessels enclosed by a nearly acellular, lamellated parallel-fibered tissue resembling poorly developed primary osteons (Fig. 2.31). The combination of woven-fibered tissue and primary osteons, which has been previously reported in crocodylians (e.g, Ricqlès, 1983; Reid, 1984, 1990; Horner et al., 2001; Ricqlès et al., 2001; Tumarkin-Deratzian, 2007), is commonly described as “fibrolamellar”. However, in this study the areas of woven tissue are patchy or discontinuous, and simple vascular canals are also scattered amongst the primary osteons. Simple vascular canals are rarely, if ever, reported in well developed “fibrolamellar” tissue. Also, primary osteons found within “fibrolamellar” tissue of many non-avian dinosaurs (Fig. 2.31F) and extant endotherms are relatively large structures made of multiple lamellae, often encompassing more than one vascular canal, and contain numerous osteocytes (Padian and Horner, 2004). This contrasts with the small size of the alligator primary osteons, with few lamellae and infrequent osteocytes. Perhaps these structures in the alligators are simply poorly developed primary osteons comprising a

kind of “pseudo-fibrolamellar” tissue resulting from temporary, unsustained rapid growth in these individuals.

Regardless, it is important to distinguish characteristics of tissue associated with temporary rapid growth in alligators from those associated with sustained rapid growth such as that found in extant endotherms. Unfortunately, use of the term “fibrolamellar” has become quite subjective in recent years as authors infrequently define or provide images to explain their intended meaning. Thus, as done here, and for future histological descriptions, it is probably best to refrain from subjective terms such as “fibrolamellar” in favor of simply describing tissue organization and structures present.

Although tissue organization and growth mark counts varied amongst elements, the presence of periosteal attachment fibers was prevalent in every specimen. The fibers were especially dense near growth marks. Because growth marks indicate a period of slowed or arrested growth, the periosteum as well as tendon insertions were not moving away from the mineralization front for a prolonged time period. Since periosteal attachment fibers anchor the periosteum and tendons to the bone matrix (Hall, 2005), it follows that there would be a concentration of these fibers if the periosteum was periodically stationary. Therefore, locating these dense perpendicular bands of periosteal attachment fibers may aid in identifying vague or faint growth marks both in extant or extinct animals.

Growth Marks

Assuming annual growth mark deposition, MOR 2007-05RC4 and MOR 2007-05RC5 were entering their sixth year of life and MOR 2007-05RC3 was one year

younger. Comparing skeletal apposition rates, annual cortical thickness, and annual cortical area from the three alligators shows the growth rate of wild-captured MOR 2007-05RC3 was in general lower than either captive raised MOR 2007-05RC4 or MOR 2007-05RC5. This is unsurprising, as captive alligators grow more rapidly than their wild counterparts, often attaining twice the length of a wild alligator of the same age in a single year (Ricqlès, 1983).

Although the spacing of the third and fourth growth marks within elements of MOR 2007-05RC5 is unusual, this is not considered an instance of a single hiatus represented by a “double LAG” (*sensu* Snover and Hohn, 2004). Because both MOR 2007-05RC4 and MOR 2007-05RC5 were captive animals with similar tissue organization in their elements, it is unlikely MOR 2007-05RC5 would be a year younger and still slightly larger than MOR 2007-05RC4. Additionally, the tissue within the narrow zone is no different from the tissue organization of adjacent zones, indicating no significant decrease in apposition occurred that year (Fig. 2.32). This relatively small zone may instead indicate a particularly long pause before growth resumed.

Generally, the kind of growth mark present (i.e., LAG, annulus, or annulus followed by a LAG) seems to vary individually and by year rather than correspond to a particular element, tissue arrangement, or even captivity status. Despite variation in the kinds of growth marks present within the cortex, the number of growth marks observed was consistent across multiple samples taken from the diaphysis of an element. However, variation in growth mark number was observed between different elements in each individual. Interestingly, elements from each alligator displayed a LAG very close to, and

often merging with, the periosteal surface while other elements lacked this extra mark. This particular discrepancy likely has a simple explanation. Alligators in Louisiana annually enter a state of greatly decreased activity and appetite beginning in October and ending in March regardless of captivity status (Coulson et al., 1973; Chabreck and Joanen, 1979; Joanen and McNease, 1987). Individuals obtained for this study were terminated in February. Therefore, it is possible that as the alligators began to emerge from their torpid state some elements resumed apposition before others, making an extra LAG visible due to the small addition of new tissue (Fig. 2.33).

Any other growth mark count discrepancies within the cortex can be attributed to medullary expansion. To determine if enough expansion occurred to fully destroy the oldest growth marks in an element, perimeters of hatchling alligator thin sections were compared with the medullary perimeters of homologous elements (Table 2.1). It was only in instances where the medullary cavity perimeter was a great deal larger than the hatchling cortical perimeter that fewer growth marks were counted.

The hypothesis that each skeletal element possesses the same number of growth marks prior to medullary expansion was independently tested using the bone microstructure of MOR 2007-05RC5. As previously described, in every element a narrow zone could be identified between two thicker zones (Fig. 2.32). This narrow zone provided a natural “label” so that even if medullary expansion had obliterated inner growth marks the number missing could be determined using this narrow zone as a landmark and complete growth records from other elements as guides. Therefore, MOR 2007-05RC5 provides strong additional evidence that it is only the action of medullary

expansion causing growth mark counts to vary within the cortex, and it is not because some elements form marks yearly while others do not.

One final source of growth mark count variability must be mentioned. Because thin section processing was done by hand, sometimes one thin section out of several made from an element was polished thinner than the others. It was only in sections polished too thin that growth marks became vague and difficult to trace, seeming to disappear into the surrounding bone fabric. When this occurred, often slides stained with Toluidine blue would aid in locating the faint marks, which did not take up the stain as readily. These results suggest that if medullary expansion is not evident, inconsistent growth mark counts within the diaphyses of long bones from an individual could be due to errors in procedure.

Statistical Analyses and Growth Curves

Because of the immature nature of the alligator specimens, the number of paired measurements (i.e., growth mark and surface perimeters) included in the Student's *t*-test of each skeletal element was fairly low (the largest sample set was $n = 6$), making the chance for statistical errors (both Type I and Type II) quite high. However, out of twenty paired elements tested only the tibia and ulna from MOR 2007-05RC5 rejected the null hypothesis of no difference between growth mark and cortical perimeters (Table 2.2). Because eighteen of the twenty pairs statistically demonstrated a result of no difference, it is highly possible that rejection of the null hypothesis for the tibia and ulna of MOR 2007-05RC5 is the result of a Type II error.

In addition to a *t*-test, the growth mark and surface perimeter measurements for left and right homologous elements from each alligator were plotted against each other to both statistically and visually assess any relationship between the measurements. The result is striking (Fig. 2.1), and these analyses ($R^2=0.984$) provide strong evidence that there is no appreciable variation in growth mark perimeters or bone surface perimeters between homologous elements in an individual if sampled from roughly the same location. Applied to paleohistology, the implication is that one can safely expect the same quantitative results regardless of which homologous element is chosen from a single individual.

Growth data were successfully collected from every element examined except osteoderms. Although Tucker (1997) achieved success by using osteoderms for skeletochronology in older, larger, and less actively growing crocodylians, they were poor indicators of age in the present study because the disorganization of the tissue obscured growth marks. This was likely due in part to the immature status of the individuals. In addition, the histology demonstrates the highly porous and richly vascularized nature of osteoderms, which further supports the hypothesis that they serve functions in addition to armored defense (e.g. Frey, 1988; Farlow et al., 2010).

From the appendicular elements sampled, average daily apposition rates were determined for each individual (Table 2.3) assuming a 214 day growing season (Joanen and McNease, 1987). In addition, annual cortical thicknesses as well as annual cortical areas are plotted in Figure 2.2 and Figure 2.3, respectively. As previously discussed, some elements showed an extra growth mark in the outer cortex. Because the zone

between the extra mark and the periosteal surface represents less than a year of growth (likely a month or two at the most), the measurements of these extra zones are omitted from the graphs and tables. For those elements, corresponding measurements representing the thickness between the next-to-last growth mark to the surface will therefore be slightly overestimated. Unfortunately, only data from the third and fourth years could be compared across all three individuals.

Regarding apposition, the femur, humerus, and tibia achieved the highest annual rates within each individual. The highest apposition rate recorded was $6.8 \mu\text{m/day}$, from the femur of MOR 2007-05RC5 during its first year of life. There was however variability in rates both within and across individuals. Apposition rates for all elements of wild-caught MOR 2007-05RC3 at the end of the third year of growth were lower than those for either captive alligator. This is unsurprising as captive alligators often grow faster than wild ones. However, at the end of the fourth year, several elements of MOR 2007-05RC3 had higher apposition rates than corresponding elements of MOR 2007-05RC5. This is likely due to the unusually long growth hiatus experienced by MOR 2007-05RC5 during the fourth year, as this would artificially decrease the apposition rate.

In each alligator, at least one element preserved every growth mark so that growth could be tracked from the end of the first year until termination. In these elements apposition rates appear to be highest during the first year of growth and generally did not return to those rates during the time interval recorded in this study. The exception to this observation is the radius of MOR 2007-05RC3, which had its highest apposition rate during its second year of life. This was the same period that woven tissue was present in

many elements of MOR 2007-05RC3, although not in the radius. Unfortunately, medullary expansion prevented the estimation of the first growth mark perimeters in the other elements of MOR 2007-05RC3 so no comparison of apposition rates for the first or second year of life could be made.

Despite the general trend of femur, humerus, and tibia having the highest annual apposition rates amongst all sampled elements in each alligator, annual average cortical thicknesses for all homologous elements varied considerably across alligators and from year to year. For example, during the fourth year of growth, cortical thickness for all elements increased in MOR 2007-05RC3. However, cortical thickness decreased from the fourth to fifth years of growth in nearly every element of MOR 2007-05RC4. And in MOR 2007-05RC5, there are virtually yearly fluctuations in the amount of cortex added. These apparent fluctuations in MOR 2007-05RC5 may be due in part to the prolonged growth hiatus during the fourth year of life. As cortical thickness (as well as apposition rates) for all elements of MOR 2007-05RC5 during the fifth year are nearly double those of the previous year, it is possible this individual was rebounding from its period of delayed growth.

Trends in annual average cortical area often mirrored those observed in annual average cortical thickness measurements. For example, elements with high annual average cortical thicknesses also had higher annual average cortical areas. However, this relationship does not always hold true. Although cortical thickness decreased in every element except the ulna during the fifth year of growth in MOR 2007-05RC4, cortical area was still higher than the previous year for every element except the radius. This is

because elements with larger perimeters (e.g., femur, humerus, tibia) require only a small addition of cortex to increase in cortical area while elements with smaller perimeters, such as the radius, require a large addition to cortical thickness to greatly increase cortical area.

These quantitative data are supported by bone fiber organization. The femur, tibia, and humerus had the highest apposition rates and were the most vascularized with either parallel-fibered tissue or a combination of parallel-fibered and woven tissue. The radius and fibula had the lowest yearly apposition rates in each individual, correlating with reduced vascularity and highly organized lamellar or parallel-fibered tissue. Therefore, to determine the maximum growth rates of alligators based on osteohistology, the femur, tibia, and humerus are most useful. However, these elements did tend to have considerable medullary expansion, making them less desirable for skeletochronology. On the other hand, comparatively less medullary expansion and drift resulted in a consistently high number of complete growth marks in the radius, likely making it preferable for alligator skeletochronology studies. The ulna, scapula, and coracoid also resembled the radius in tissue organization, but there was considerable medullary expansion and drift which often obscured or obliterated inner growth marks, making these elements less desirable for any osteohistological analyses. However, as shown by MOR 2007-05RC5, it is not impossible for all elements to retain inner growth marks for the same amount of time, suggesting rates of medullary expansion also vary considerably between homologous elements in different individuals. Ultimately, the variability in annual apposition and cortical area in homologous elements across individuals

demonstrates that a sample size of three young alligators is not appropriate for drawing solid conclusions concerning general skeletal element growth rates in this taxon.

Additionally, comparing the curves of linear cortical thicknesses (Fig. 2.2) with curves of two-dimensional cortical areas (Fig. 2.3) highlights potential sources of error in growth curves of extinct taxa. Ontogenetic mass estimates, often used to reconstruct the growth history of a dinosaur taxon, are frequently extrapolated from equations based on bone length or circumference (Anderson et al., 1985; Erickson and Tumanova, 2000). The data here illustrate that small changes (or errors) in linear measurements can lead to considerable changes (or errors) in two and three dimensions. For example, annual cortical apposition may become virtually negligible as growth in length of the element ceases and skeletal maturity is attained, resulting in a curve of cortical thickness that plateaus. However, even an incremental thickening of the cortex after attainment of skeletal maturity would result in a noticeable mass increase in a large animal. Despite the increased risk of cubing error when extrapolating mass from a linear measurement, mass curves provide the greatest amount of ontogenetic information. Thus, to account for both possible error and the three-dimensional nature of a taxon it is best to display not only mass curves but also the linear curves from which the mass estimates are obtained.

Conclusions

This report quantifies alligator intraskeletal growth rates and tissue variability by using the largest crocodylian osteohistological sample size to date. It also represents the most extensive comparative intraskeletal study on zonal bone tissue variability within

extant archosaurs. Thin section analyses confirm that growth mark count, bone tissue organization, and growth rates vary between different elements as well as between individual alligators, regardless of captivity status. Although growth mark count varied intraskeletally, there is good evidence this inconsistency is due to the action of medullary expansion rather than because some elements fail to form growth marks every year. Only in elements with medullary cavities considerably larger than the perimeters of homologous hatchling elements was growth mark count reduced.

Generally, it appears that the femur, tibia, and humerus have the highest growth rates, and would therefore be the preferred elements to examine for growth studies. Pectoral girdle elements appear to be the least desirable for growth studies due to their considerable medullary drift. Skeletochronological studies would likely benefit most from analyzing the radius, as medullary expansion generally did not affect the innermost growth marks as quickly in this element.

However, it remains to be seen if these observations hold true for alligators in general and also for other tetrapods. In other words, this study reveals intraskeletal consistency in growth mark counts and homologous cortical perimeters, but also demonstrates that to compare intraskeletal trends across individuals, more than three specimens are required before any meaningful conclusions can be made. Additionally, the individuals in this study were very young, so the number of years available to sample was a constraint. Despite these shortcomings, inter- and intraskeletal results strongly support the observations of Castanet et al. (2000): enough individual intraskeletal variability is present within extant archosaurs that any interpretations of dinosaur

physiology based on small sample sizes should be considered cautiously. If so much skeletal element variation in growth is observed between three extant alligators- two of which were captive compatriots- this calls into question any generalized conclusions regarding the growth of extinct taxa drawn from equivalent or smaller sample sizes.

Acknowledgements

We thank R. Elsey (Rockefeller Wildlife Refuge, Grand Chenier, LA) and the Louisiana Department of Wildlife and Fisheries for providing specimens for analysis and E.-T. Lamm (Museum of the Rockies) for assisting with thin section preparation. The Museum of the Rockies provided funding as well as facilities for histological analyses and microphotograph digital imaging equipment. Thanks to T. Ballard and D. Fowler for helpful discussions on earlier manuscript drafts.

Table 2.1. Data collected for the three alligator individuals included in this study. An asterisk appears next to measurements in which the outermost growth mark was omitted from measurements. MOR: Museum of the Rockies, Montana, USA.

Specimen Number	Element	Location	Left Cortical Area (mm ²)	Right Cortical Area (mm ²)	Left Perimeter (mm ²)	Right Perimeter (mm ²)	Average Cortical Thickness of Left Element (mm)
MOR 2007-05RC 3	Coracoid	Medullary Cavity	4.79	4.45	14.95	9.90	
		Hatchling	1.24	1.24	4.81	4.81	
		Mark 1	Not Observed	Not Observed	Not Observed	Not Observed	
		Mark 2	8.75	8.06	13.41	12.20	
		Mark 3	10.69	10.58	13.62	13.60	
		Mark 4	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	13.93	13.09	15.63	15.08	
	Fibula	Medullary Cavity	0.06	0.72	2.99	3.62	
		Hatchling	0.43	0.43	3.55	3.55	0.37
		Mark 1	Not Fully Traceable	Not Observed	Not Fully Traceable	Not Observed	
		Mark 2	Not Fully Traceable	Not Observed	Not Fully Traceable	Not Observed	1.05
		Mark 3	4.24	4.29	7.72	7.85	1.17
		Mark 4	Not Fully Traceable	Not Observed	Not Fully Traceable	Not Observed	
		Surface	5.27	5.40	8.63	8.81	1.30*
	Femur	Medullary Cavity	2.55	3.40	6.08	7.01	
		Hatchling	1.61	1.61	6.74	6.74	0.71
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	15.12	15.12	14.82	14.77	2.20
		Mark 3	18.52	18.34	16.45	16.29	2.43
		Mark 4	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	24.70	24.42	18.97	18.81	2.81
	Humerus	Medullary Cavity	2.06	1.91	5.41	5.22	
		Hatchling	1.02	1.02	4.06	4.06	0.57
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 3	12.90	12.94	13.47	13.51	2.01
		Mark 4	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	17.31	17.67	15.61	15.76	2.35
	Radius	Medullary Cavity	0.71	0.67	3.17	3.10	
		Hatchling	0.42	0.42	3.42	3.42	0.37

Table 2.1 continued.

		Mark 1	1.21	1.12	4.13	4.00	0.61
		Mark 2	3.21	3.16	6.76	6.82	0.99
		Mark 3	4.22	4.14	7.78	7.79	1.14
		Mark 4	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	5.47	5.45	8.86	8.98	1.30
	Scapula	Medullary Cavity	1.85	4.08	8.70	9.46	
		Hatchling	0.70	0.70	5.73	5.73	
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	5.56	Not Fully	12.17	Not Fully	
		Mark 3	7.40	Not Fully	13.70	Not Fully	
		Mark 4	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	10.04	16.33	15.33	16.55	
	Tibia	Medullary Cavity	2.41	2.07	5.89	5.54	
		Hatchling	0.96	0.96	3.95	3.95	0.55
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	10.10	9.92	11.99	11.90	1.76
		Mark 3	12.45	12.32	13.32	13.20	1.96
		Mark 4	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	16.13	16.08	15.11	15.10	2.24
	Ulna	Medullary Cavity	0.85	0.99	3.63	3.96	
		Hatchling	0.39	0.39	2.61	2.61	0.36
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	3.88	3.91	7.62	7.61	1.12
		Mark 3	5.28	5.35	8.89	8.91	1.31
		Mark 4	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	7.22	7.33	10.41	10.49	1.52
MOR 2007-05RC4	Coracoid	Medullary Cavity	5.41	5.42	15.61	16.67	
		Hatchling	1.24	1.24	4.81	4.81	
		Mark 1	5.29	Not Fully	11.27	Not Fully	
		Mark 2	9.52	9.91	13.37	13.35	
		Mark 3	15.08	15.85	16.07	16.18	
		Mark 4	18.38	19.52	17.58	17.94	
		Mark 5	Not Fully Traceable	Not Observed	Not Fully Traceable	Not Observed	
		Surface	22.40	23.94	19.26	19.73	
	Fibula	Medullary Cavity	0.68	0.74	3.12	3.28	
		Hatchling	0.43	0.43	3.55	3.55	0.37
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	

Table 2.1 continued.

		Mark 2	3.51	3.84	7.03	7.37	1.04
		Mark 3	5.60	5.67	8.91	8.96	1.33
		Mark 4	8.21	8.16	10.83	10.81	1.61
		Mark 5	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Surface	11.06	11.02	12.57	12.63	1.86*
	Femur	Medullary Cavity	6.47	5.03	12.05	11.43	
		Hatchling	1.61	1.61	6.74	6.74	0.71
		Mark 1	Not Observed	Not Observed	Not Observed	Not Observed	
		Mark 2	11.58	13.68	12.87	14.06	1.93
		Mark 3	22.54	24.65	17.99	18.78	2.71
		Mark 4	35.20	36.23	22.56	22.75	3.39
		Mark 5	Not Fully Traceable	Not Observed	Not Fully Traceable	Not Observed	
		Surface	47.93	48.57	26.22	26.34	3.89*
	Humerus	Medullary Cavity	3.47	3.01	7.35	6.92	
		Hatchling	1.02	1.02	4.06	4.06	0.57
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	7.48	7.13	10.31	10.07	1.53
		Mark 3	14.44	14.42	14.29	14.26	2.14
		Mark 4	23.12	23.44	18.09	18.26	2.70
		Mark 5	31.25	Not Observed	21.01	Not Observed	3.14
		Surface	32.53	32.86	21.44	21.55	3.2*
	Radius	Medullary Cavity	0.51	N/A	2.82	N/A	
		Hatchling	0.42	N/A	3.42	N/A	0.37
		Mark 1	3.07	N/A	6.67	N/A	0.96
		Mark 2	4.95	N/A	8.48	N/A	1.26
		Mark 3	7.22	N/A	10.20	N/A	1.52
		Mark 4	10.12	N/A	12.02	N/A	1.78
		Mark 5	Not Observed	N/A	Not Observed	N/A	
		Surface	10.91	N/A	12.49	N/A	1.84
	Scapula	Medullary Cavity	2.26	5.73	12.03	16.12	
		Hatchling	0.70	0.70	5.73	5.73	
		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	4.30	Not Fully	14.01	Not Fully	
		Mark 3	8.35	Not Fully	16.79	Not Fully	
		Mark 4	13.49	14.89	19.58	17.88	
		Mark 5	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Surface	20.51	22.31	22.10	21.05	
	Tibia	Medullary Cavity	3.90	4.58	8.39	8.85	
		Hatchling	0.96	0.96	3.95	3.95	0.55

Table 2.1 continued.

		Mark 1	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Mark 2	7.98	7.56	10.87	10.47	1.63
		Mark 3	14.78	14.70	14.56	14.47	2.22
		Mark 4	22.28	22.72	17.81	17.98	2.70
		Mark 5	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	Not Fully Traceable	
		Surface	30.24	30.58	20.73	20.86	3.15*
	Ulna	Medullary Cavity	1.11	1.11	4.52	4.32	
		Hatchling	0.39	0.39	2.61	2.61	0.36
		Mark 1	Not Observed	Not Observed	Not Observed	Not Observed	
		Mark 2	Not Observed	Not Observed	Not Observed	Not Observed	
		Mark 3	6.26	6.06	9.74	9.53	1.43
		Mark 4	9.45	9.30	11.91	11.90	1.76
		Mark 5	12.90	12.91	13.94	14.06	2.05
		Surface	14.05	14.05	14.51	14.59	2.14*
MOR 2007-05RC5	Coracoid	Medullary Cavity	N/A	6.90	N/A	16.27	
		Hatchling	N/A	1.24	N/A	4.81	
		Mark 1	N/A	Not Fully	N/A	Not Fully	
		Mark 2	N/A	6.01	N/A	11.67	
		Mark 3	N/A	15.33	N/A	15.97	
		Mark 4	N/A	17.47	N/A	16.88	
		Mark 5	N/A	Not Observed	N/A	Not Observed	
		Surface	N/A	28.93	N/A	21.50	
	Fibula	Medullary Cavity	1.43	1.31	4.67	4.41	
		Hatchling	0.43	0.43	3.55	3.55	0.37
		Mark 1	3.48	3.87	7.08	7.45	1.04
		Mark 2	5.23	5.50	8.66	8.88	1.29
		Mark 3	7.89	8.00	10.62	10.71	1.60
		Mark 4	9.22	9.43	11.56	11.69	1.73
		Mark 5	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	14.31	14.27	14.47	14.37	2.17
	Femur	Medullary Cavity	5.56	4.47	9.10	8.50	
		Hatchling	1.61	1.61	6.74	6.74	0.71
		Mark 1	15.18	12.46	15.20	13.60	2.16
		Mark 2	21.45	19.82	17.73	16.86	2.57
		Mark 3	31.63	31.49	21.30	21.21	3.11
		Mark 4	37.36	38.03	23.13	23.26	3.39
		Mark 5	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	56.97	59.47	28.50	29.11	4.16
	Humerus	Medullary Cavity	3.23	2.09	7.33	5.55	
		Hatchling	1.02	1.02	4.06	4.06	0.57

Table 2.1 continued.

		Mark 1	6.78	6.78	9.91	9.78	1.46
		Mark 2	12.67	12.48	13.37	13.28	2.02
		Mark 3	21.07	21.27	17.25	17.36	2.61
		Mark 4	25.05	24.68	18.82	18.69	2.83
		Mark 5	Not Observed	Not Observed	Not Observed	Not Observed	
	Radius	Surface	41.56	41.02	24.25	24.04	3.65
		Medullary Cavity	0.68	0.42	3.28	2.54	
		Hatchling	0.42	0.42	3.42	3.42	0.37
		Mark 1	3.26	2.50	6.92	5.97	1.04
		Mark 2	4.72	4.49	8.30	8.03	1.25
		Mark 3	7.22	7.19	10.20	10.16	1.52
		Mark 4	8.68	8.71	11.18	11.17	1.66
		Mark 5	12.28	14.05	13.88	14.26	2.04
		Surface	13.78	15.65	14.10	14.99	2.09*
	Scapula	Medullary Cavity	N/A	1.06	N/A	11.40	
		Hatchling	N/A	0.70	N/A	5.73	
		Mark 1	N/A	5.50	N/A	16.59	
		Mark 2	N/A	7.66	N/A	17.89	
		Mark 3	N/A	12.43	N/A	21.52	
		Mark 4	N/A	15.39	N/A	22.58	
		Mark 5	N/A	Not Observed	N/A	Not Observed	
		Surface	N/A	26.25	N/A	26.87	
	Tibia	Medullary Cavity	4.22	3.98	7.83	7.63	
		Hatchling	0.96	0.96	3.95	3.95	0.55
		Mark 1	7.24	Not Fully	10.17	Not Fully	1.50
		Mark 2	13.16	13.60	13.71	13.99	2.06
		Mark 3	21.65	22.96	17.70	18.04	2.64
		Mark 4	25.24	27.10	18.94	19.58	2.85
		Mark 5	Not Observed	Not Observed	Not Observed	Not Observed	
		Surface	40.24	43.56	23.93	24.87	3.57
	Ulna	Medullary Cavity	2.47	2.00	6.44	5.71	
		Hatchling	0.39	0.39	2.61	2.61	0.36
		Mark 1	Not Observed	Not Observed	Not Observed	Not Observed	
		Mark 2	6.05	5.73	9.60	9.38	1.35
		Mark 3	9.50	9.05	11.83	11.62	1.74
		Mark 4	11.37	10.82	12.96	12.66	1.87
		Mark 5	18.53	17.57	16.46	16.04	2.45
		Surface	19.27	18.58	16.74	16.57	2.5*

Table 2.2. Results of Student's *t*-tests performed on growth mark and surface perimeter measurements of paired homologous elements. MOR: Museum of the Rockies, Montana, USA.

Specimen Number	Skeletal Element	Mean Difference Between Perimeters (mm)	Standard Deviation	Number of Paired Perimeters	P-Value	Confidence Level	Reject Null Hypothesis?
MOR 2007-05RC3	Coracoid	0.59	0.60	3	0.23	1.48	No
	Fibula	0.16	0.04	2	0.10	0.33	No
	Femur	0.16	0.00	2	0.01	0.03	No
	Humerus	0.10	0.08	2	0.34	0.75	No
	Radius	0.02	0.11	4	0.77	0.17	No
	Tibia	0.07	0.06	3	0.15	0.14	No
	Ulna	0.03	0.05	3	0.35	0.11	No
MOR 2007-05RC4	Coracoid	0.21	0.24	3	0.28	0.60	No
	Fibula	0.11	0.16	4	0.27	0.25	No
	Femur	0.57	0.51	4	0.11	0.81	No
	Humerus	0.00	0.18	4	0.99	0.29	No
	Scapula	1.38	0.46	2	0.15	4.11	No
	Tibia	0.05	0.26	4	0.74	0.42	No
	Ulna	0.00	0.15	4	0.99	0.23	No
MOR 2007-05RC5	Fibula	0.14	0.17	5	0.14	0.22	No
	Femur	0.36	0.88	5	0.41	1.09	No
	Humerus	0.09	0.12	5	0.17	0.15	No
	Radius	0.00	0.62	6	1.00	0.58	No
	Tibia	0.55	0.30	4	0.04	0.48	Yes
	Ulna	0.27	0.10	5	0.00	0.12	Yes

Table 2.3. Annual average skeletal apposition rates for the three alligator individuals included in this study. An asterisk appears next to measurements in which the outermost growth mark was omitted from measurements. MOR: Museum of the Rockies, Bozeman, Montana, USA.

Specimen Number	Element	Location	Average Cumulative Cortical Thickness (mm)	Average Annual Cortical Thickness (mm)	Average Apposition Rate ($\mu\text{m/day}$)
MOR 2007-05RC 3	Fibula	Hatchling	0.37	0.37	
		Mark 1			
		Mark 2	1.05		
		Mark 3	1.17	0.12	0.56
		Mark 4			
		Surface	1.30	0.13	0.61*
	Femur	Hatchling	0.71	0.71	
		Mark 1			
		Mark 2	2.20		
		Mark 3	2.43	0.23	1.05
		Mark 4			
		Surface	2.81	0.38	1.78
	Humerus	Hatchling	0.57	0.57	
		Mark 1			
		Mark 2			
		Mark 3	2.01		
		Mark 4			
		Surface	2.35	0.34	1.57
	Radius	Hatchling	0.37	0.36	
		Mark 1	0.61	0.25	1.14
		Mark 2	0.99	0.38	1.78
		Mark 3	1.14	0.15	0.68
		Mark 4			
		Surface	1.30	0.17	0.77
	Tibia	Hatchling	0.55	0.55	
		Mark 1			
		Mark 2	1.76		
		Mark 3	1.96	0.20	0.93
		Mark 4			
		Surface	2.24	0.28	1.31
	Ulna	Hatchling	0.36	0.36	
		Mark 1			
		Mark 2	1.12		
		Mark 3	1.31	0.20	0.91
		Mark 4			
		Surface	1.52	0.21	0.98
MOR 2007-05RC4	Fibula	Hatchling	0.37	0.37	
		Mark 1			
		Mark 2	1.04		
		Mark 3	1.33	0.29	1.36
		Mark 4	1.61	0.28	1.29
		Mark 5			
		Surface	1.86	0.26	1.19*
	Femur	Hatchling	0.71	0.71	
		Mark 1			
		Mark 2	1.93		
		Mark 3	2.71	0.79	3.67
		Mark 4	3.39	0.68	3.18

Table 2.3 continued.

	Humerus	Mark 5			
		Surface	3.89	0.50	2.34*
		Hatchling	0.57	0.57	
		Mark 1			
		Mark 2	1.53		
		Mark 3	2.14	0.61	2.85
		Mark 4	2.70	0.56	2.62
		Mark 5	3.14		
		Surface	3.20	0.50	2.36*
	Radius	Hatchling	0.37	0.36	
		Mark 1	0.96	0.59	2.76
		Mark 2	1.26	0.30	1.40
		Mark 3	1.52	0.26	1.21
		Mark 4	1.78	0.26	1.21
		Mark 5			
		Surface	1.84	0.06	0.30
	Tibia	Hatchling	0.55	0.55	
		Mark 1			
		Mark 2	1.63		
		Mark 3	2.22	0.59	2.76
		Mark 4	2.70	0.48	2.24
		Mark 5			
		Surface	3.15	0.45	2.10*
	Ulna	Hatchling	0.36	0.36	
		Mark 1			
		Mark 2			
		Mark 3	1.43		
		Mark 4	1.76	0.34	1.57
		Mark 5	2.05		
		Surface	2.14	0.38	1.78*
MOR 2007-05RC5	Fibula	Hatchling	0.37	0.37	
		Mark 1	1.04	0.66	3.10
		Mark 2	1.29	0.26	1.19
		Mark 3	1.60	0.31	1.46
		Mark 4	1.73	0.13	0.60
		Mark 5			
		Surface	2.17	0.44	2.04
	Femur	Hatchling	0.71	0.71	
		Mark 1	2.16	1.46	6.80
		Mark 2	2.57	0.41	1.93
		Mark 3	3.11	0.53	2.49
		Mark 4	3.39	0.28	1.31
		Mark 5			
		Surface	4.16	0.78	3.63
	Humerus	Hatchling	0.57	0.57	
		Mark 1	1.46	0.89	4.16
		Mark 2	2.02	0.56	2.59
		Mark 3	2.61	0.59	2.76
		Mark 4	2.83	0.23	1.05
		Mark 5			
		Surface	3.65	0.82	3.83
	Radius	Hatchling	0.37	0.36	

Table 2.3 continued.

		Mark 1	1.04	0.67	3.14
		Mark 2	1.25	0.21	0.97
		Mark 3	1.52	0.28	1.30
		Mark 4	1.66	0.14	0.65
		Mark 5	2.04		
		Surface	2.09	0.42	1.97*
	Tibia	Hatchling	0.55	0.55	
		Mark 1	1.50	0.95	4.44
		Mark 2	2.06	0.56	2.59
		Mark 3	2.64	0.58	2.71
		Mark 4	2.85	0.21	0.98
		Mark 5			
		Surface	3.57	0.72	3.36
	Ulna	Hatchling	0.36	0.36	
		Mark 1			
		Mark 2	1.35		
		Mark 3	1.74	0.39	1.81
		Mark 4	1.87	0.13	0.58
		Mark 5	2.45		
		Surface	2.50	0.63	2.96*

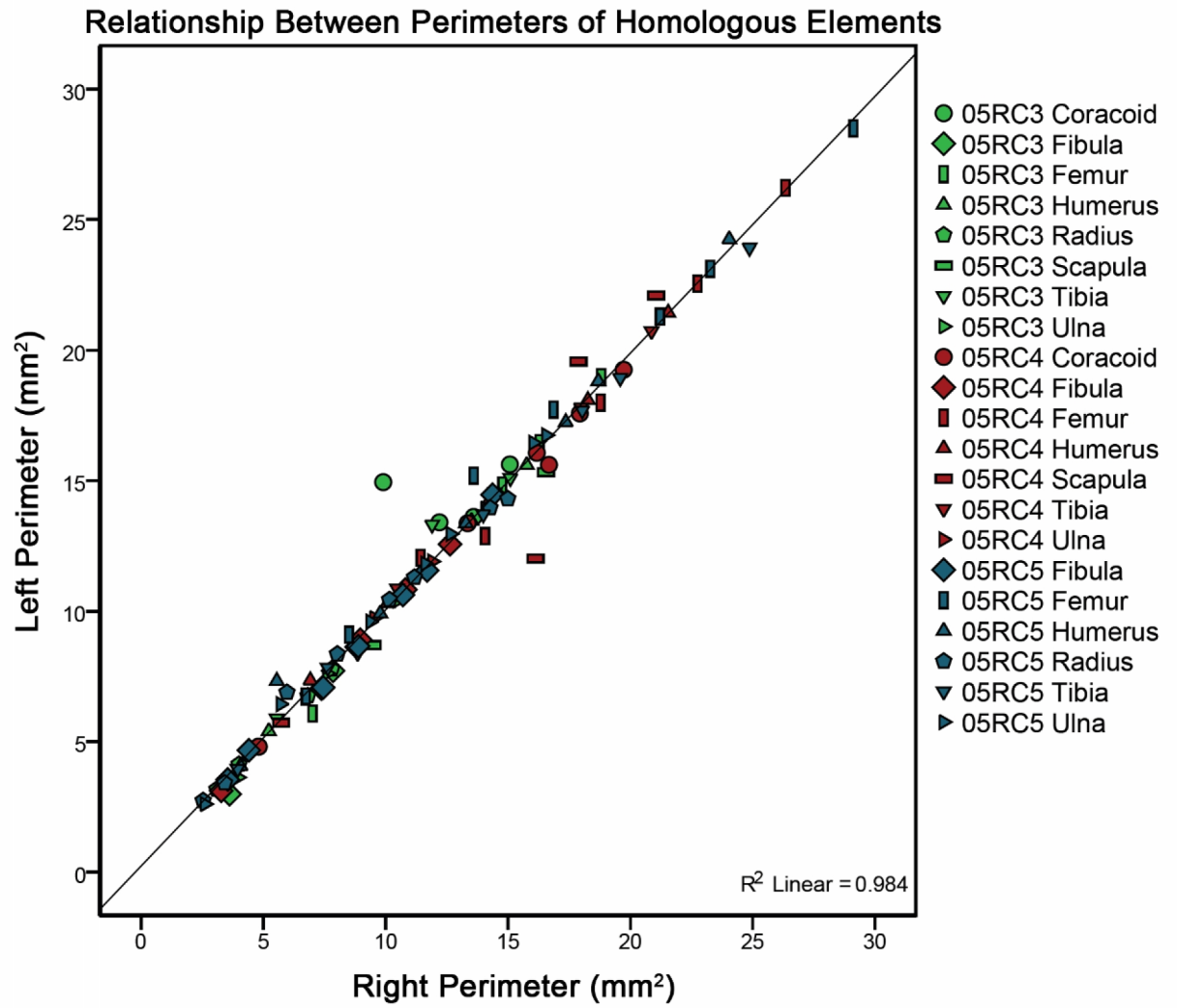


Figure 2.1. A plot comparing bone growth mark and surface perimeters of left and right homologous elements. The fitted linear regression with an R^2 value of 0.984 and a slope of 1 strongly suggests there is no significant difference between either growth mark or surface perimeters across left and right homologous elements from a single individual. Two potential outliers are observed (a scapula and a coracoid), which may be the result of dissimilar sampling locations between left and right elements.

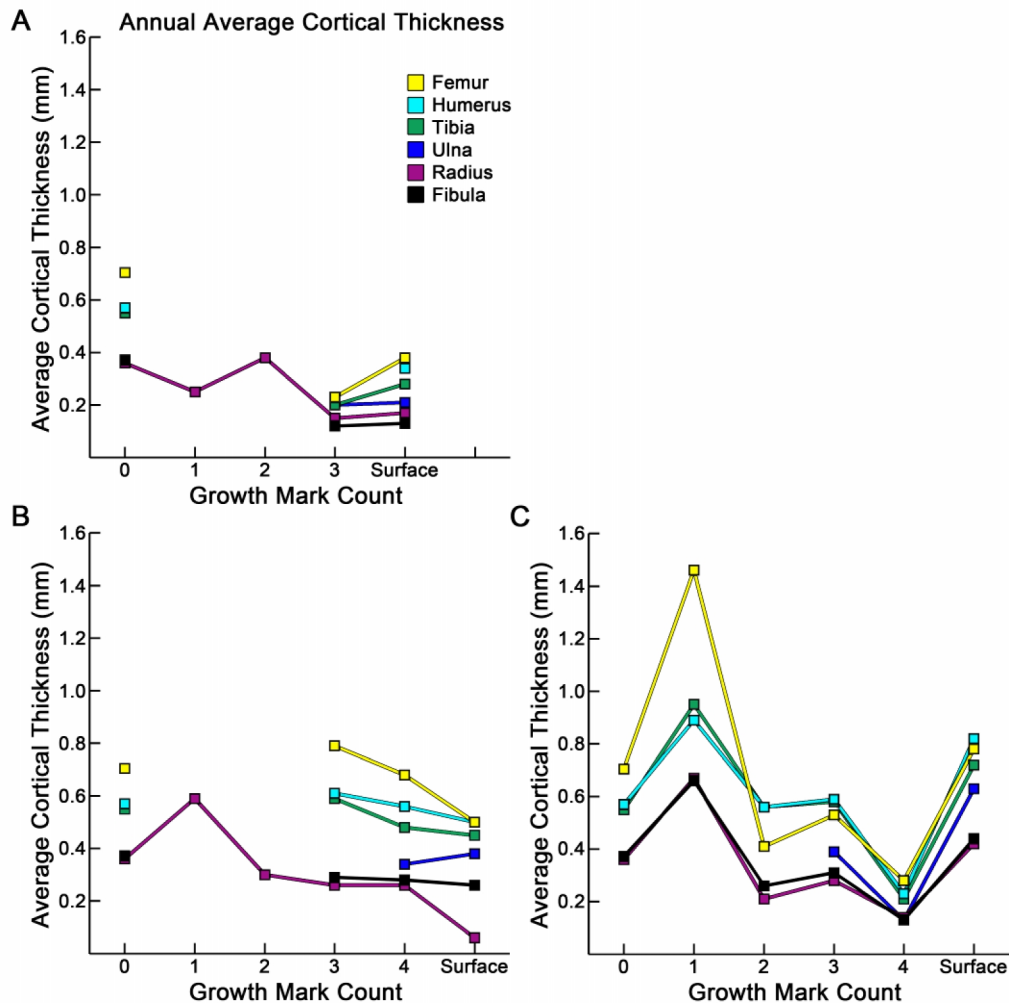


Figure 2.2. Intraskeletal annual average cortical thicknesses. The numbers in the axis “Growth Mark Count” correspond to the animal’s year of life. Growth Mark 0 indicates hatching, Growth Mark 1 the end of the first year of life, and so on. The average cortical thickness associated with each growth mark is the amount deposited during that year of life. Thus the average cortical thickness associated with Growth Mark 1 is that deposited in the first year of life, measured between Growth Marks 0 and 1. The annual addition of cortical area was in general greatest in the femur, humerus, and tibia of A) MOR 2007-05RC3, B) MOR 2007-05RC, and C) MOR 2007-05RC5. However, considerable variability exists with regards to how much cortical area was added yearly when comparing homologous elements, especially between B) MOR 2007-05RC and C) MOR 2007-05RC5, the two captive alligators. Lines with no connection to the origin indicate growth marks that were either wholly or partially obliterated by medullary cavity expansion and could not be accurately measured.

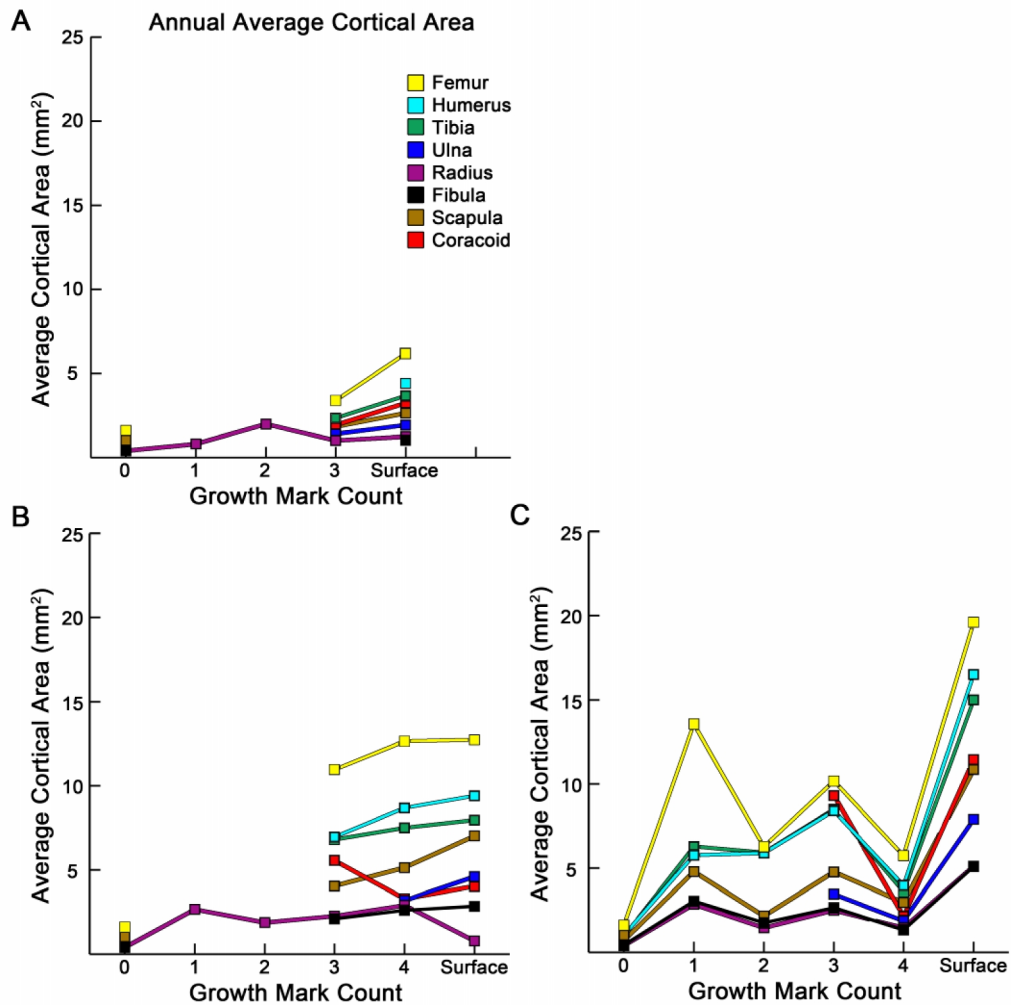


Figure 2.3. Intraskeletal annual average cortical area. Interpretation of growth mark counts and their associated annual bone deposition is as in Figure 2.2. In general, skeletal elements of A) MOR 2007-05RC3 grew at lower rates than either B) MOR 2007-05RC4 or C) MOR 2007-05RC5. However, the femur, tibia, and humerus consistently had the highest annual growth rates in each alligator. Lines with no connection to the origin indicate growth marks that were either wholly or partially obliterated by medullary cavity expansion and could not be accurately measured.

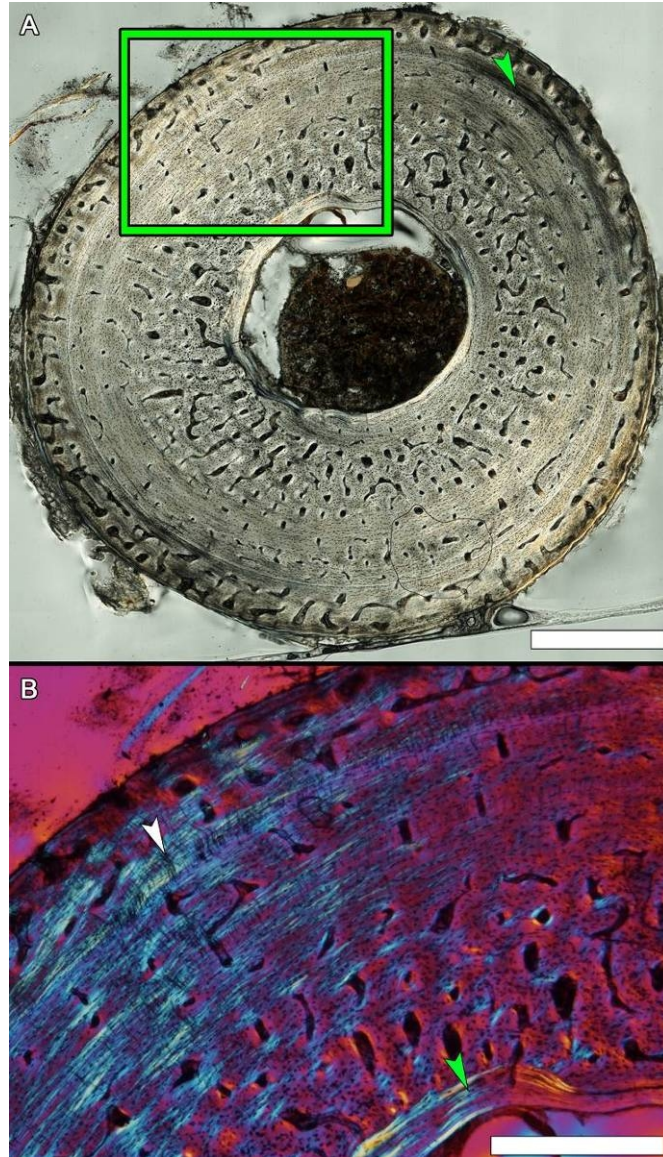


Figure 2.4. Osteohistology of the mid-diaphyseal humerus of alligator MOR 2007-05RC3. A) Transverse section. Vascularity decreases mid cortex but increases again in the outer cortex. Periosteal attachment fibers can be seen running parallel to a growth mark (arrow). Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. The endosteal layer (green arrow) cuts across the primary tissue of the inner cortex, which is well vascularized and woven. The mid-cortex becomes less well vascularized and is parallel-fibered, while the outer cortex is again well vascularized but remains parallel-fibered. Hair-like periosteal attachment fibers (white arrow) are arranged perpendicular to bone tissue orientation. Scale bar, 500 μ m.

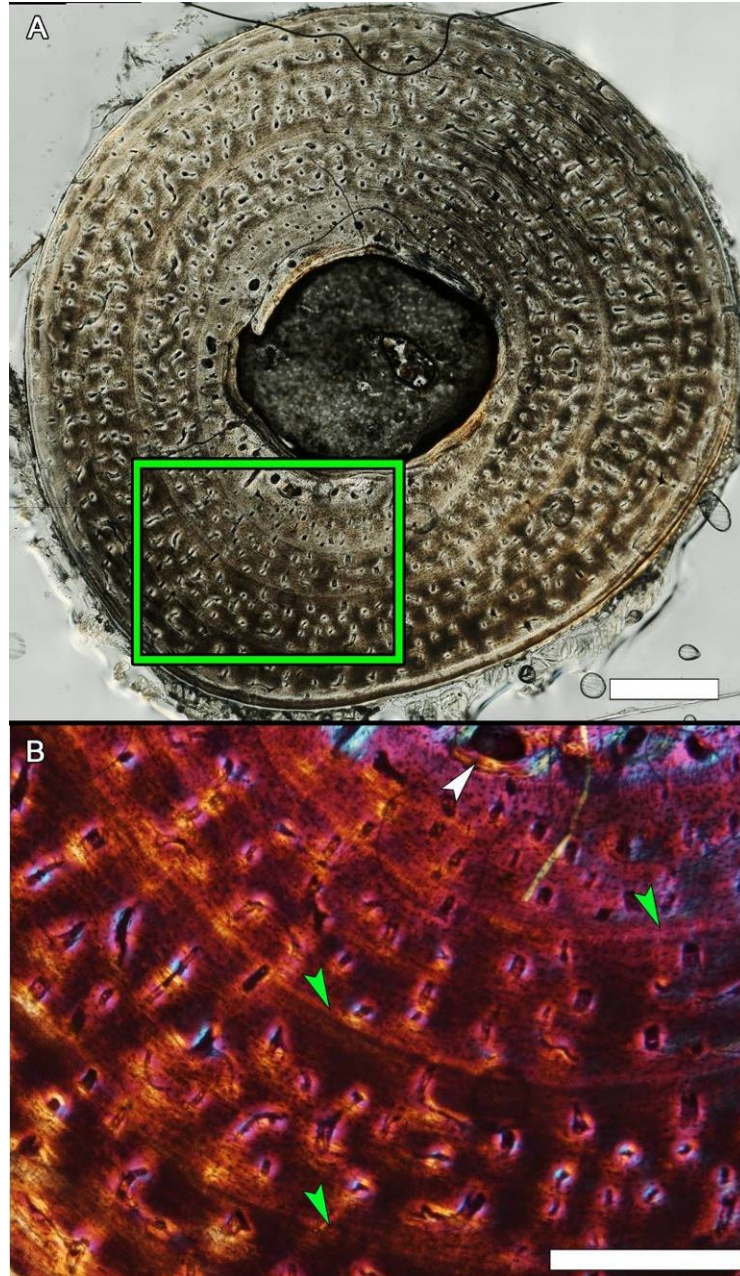


Figure 2.5. Osteohistology of the mid-diaphyseal humerus of alligator MOR 2007-05RC4. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. Three growth marks are in view (green arrows). Resorption cavities (white arrow) are present near the medullary cavity, and tissue is parallel-fibered. Vascularity is uniform throughout, consisting of longitudinal as well as obliquely anastomosing canals. Scale bar, 500 μ m.

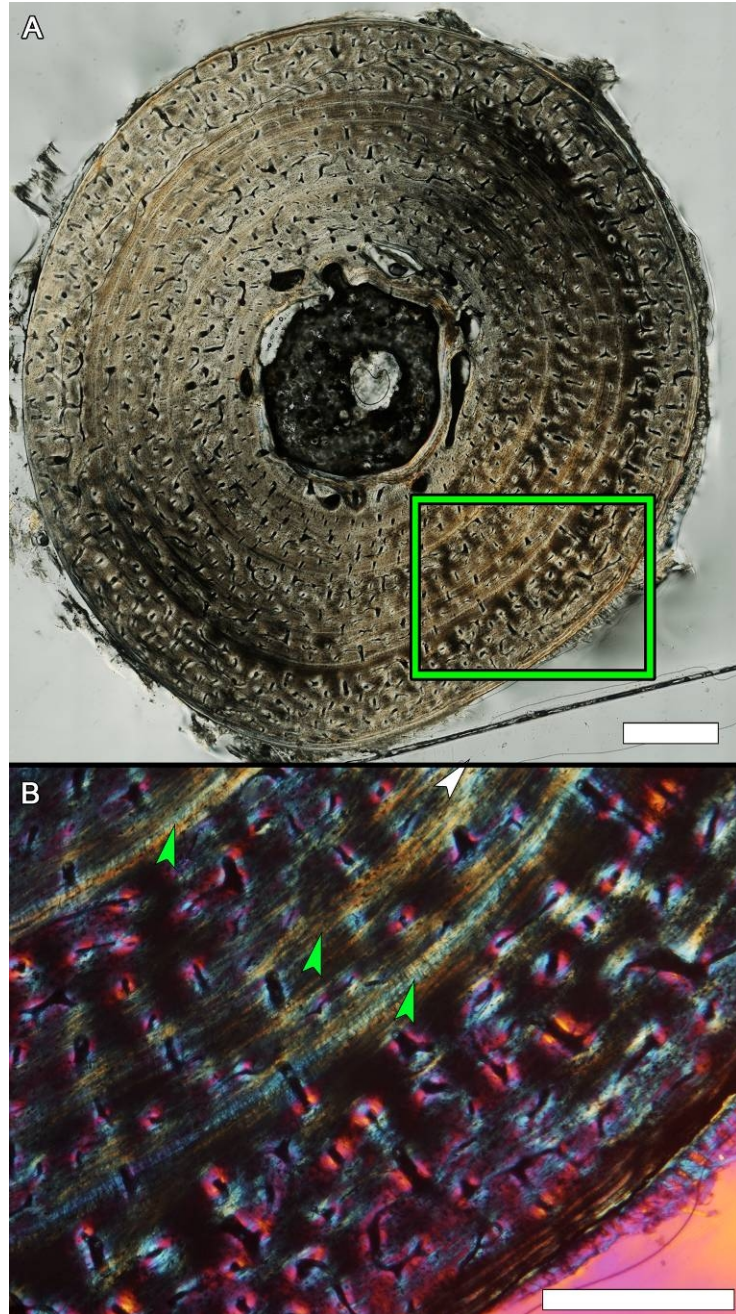


Figure 2.6. Osteohistology of the mid-diaphyseal humerus of alligator MOR 2007-05RC5. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Still, three growth marks (arrows) are visible in this portion of the cortex. The cortex is parallel-fibered throughout, and vascular density is uniform. Vascular orientation changes from mostly longitudinal within the inner cortex to predominately obliquely anastomosing. Scale bar, 500 μ m.

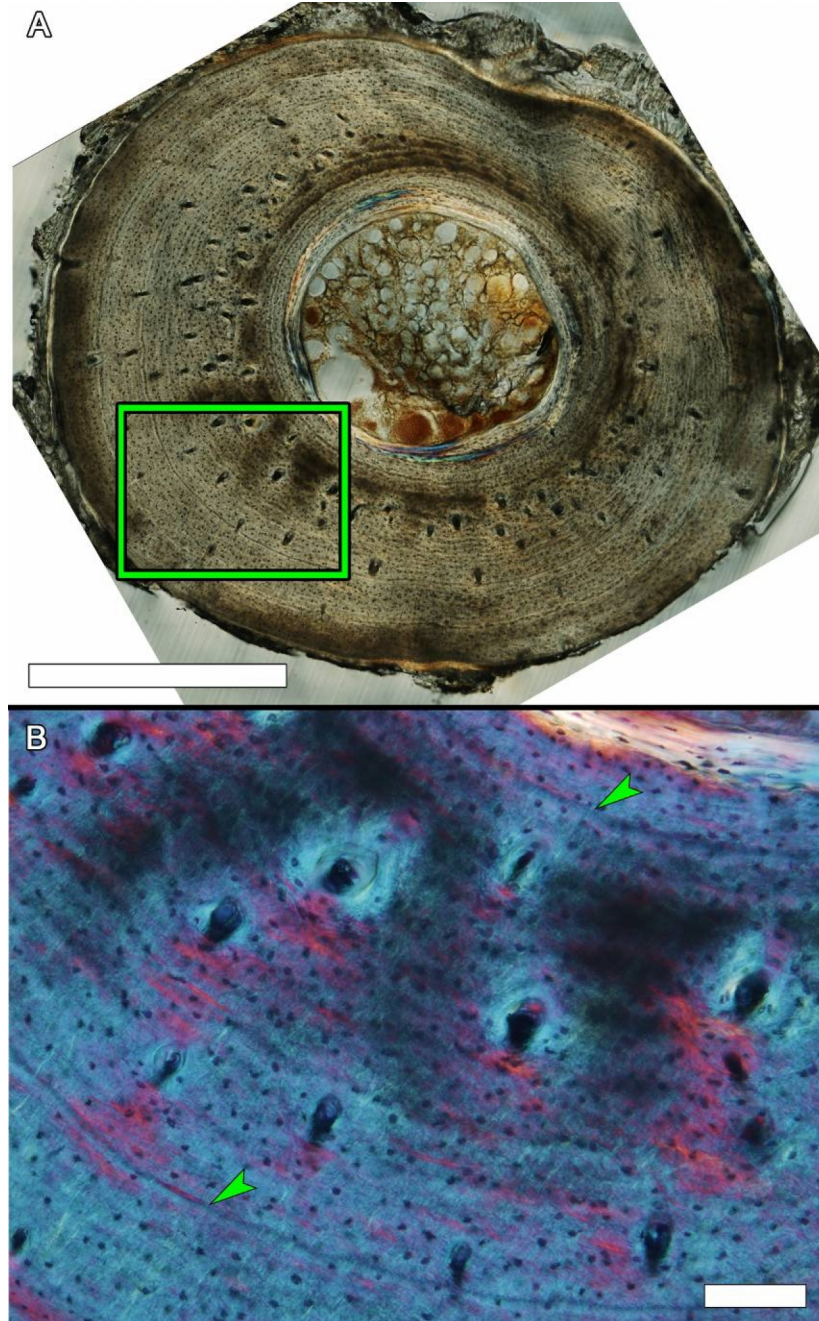


Figure 2.7. Osteohistology of the mid-diaphyseal radius of alligator MOR 2007-05RC3. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. However, two growth marks can still be made out in this region (arrows). The cortex is made of highly organized lamellar tissue with a sparse scattering of longitudinal vascular canals. Scale bar, 100 μ m.

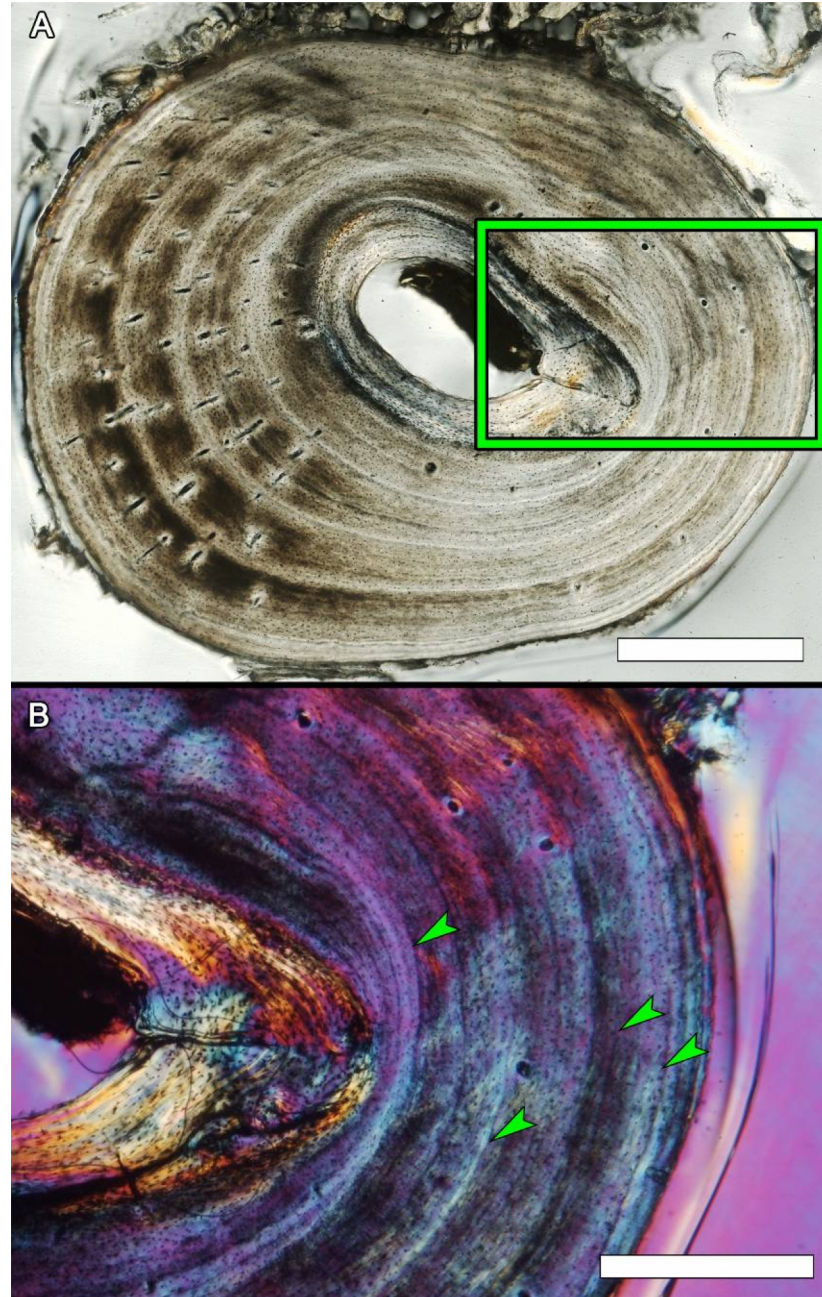


Figure 2.8. Osteohistology of the mid-diaphyseal radius of alligator MOR 2007-05RC4. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. The cortex is made of highly organized lamellar tissue and is nearly avascular. Four LAGs are shown with green arrows. The well developed endosteal layer (left) cuts across primary tissue and in places consists of secondary osteons, indicating medullary drift. Scale bar, 500 μm .

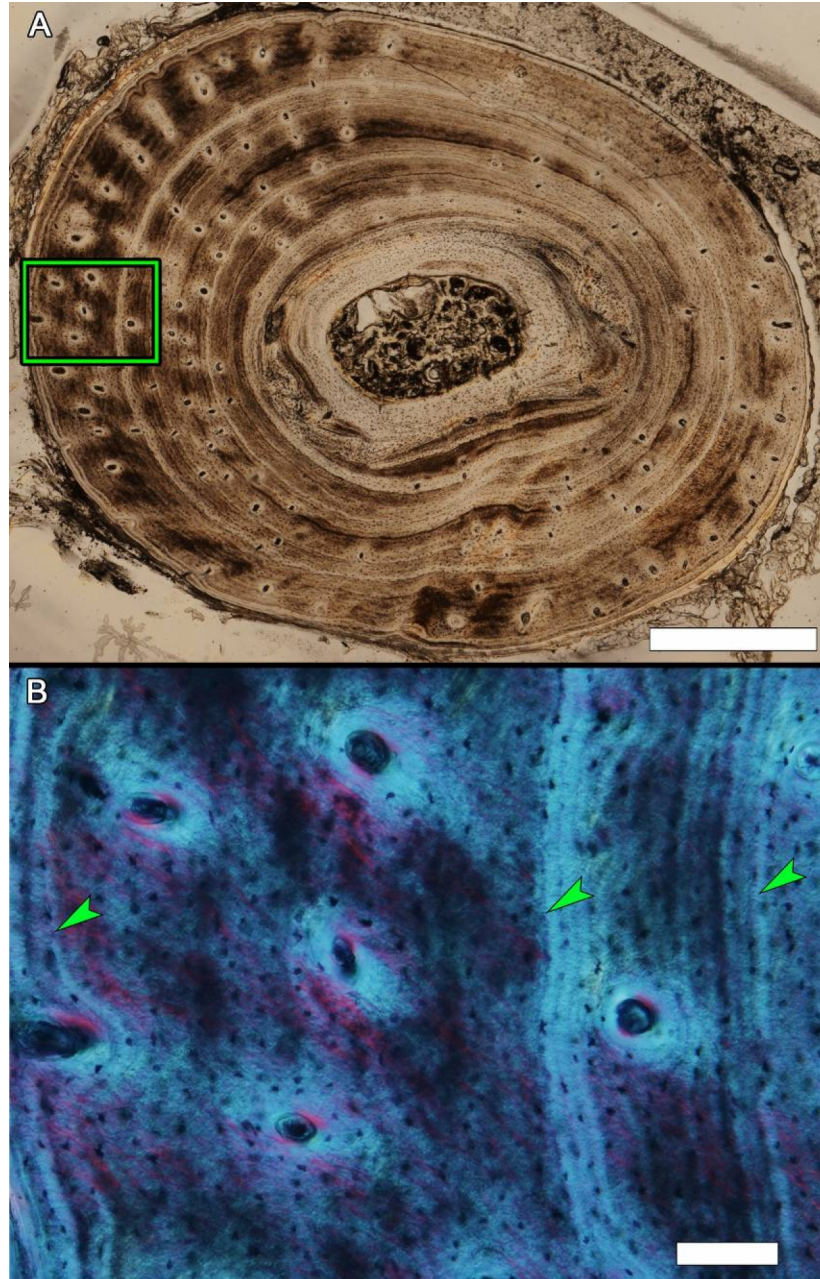


Figure 2.9. Osteohistology of the mid-diaphyseal radius of alligator MOR 2007-05RC5. A) Transverse section. The well developed endosteal layer cuts across primary tissue and in places consists of secondary osteons, indicating medullary drift. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. The cortex is made of highly organized lamellar tissue containing scattered longitudinal vascular canals. Three LAGs are evident (arrows). Scale bar, 100 μ m.

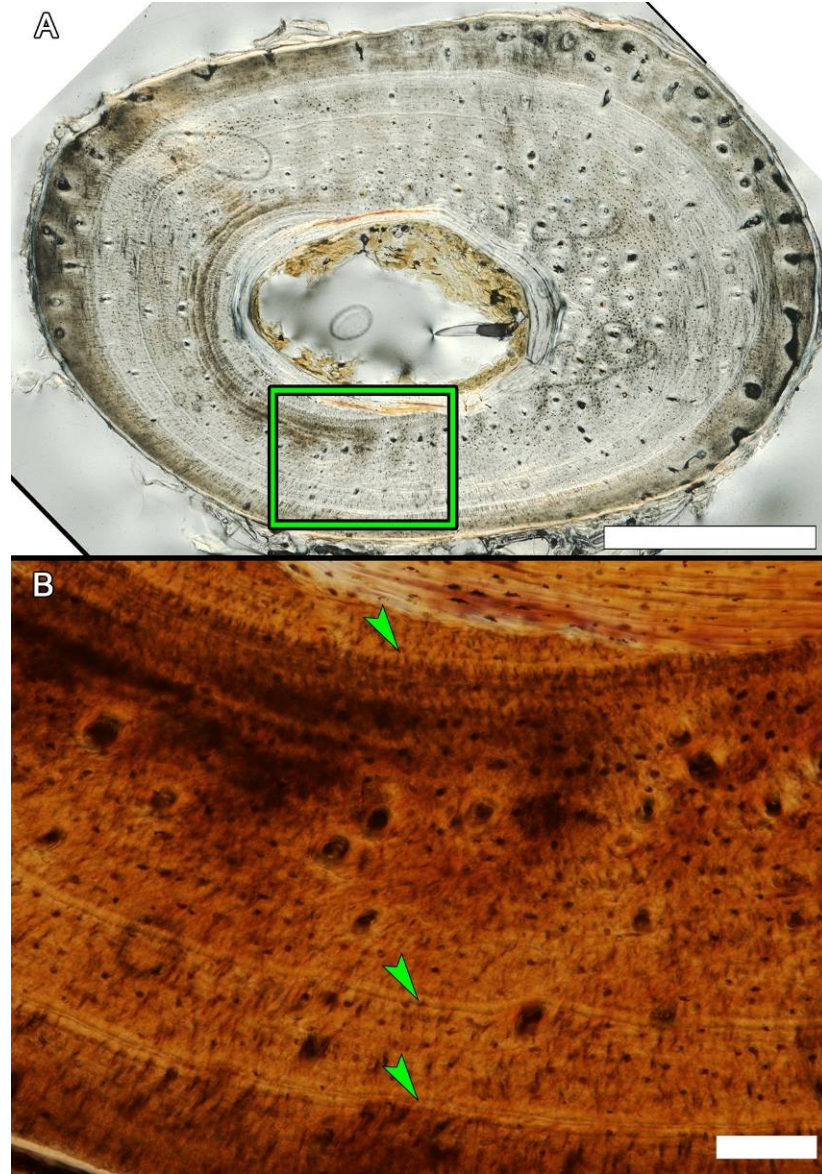


Figure 2.10. Osteohistology of the mid-diaphyseal ulna of alligator MOR 2007-05RC3. A) Transverse section. Periosteal attachment fibers (arrow) are evident even at low magnification. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Here the cortex is lamellar, with sparsely scattered longitudinal vascular canals. The innermost LAG is partially destroyed by medullary expansion and two more are visible within the cortex (green arrows). Periosteal attachment fibers are largely perpendicular to bone fiber orientation and appear black and hair-like. Scale bar, 100 μ m.

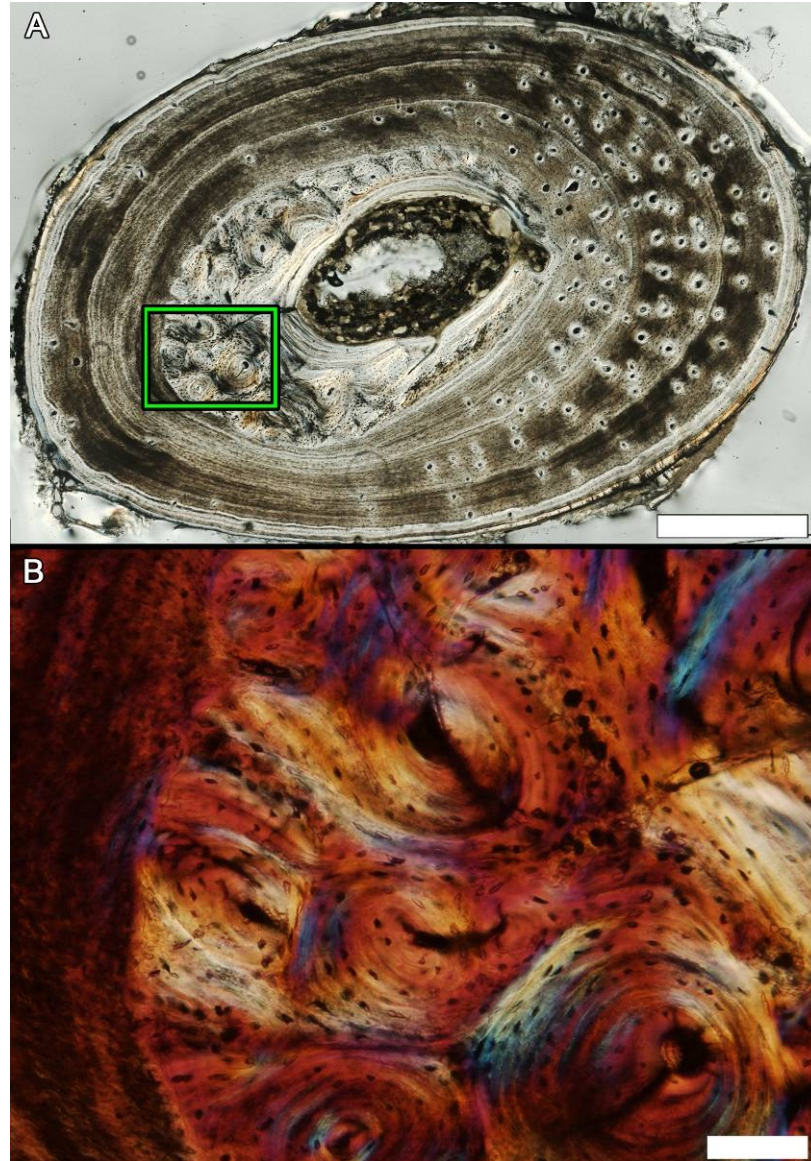


Figure 2.11. Osteohistology of the mid-diaphyseal ulna of alligator MOR 2007-05RC4. A) Transverse section. The high degree of endosteal remodeling is apparent even at low magnification, especially in the region of the green box. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. A considerable amount of secondary reconstruction occurred within the inner cortex due to medullary drift, resulting in large, overlapping secondary osteons. Scale bar, 100 μ m.

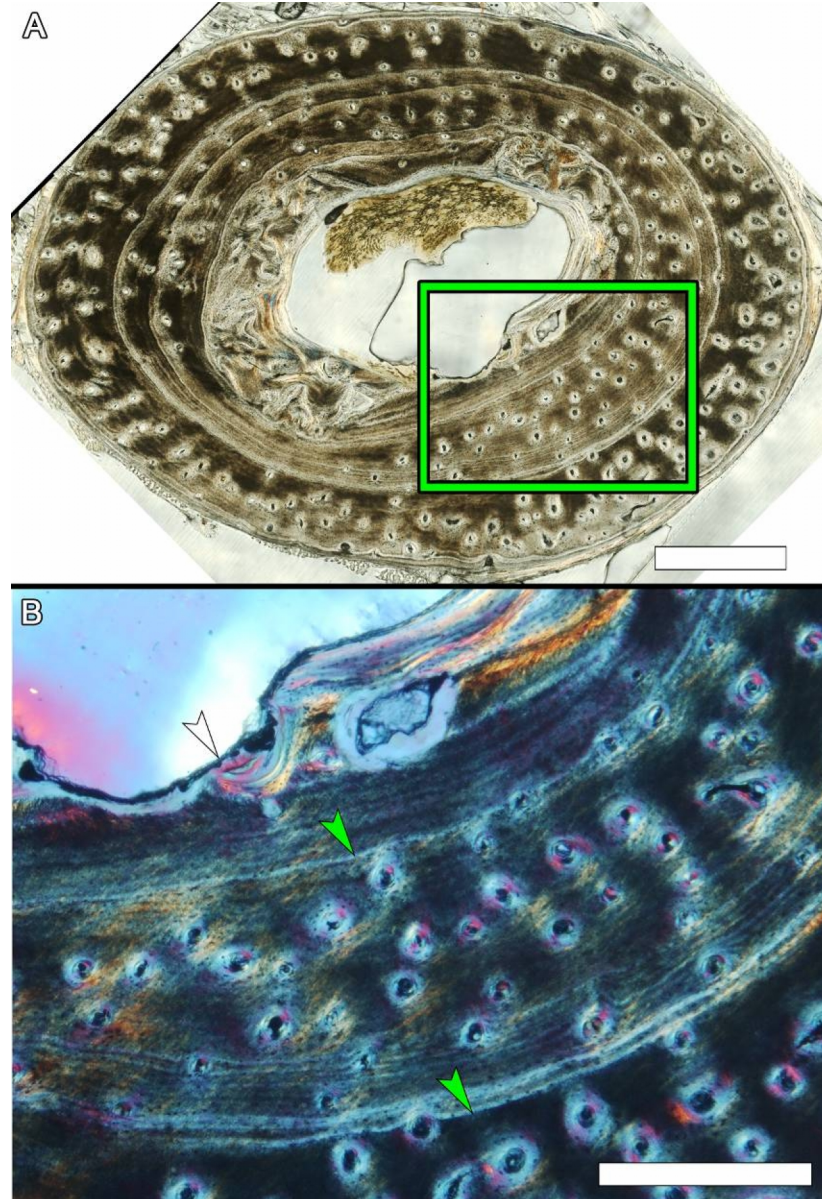


Figure 2.12. Osteohistology of the mid-diaphyseal ulna of alligator MOR 2007-05RC5. A) Transverse section. The high degree of endosteal remodeling is apparent even at low magnification. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. Two LAGs are marked by green arrows. Medullary expansion was active in this region as indicated by the partially resorbed endosteal layer (white arrow) and resorption cavity within the inner cortex. Tissue organization within the cortex is lamellar, and vascularity is longitudinal. Scale bar, 500 μm .



Figure 2.13. Osteohistology of the mid-diaphyseal femur of alligator MOR 2007-05RC3. A) Transverse section shows vascularity is a combination of anastomosing and radial canals. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Tissue within the innermost cortex is woven but becomes more parallel-fibered by the mid to outer cortex. Scale bar, 500 μ m.



Figure 2.14. Osteohistology of the mid-diaphyseal femur of alligator MOR 2007-05RC4. A) Transverse section, stained with Toluidine chloride. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. The cortical tissue is somewhat disorganized, but not to the degree that defines woven tissue; there remains a loosely parallel-fibered orientation. Scale bar, 500 μ m.

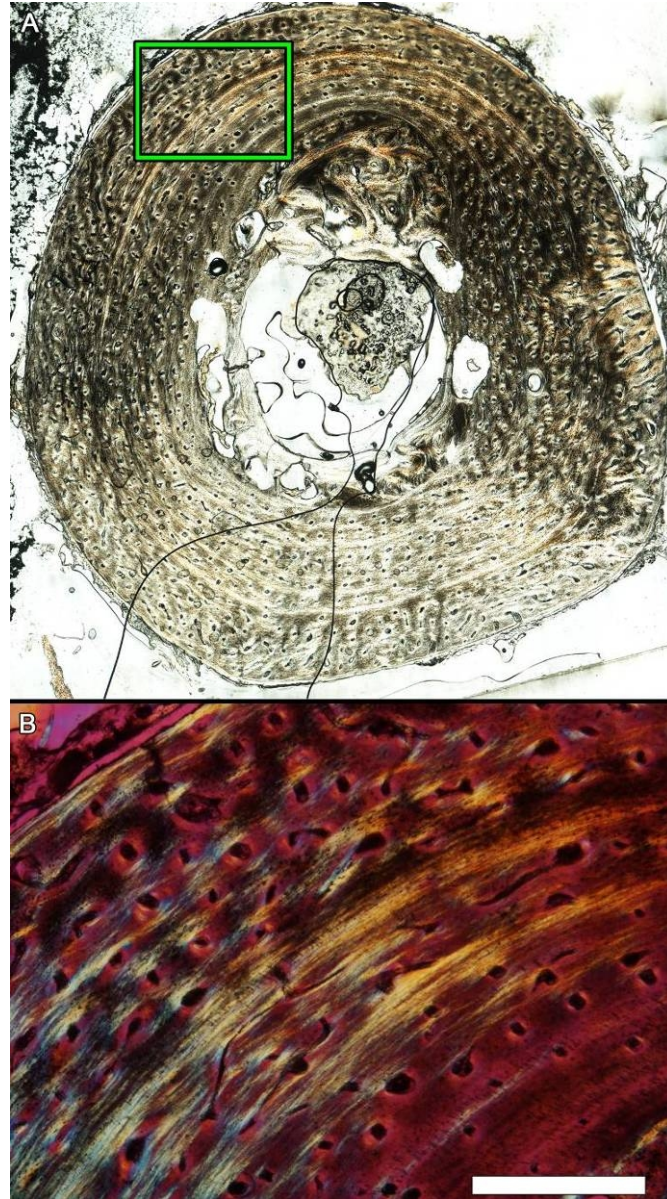


Figure 2.15. Osteohistology of the mid-diaphyseal femur of alligator MOR 2007-05RC5. A) Transverse section photographed using a circular polarizer. Medullary drift is evident by the concentration of secondary tissue endosteally, and active medullary expansion is revealed by large resorption cavities within the inner cortex. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. The cortical tissue is somewhat disorganized, but not to the degree that defines woven tissue; there remains a loosely parallel-fibered orientation. Scale bar, 500 μ m.

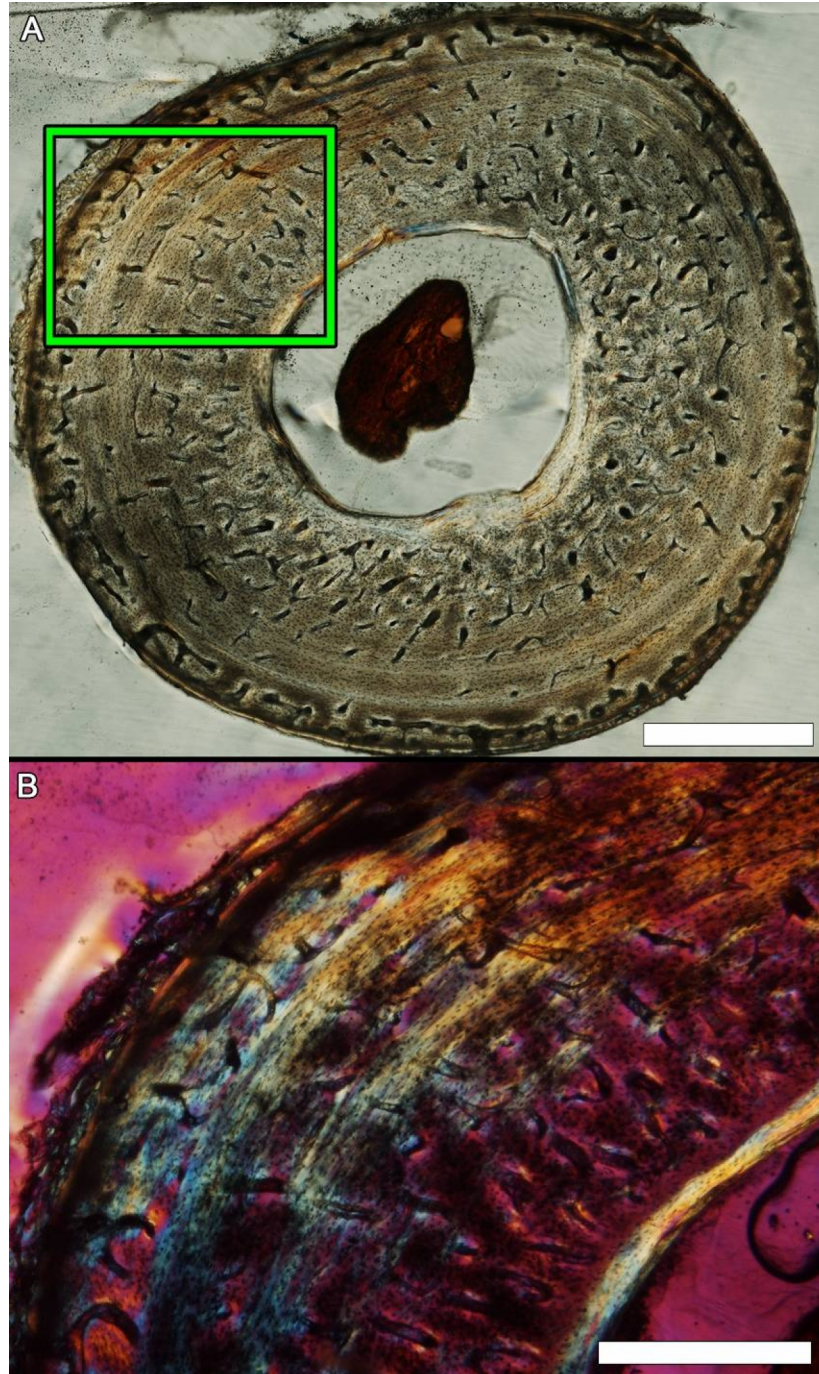


Figure 2.16. Osteohistology of the mid-diaphyseal tibia of alligator MOR 2007-05RC3. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Fiber orientation is woven within the inner cortex but changes to parallel-fibered by mid cortex. Vascular canals are obliquely anastomosing. Scale bar, 500 μm .

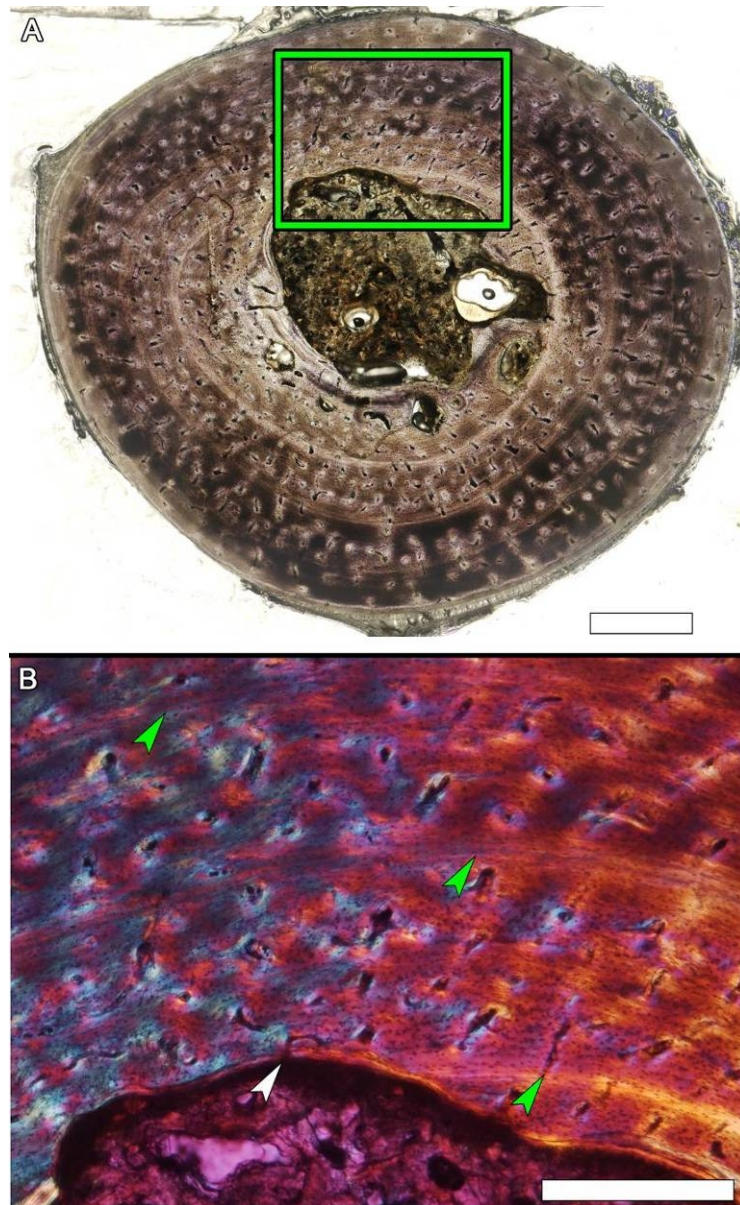


Figure 2.17. Osteohistology of the mid-diaphyseal tibia of alligator MOR 2007-05RC4. A) Transverse section, stained with Toluidine chloride. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Medullary expansion is evident by the resorbed region of inner cortex (white arrow), showing the partial destruction of the innermost annulus (green arrow). Two more annuli (green arrows) are present but difficult to distinguish within the cortex. Tissue is parallel-fibered and most vascular canals are either longitudinal or somewhat anastomosing. Scale bar, 500 μ m.

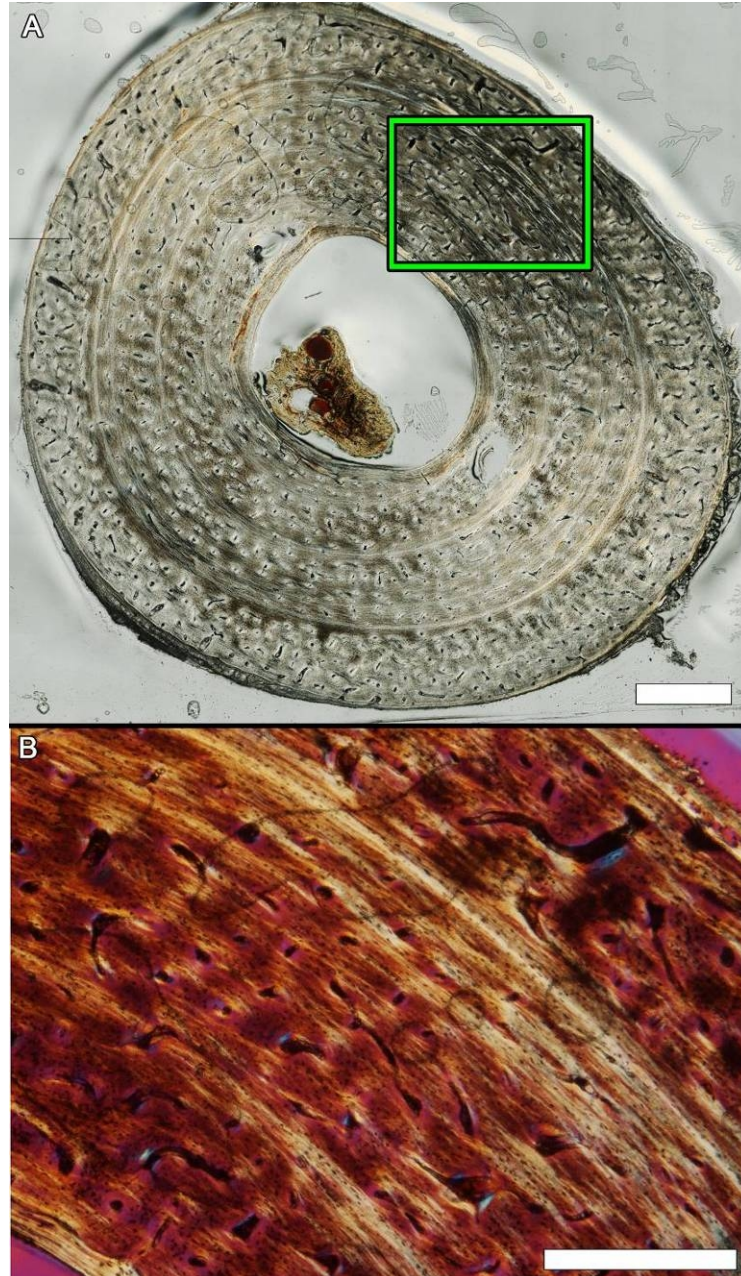


Figure 2.18. Osteohistology of the mid-diaphyseal tibia of alligator MOR 2007-05RC5. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. In this area, tissue fibers are only somewhat disorganized, arranged loosely in parallel. Vascularity is a combination of longitudinal and anastomosing canals. Scale bar, 500 μ m.

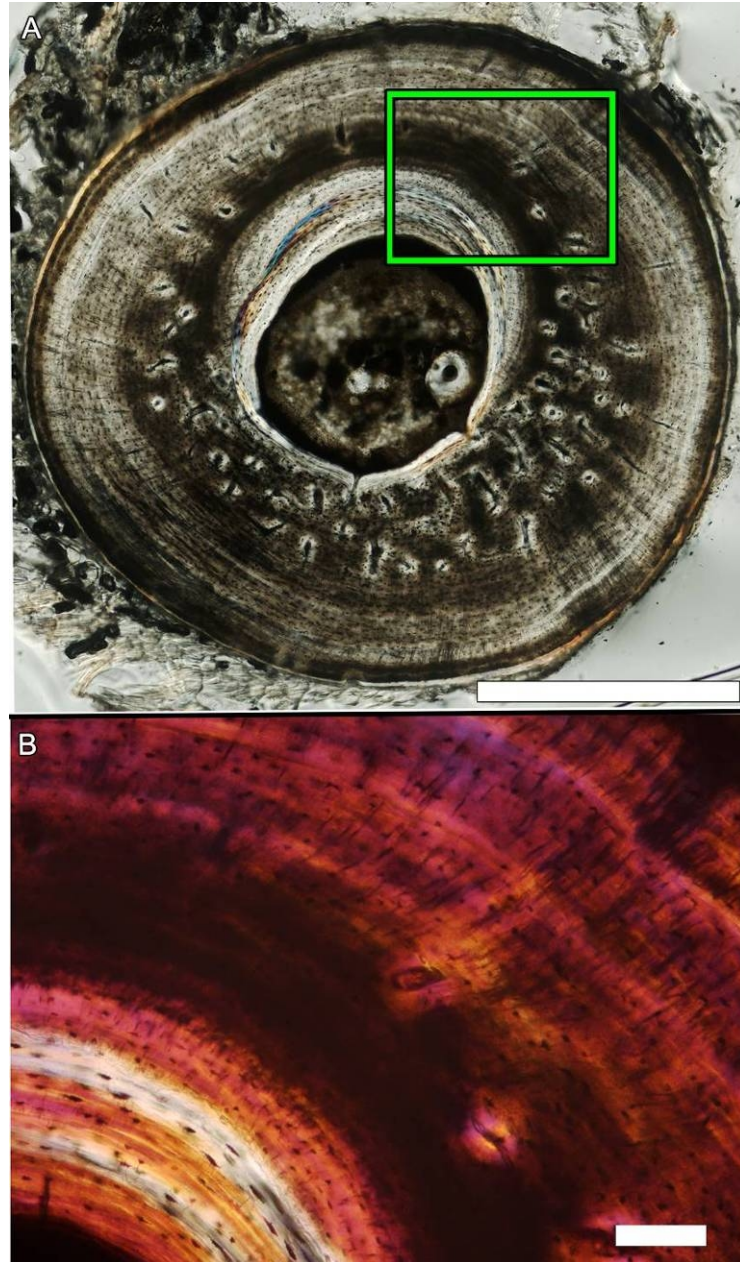


Figure 2.19. Osteohistology of the mid-diaphyseal fibula of alligator MOR 2007-05RC3. A) Transverse section. Medullary drift has partially destroyed the innermost lamellar cortex. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. This region is largely avascular and the lamellar tissue contains numerous black periosteal attachment fibers. Scale bar, 100 μm .

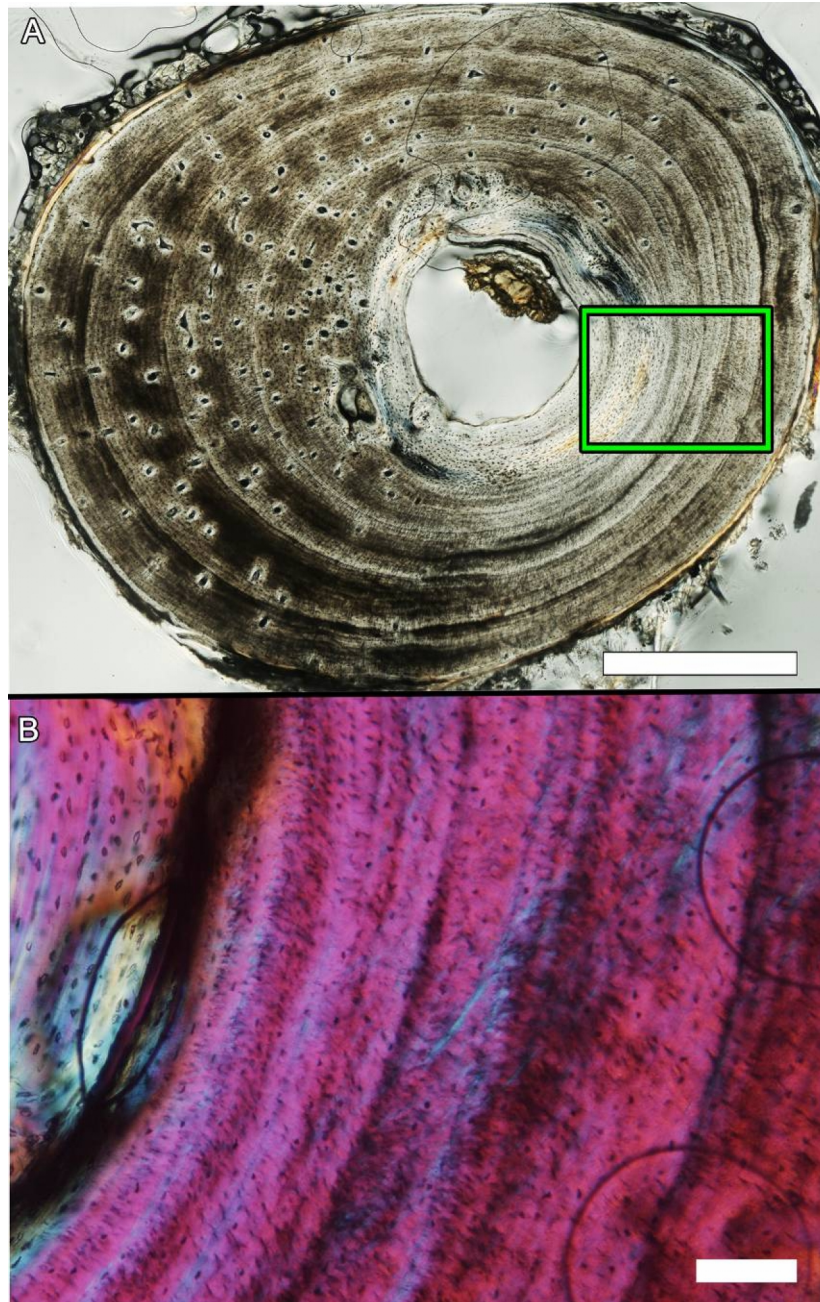


Figure 2.20. Osteohistology of the mid-diaphyseal fibula of alligator MOR 2007-05RC4. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Bone tissue is of highly organized lamellar fibers containing scattered osteocytes. Periosteal attachment fibers are abundant. The circles on the right of the image are preparation artifacts. Scale bar, 100 μm .

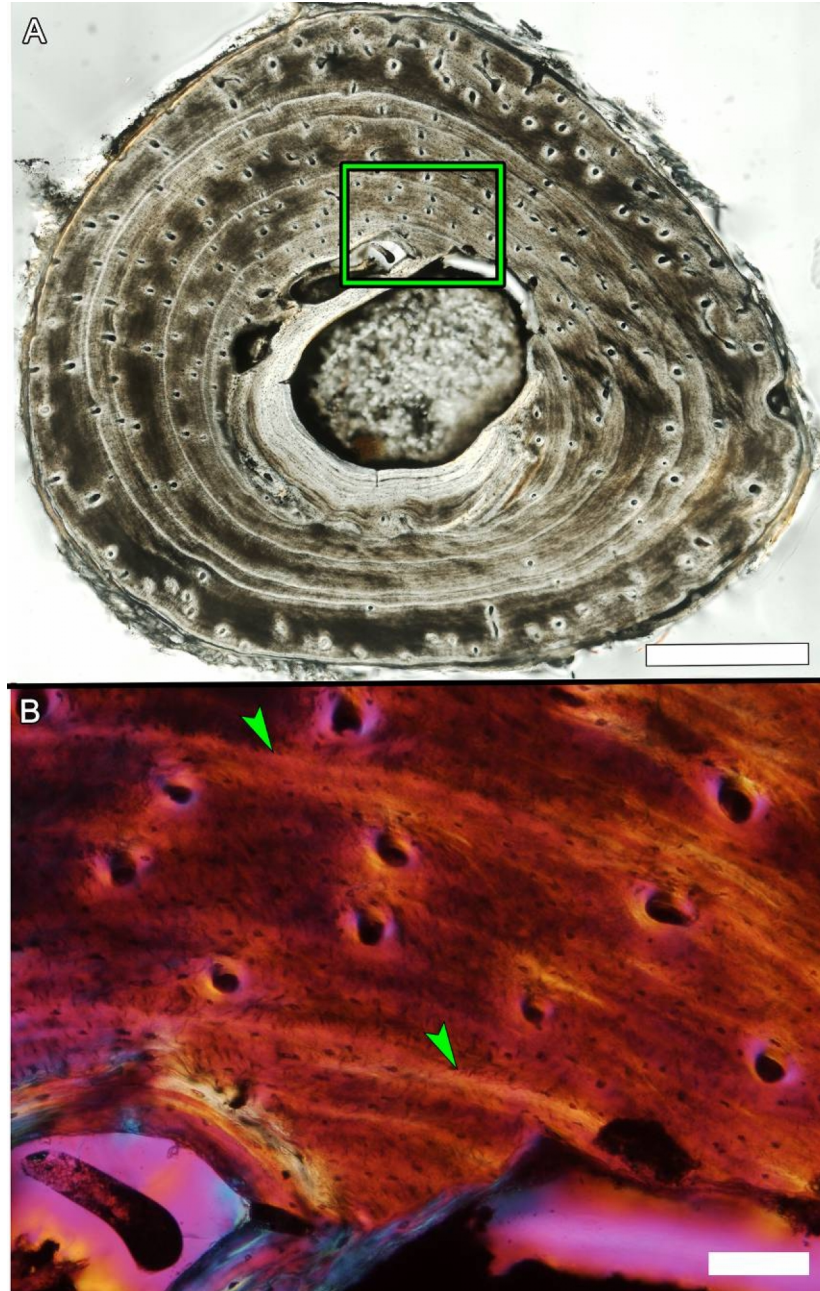


Figure 2.21. Osteohistology of the mid-diaphyseal fibula of alligator MOR 2007-05RC5. A) Transverse section. Medullary expansion and resorption cavities have cut into the innermost primary cortex. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Two LAGs are visible here (arrows), with the innermost being destroyed by medullary expansion. Tissue within the cortex is lamellar to somewhat parallel-fibred and vascular canals are longitudinal. Scale bar, 100 μ m.

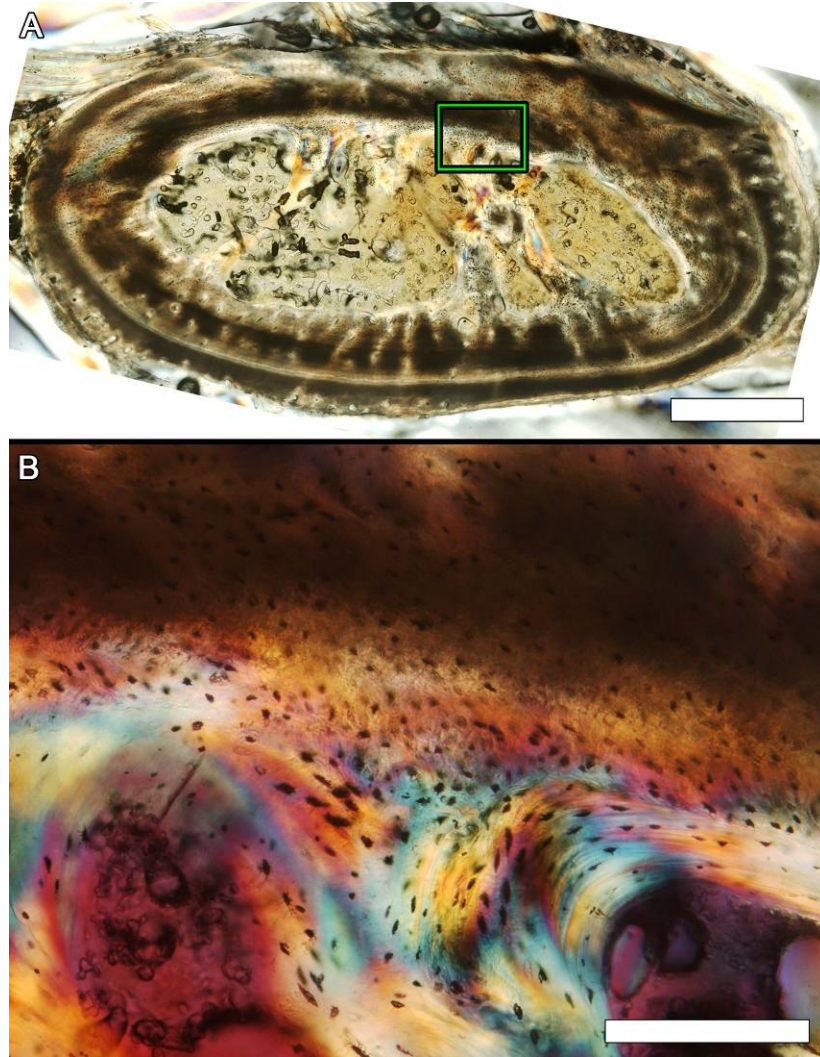


Figure 2.22. Osteohistology of the coracoid blade of alligator MOR 2007-05RC3. A) Transverse section. Due to the size of the medullary cavity, it is likely growth marks were lost to resorption. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. This region shows the boundary between the medullary endosteal layer with a strut of remodeled bone (lower half) and the primary inner cortex (upper half). Scale bar, 200 μm .

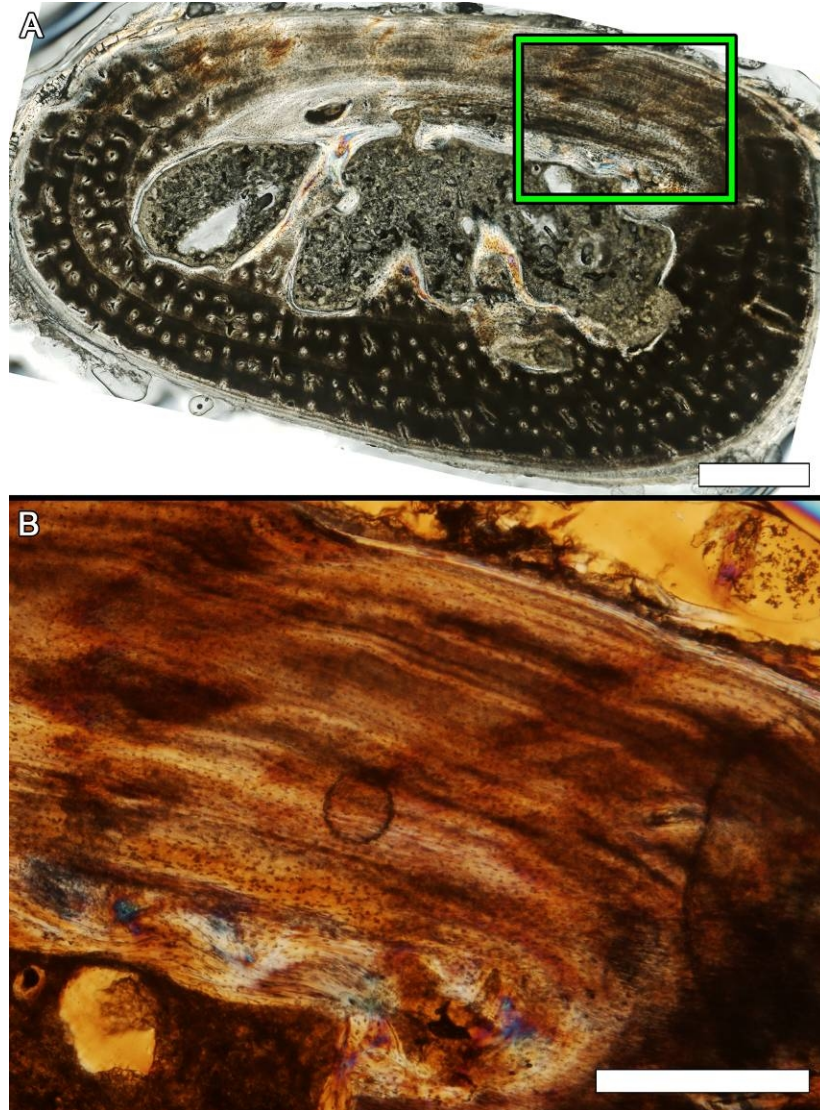


Figure 2.23. Osteohistology of the coracoid blade of alligator MOR 2007-05RC4. A) Transverse section. The innermost annulus (arrow) is partially destroyed by expansion of the large medullary cavity. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Tissue in the region is avascular and lamellar. Periosteal attachment fibers radiate outward within the cortex. Scale bar, 500 μ m.

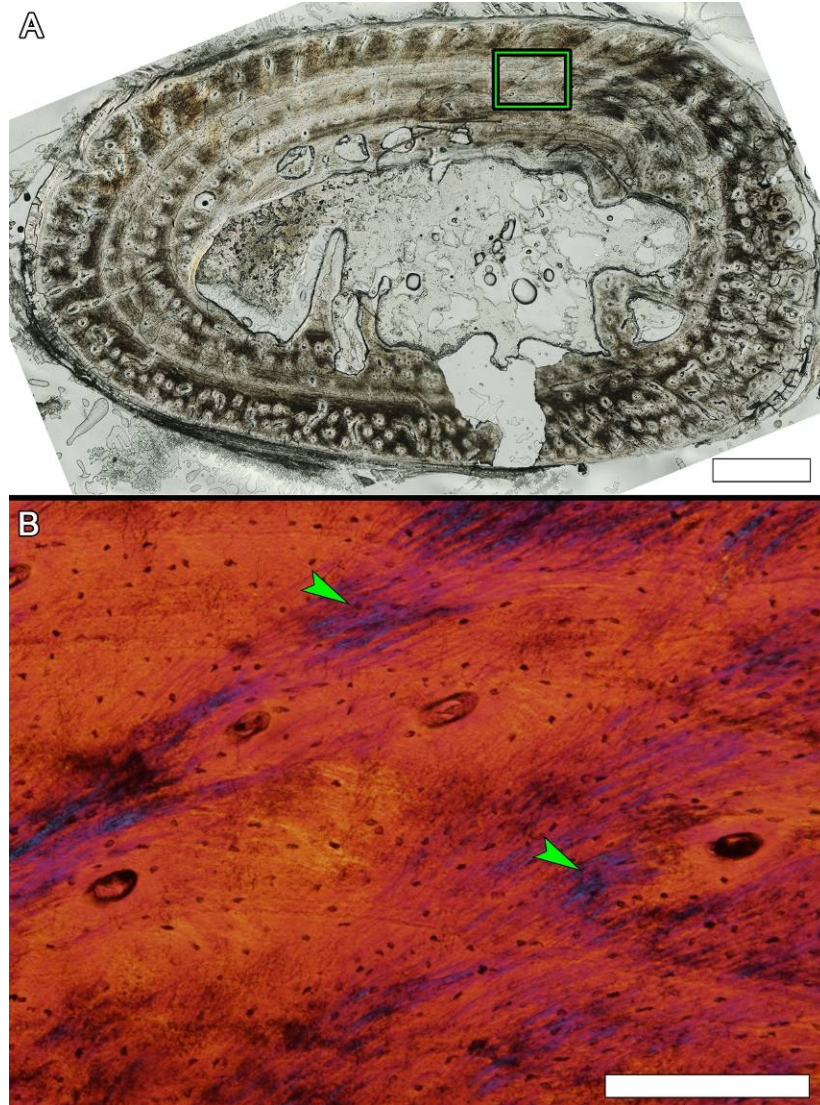


Figure 2.24. Osteohistology of the coracoid blade of alligator MOR 2007-05RC5. A) Transverse section. The large marrow cavity and surrounding resorption cavities have nearly destroyed the innermost growth mark. Missing cortex in the bottom of the image is an artifact of preparation. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Tissue here is lamellar to somewhat parallel-fibered. Periosteal attachment fibers (arrows) frequently radiate within the cortex. Scale bar, 200 μ m.

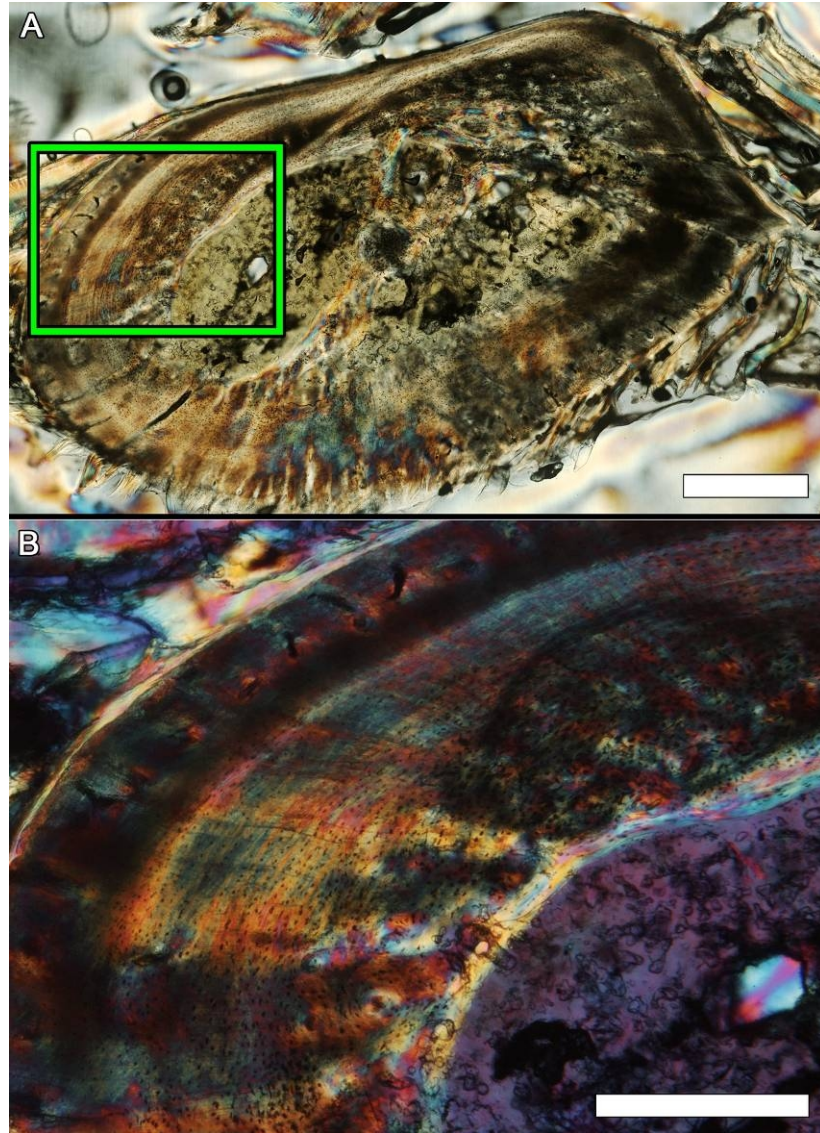


Figure 2.25. Osteohistology of the scapula blade of alligator MOR 2007-05RC3. A) Transverse section. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation, but this tends to obscure growth marks. Lamellar cortex contains scattered vascular canals but the mid cortex is largely avascular. Scale bar, 500 μ m.

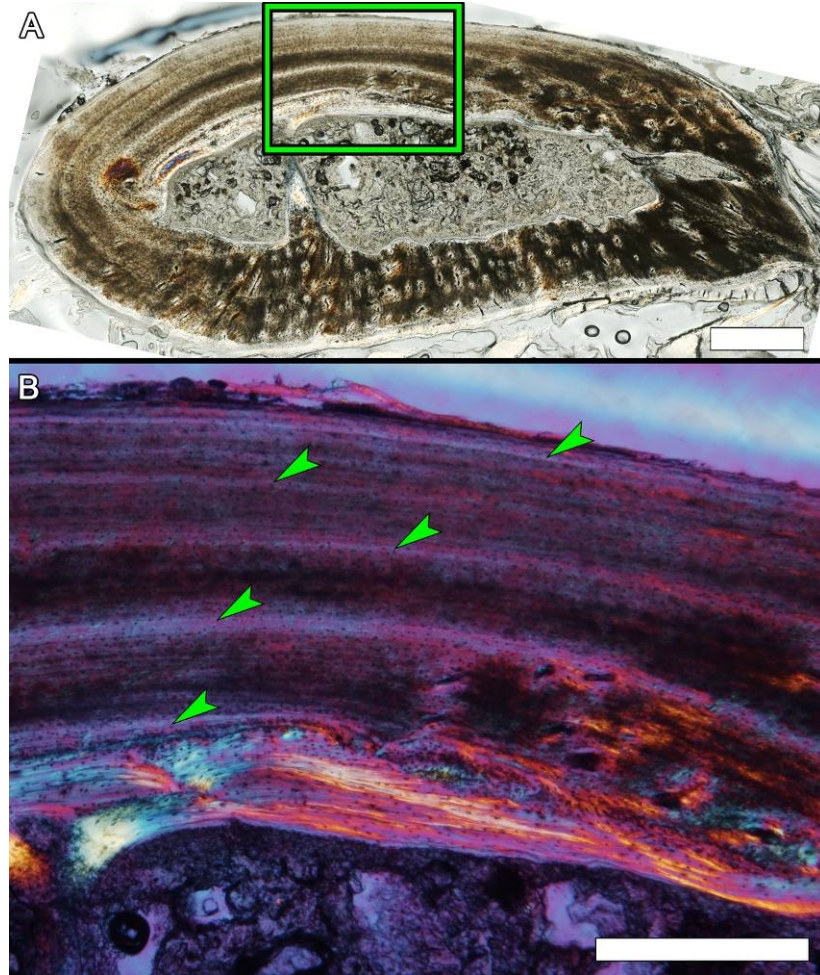


Figure 2.26. Osteohistology of the scapula blade of alligator MOR 2007-05RC4. A) Transverse section. Medullary expansion has partially destroyed several growth marks within the cortex. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. This region is predominately avascular and lamellar with scattered osteocytes. Five annuli followed by LAGs (arrows) appear as lighter bands within the cortex. Scale bar, 500 μm .

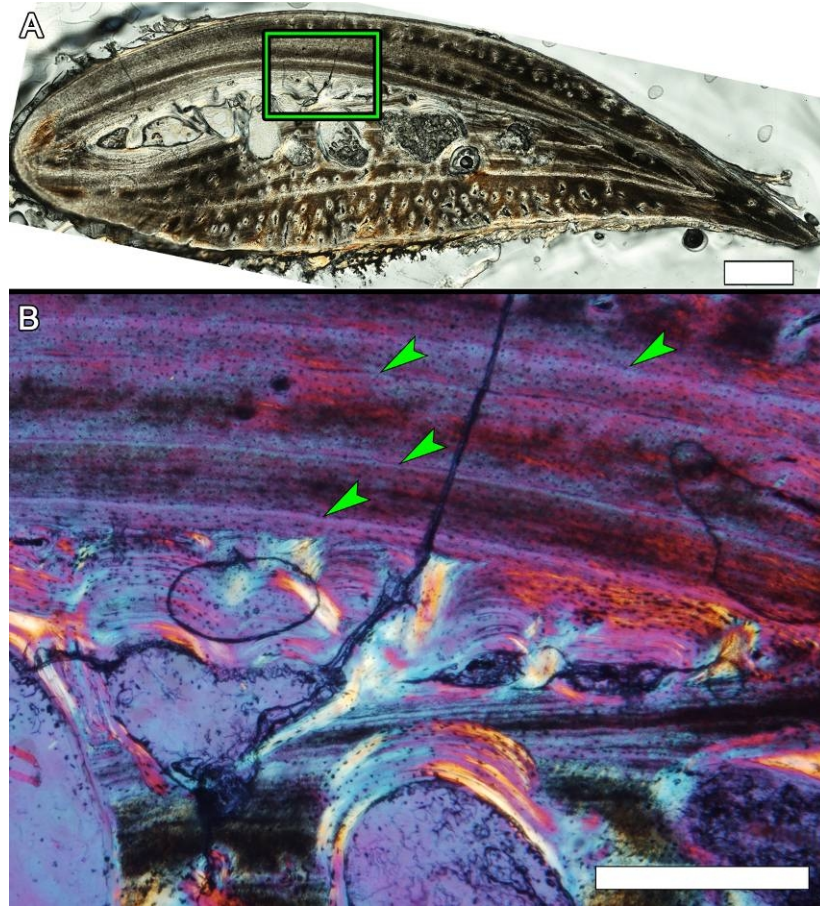


Figure 2.27. Osteohistology of the scapula blade of alligator MOR 2007-05RC5. A) Transverse section. Large resorption cavities have partially destroyed growth marks in several regions. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. Near the center of the image, remodeled secondary tissue borders the medullary cavity and resorption cavities can be observed near the bottom. Primary tissue in this area is fairly avascular and lamellar. Four annuli followed by LAGs (arrows) appear as light bands within the cortex. Scale bar, 500 μm .

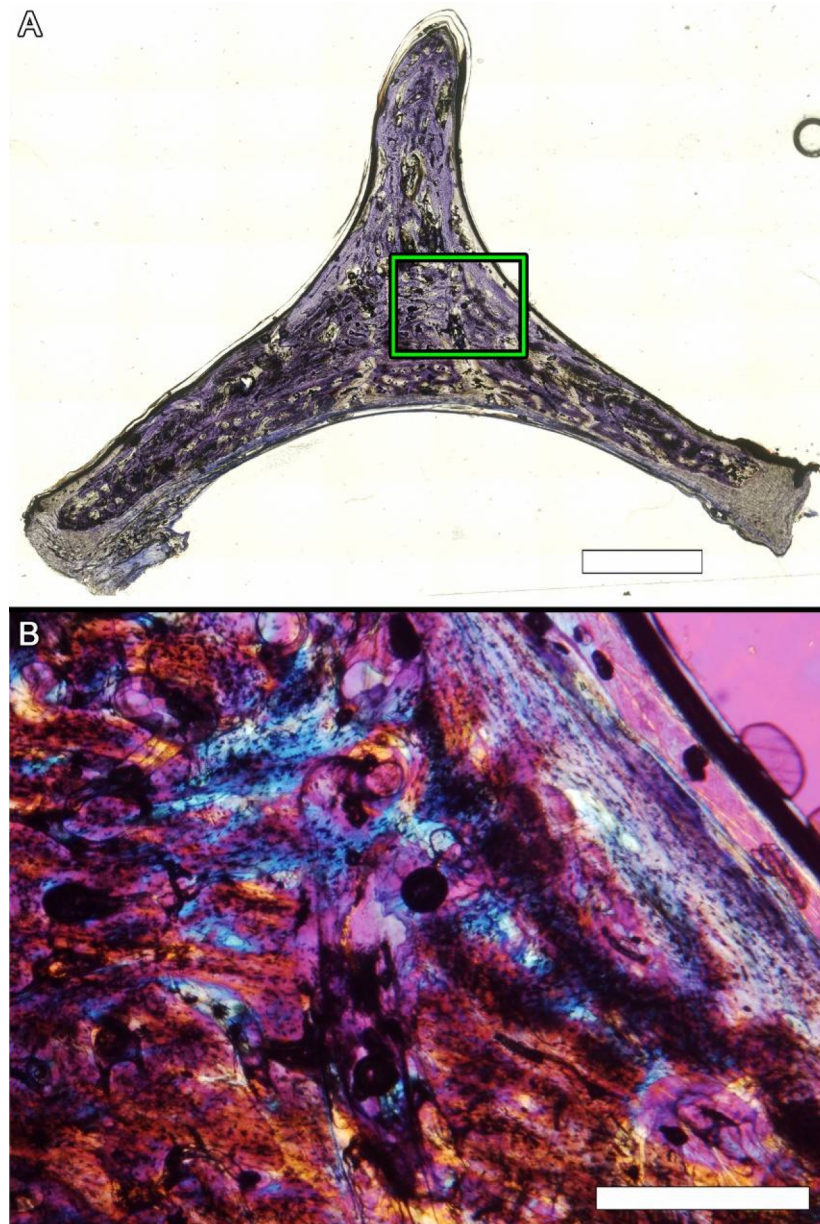


Figure 2.28. Osteohistology of an osteoderm from alligator MOR 2007-05RC3. A) Transverse section, stained with Toluidine chloride. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. Tissue is highly disorganized and richly vascularized. The vascular canals appear transparent and do not have a common orientation. Periosteal attachment fibers are also frequent and disorganized. Scale bar, 500 μ m.

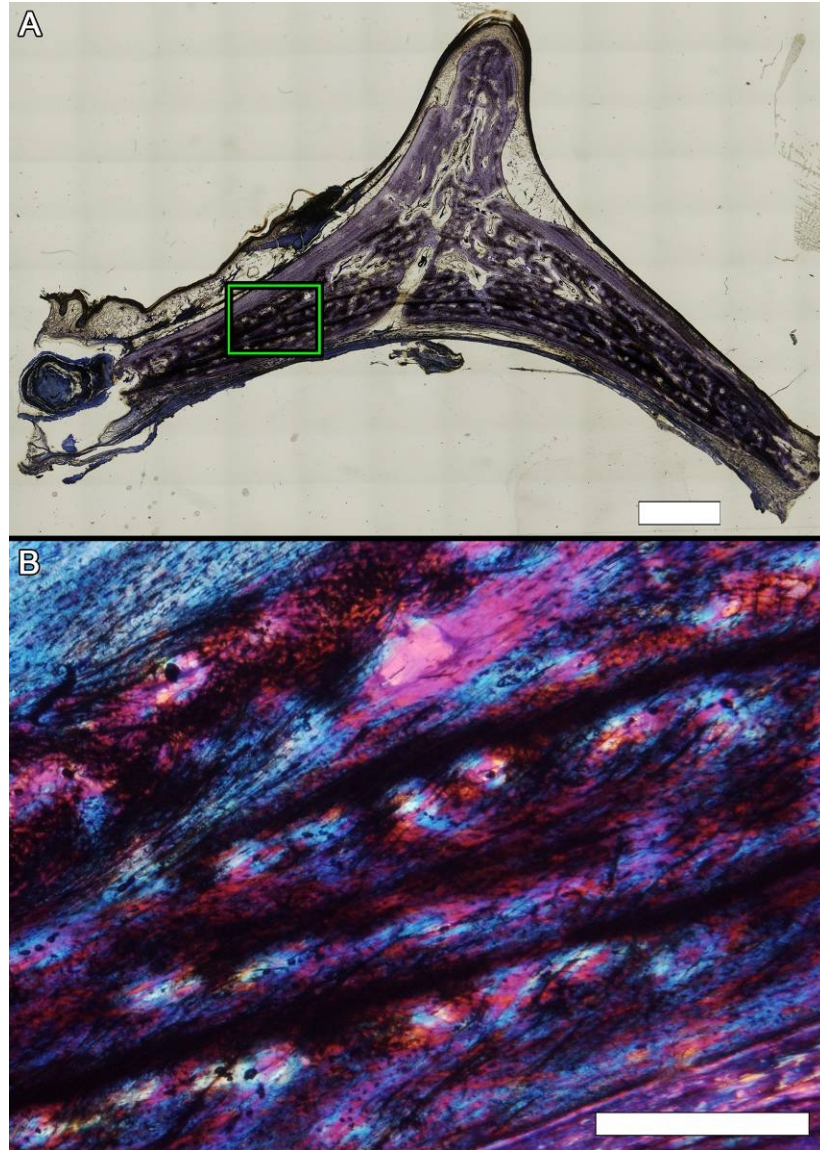


Figure 2.29. Osteohistology of an osteoderm from alligator MOR 2007-05RC4. A) Transverse section, stained with Toluidine chloride. Three, possibly four, growth marks are observed near the ventral surface at low magnification. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. Two growth marks are evident, apparently made black by the density of periosteal attachment fibers. These fibers are also frequent and disorganized within the tissue matrix. Scale bar, 500 μm .

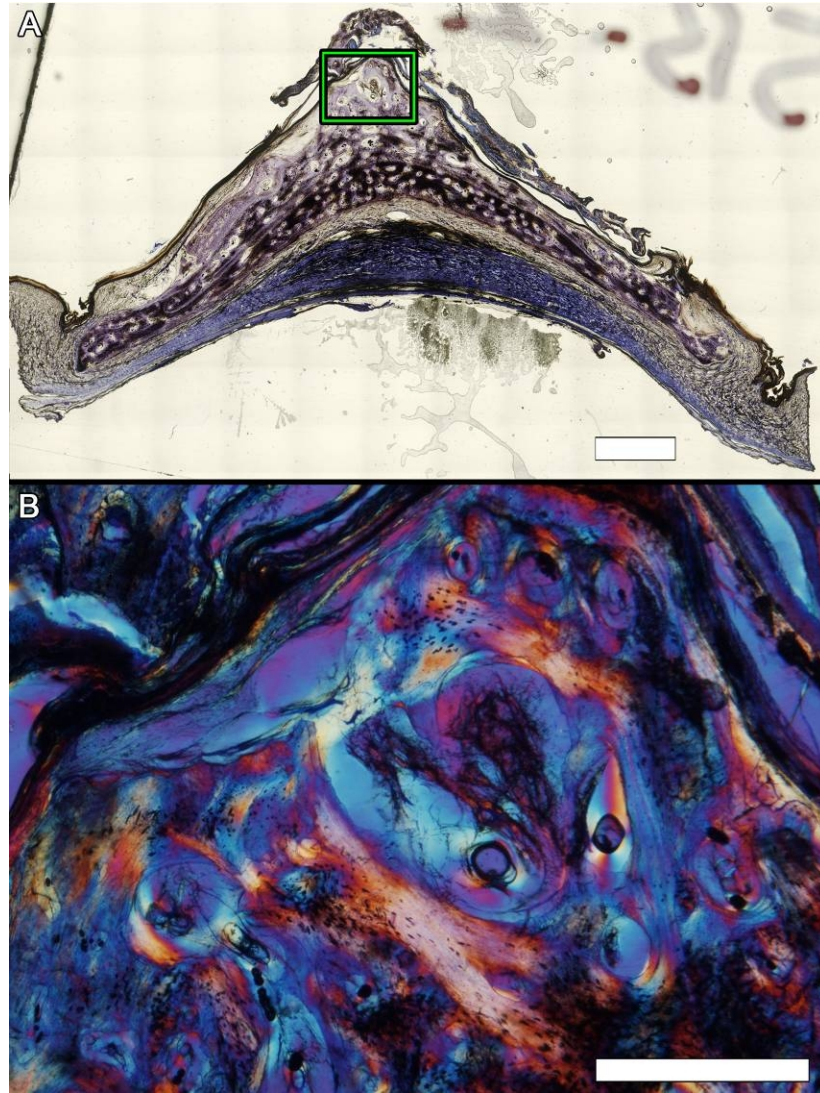


Figure 2.30. Osteohistology of an osteoderm from alligator MOR 2007-05RC5. A) Transverse section, stained with Toluidine chloride. One growth mark is observed near the ventral surface at low magnification, seeming to outline an earlier shape of the osteoderm. Scale bar, 1 mm. B) Enlargement of the area from A) within the green box. This enlargement was photographed using a full lambda (530 nm) plate to reveal fiber orientation. This region near the apex of the keel is richly vascularized and bone is more cancellous than compact. Scale bar, 500 μ m.

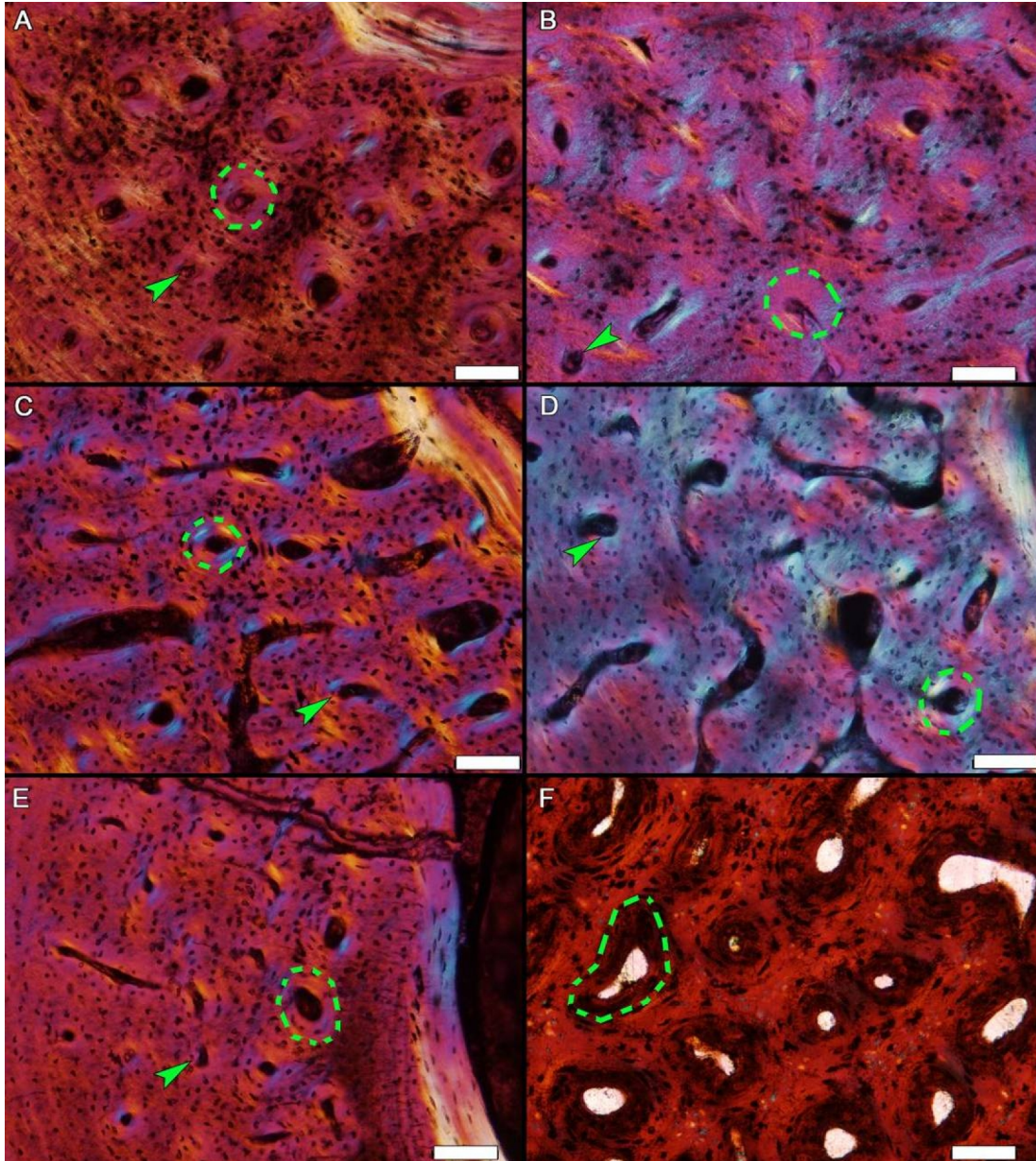


Figure 2.31. Examples of woven tissue and primary osteons within diaphyseal sections of various elements. Primary osteons (examples in dashed outlines) are embedded within a woven tissue matrix in A) the ulna of MOR 2007-05RC3, B) the tibia of MOR 2007-05RC5, C) the humerus of MOR 2007-05RC3, D) the tibia of MOR 2007-05RC3, E) the fibula of 2007-05RC3, and for comparison, F) the tibia of the non-avian dinosaur *Maiasaura* (MOR 2007-15 T11-3). The primary osteons of the alligators have fewer lamellae and are more acellular than those from the *Maiasaura*. Simple vascular canals (examples indicated by arrows) are also scattered amongst the primary osteons in the alligator tissue. Scale bars, 100 μm.

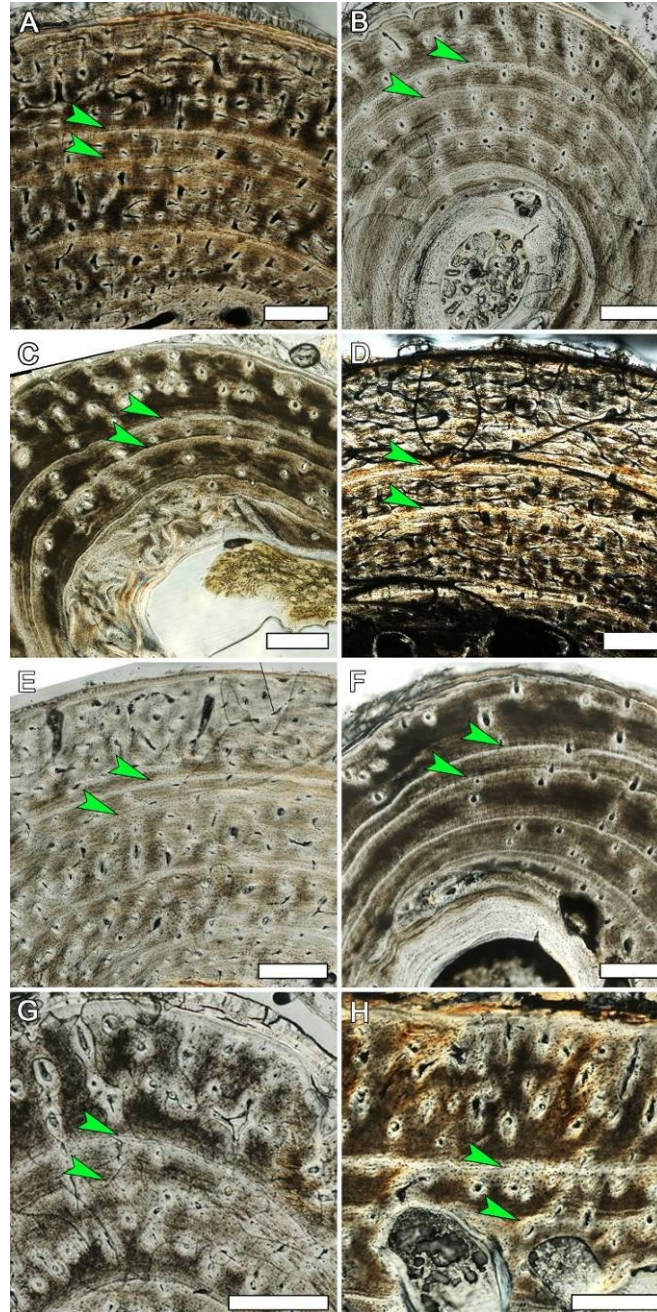


Figure 2.32. Third and fourth growth marks in every sampled element of MOR 2007-05RC5. These growth marks are unusually closely spaced in all sampled elements, providing a natural “marker” within the cortex. They are indicated by arrows in A) the humerus, B) radius, C) ulna, D) femur, E) tibia, F) fibula, G) coracoid, and H) scapula. Scale bars, 100 μm .

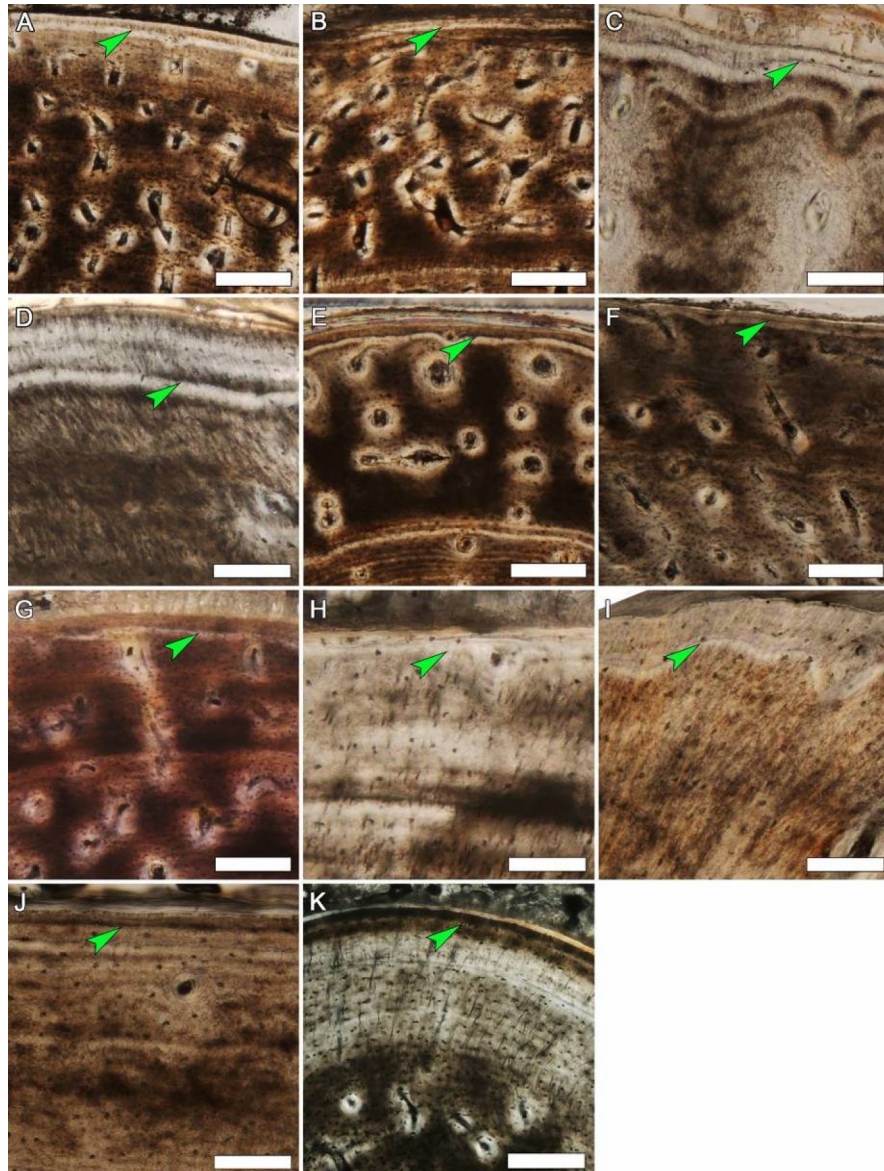


Figure 2.33. Detail of the outer cortex from elements of MOR 2007-05RC4 and MOR 2007-05RC5 showing a fifth growth mark. An arrow indicates the outermost growth mark observed in many elements of the two captive alligators. A) the humerus of MOR 2007-05RC4. Scale bar, 500 μm ; B) the humerus of MOR 2007-05RC5. Scale bar, 500 μm ; C) the radius of MOR 2007-05RC5. Scale bar, 100 μm ; D) the ulna of MOR 2007-05RC4. Scale bar, 100 μm ; E) the ulna of MOR 2007-05RC5. Scale bar, 500 μm ; F) the femur of MOR 2007-05RC4. Scale bar, 500 μm ; G) the tibia of MOR 2007-05RC4. Scale bar, 500 μm ; H) the fibula of MOR 2007-05RC4. Scale bar, 100 μm ; I) the scapula of MOR 2007-05RC4. Scale bar, 100 μm ; J) the scapula of MOR 2007-05RC5. Scale bar, 100 μm ; K) the fibula of MOR 2007-03RC5. Scale bar, 100 μm .

Literature Cited

- Anderson, J. F., A. Hall-Martin, and D. A. Russell. 1985. Long bone circumference and weight in mammals, birds, and dinosaurs. *Journal of the Zoological Society of London* 207:53-61.
- Bourdon, E., J. Castanet, A. d. Ricqlès, P. Scofield, A. Tennyson, H. Lamrous, and J. Cubo. 2009. Bone growth marks reveal protracted growth in New Zealand kiwi (Aves, Apterygidae). *Biology Letters* 5:639-642.
- Bybee, P. J., A. H. Lee, and E. Lamm. 2006. Sizing the Jurassic theropod dinosaur *Allosaurus*: assessing growth strategy and evolution of ontogenetic scaling of limbs. *Journal of Morphology* 267:347-359.
- Castanet, J., H. Francillon-Vieillot, P. J. Meunier, and A. d. Ricqlès. 1993. Bone and individual aging; pp. 245-283 in B. K. Hall (ed.), *Bone*. CRC Press, London.
- Castanet, J., A. Grandin, A. Abourachid, and A. d. Ricqlès. 1996. Expression de la dynamique de croissance dans la structure de l'os périostique chez *Anas platyrhynchos*. *Comptes Rendus de l'Académie des sciences - Series III - Sciences de la Vie* 319:301-308.
- Castanet, J., K. C. Rogers, J. Cubo, and J.-J. Boisard. 2000. Periosteal bone growth rates in extant ratites (ostriche and emu). Implications for assessing growth in dinosaurs. *Comptes Rendus de l'Académie des sciences - Series III - Sciences de la Vie* 323:543-550.
- Castanet, J., S. Croci, F. Aujard, M. Perret, J. Cubo, and E. d. Margerie. 2004. Lines of arrested growth in bone and age estimation in a small primate: *Microcebus murinus*. *Journal of Zoology, London* 263:31-39.
- Chabreck, R. H., and T. Joanen. 1979. Growth rates of American alligators in Louisiana. *Herpetologica* 35:51-57.
- Chinsamy, A. 1993. Image analysis and the physiological implications of the vascularisation of femora in archosaurs. *Modern Geology* 19:101-108.
- Coulson, T. D., R. A. Coulson, and T. Hernandez. 1973. Some observations on the growth of captive alligators. *Zoologica*:47-52.
- Doube, M., M. M. Kłosowski, I. Arganda-Carreras, F. Cordelières, R. P. Dougherty, J. Jackson, B. Schmid, J. R. Hutchinson, and S. J. Shefelbine. 2010. BoneJ: free and extensible bone image analysis in ImageJ. *Bone* 47:1076-1079.

- Erickson, G. M., and T. A. Tumanova. 2000. Growth curve of *Psittacosaurus mongoliensis* Osborn (Ceratopsia: Psittacosauridae) inferred from long bone histology. *Zoological Journal of the Linnean Society* 130:551-566.
- Farlow, J. O., S. Hayashi, and G. J. Tattersall. 2010. Internal vascularity of the dermal plates of *Stegosaurus* (Ornithischia, Thyreophora). *Swiss Journal of Geoscience* 103:173-185.
- Fowler, D. W., H. N. Woodward, E. A. Freedman, P. L. Larson, and J. R. Horner. 2011. Reanalysis of "*Raptorex kriegsteini*": a juvenile tyrannosaurid dinosaur from Mongolia. *Public Library of Science ONE* 6:e21376.
- Francillon-Vieillot, H., V. d. Buffrenil, J. Castanet, J. Geraudie, F. J. Meunier, J. Y. Sire, L. Zylberberg, and A. d. Ricqlès. 1990. Microstructure and mineralization of vertebrate skeletal tissues; pp. 471-548 in J. G. Carter (ed.), *Skeletal biomineralization patterns, processes and evolutionary trends*. Van Nostrand Reinhold, New York.
- Frey, T. v. E. 1988. Das Tragsystem der Krododile- eine biomechanische und phylogenetische Analyse. *Stuttgarter Beiträge zur Naturkunde Serie A (Biologie)* 426:1-60.
- Frylestam, B., and T. von Schantz. 1977. Age determination of European hares based on periosteal growth lines. *Mammal Review* 7:151-154.
- Hall, B. K. 2005. *Bones and Cartilage: Developmental and Evolutionary Skeletal Biology*. Elsevier Academic Press, San Diego, 760 pp.
- Hemelaar, A. S. M., and J. J. van Gelder. 1980. Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. *Netherlands Journal of Zoology* 30:129-135.
- Horner, J. R., and M. B. Goodwin. 2009. Extreme cranial ontogeny in the Upper Cretaceous dinosaur *Pachycephalosaurus*. *Public Library of Science ONE* 4:e7626.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 1999. Variation in dinosaur skeletochronology indicators: implications for age assessment and physiology. *Paleobiology* 25:295-304.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 2000. Long bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. *Journal of Vertebrate Paleontology* 20:115-129.

- Horner, J. R., K. Padian, and A. d. Ricqlès. 2001. Comparative osteohistology of some embryonic and perinatal archosaurs: developmental and behavioral implications for dinosaurs. *Paleobiology* 27:39-58.
- Hutton, J. M. 1986. Age determination of living Nile crocodiles from the cortical stratification of bone. *Copeia* 2:332-341.
- Joanen, T., and L. McNease. 1987. Alligator farming research in Louisiana, USA; pp. 329-340 in J. W. Grahame, S. Webb, C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.
- Lee, A. H., and S. Werning. 2008. Sexual maturity in growing dinosaurs does not fit reptilian growth models. *Proceedings of the National Academy of Sciences, USA* 105:582-587.
- Margerie, E. d. 2002. Laminar bone as an adaptation to torsional loads in flapping flight. *Journal of Anatomy* 201:521-526.
- Montes, L., J. Castanet, and J. Cubo. 2010. Relationship between bone growth rate and bone tissue organization in amniotes: first test of Amprino's rule in a phylogenetic context. *Animal Biology* 60:25-41.
- Padian, K., and J. R. Horner. 2002. Typology versus transformation in the origin of birds. *TRENDS in Ecology & Evolution* 17:120-124.
- Padian, K., and J. R. Horner. 2004. *Dinosaur Physiology*; pp. in D. B. Weishampel, P. Dodson, and H. Osmólska (eds.), *The Dinosauria*. University of California Press, Berkeley.
- Peabody, F. E. 1961. Annual growth zones in living and fossil vertebrates. *Journal of Morphology* 108:11-62.
- Reid, R. H. 1984. Primary bone and dinosaurian physiology. *Geological Magazine* 121:589-598.
- Reid, R. H. 1990. Zonal "growth rings" in dinosaurs. *Modern Geology* 15:19-48.
- Ricqlès, A. d. 1974. Evolution of endothermy: histological evidence. *Evolutionary Theory* 1:51-80.
- Ricqlès, A. d. 1980. Tissue structures of dinosaur bone: functional significance and possible relation to dinosaur physiology; pp. 103-138 in R. D. K. Thomas and E.

- C. Olson (eds.), *A Cold Look at the Warm Blooded Dinosaurs*. Westview Press, Boulder.
- Ricqlès, A. d. 1983. Cyclical growth in the long limb bones of a sauropod dinosaur. *Acta Palaeontologica Polonica* 28:225-232.
- Ricqlès, A. d., K. Padian, and J. R. Horner. 2001: The bone histology of basal birds in phylogenetic and ontogenetic perspectives. *New Perspectives on the Origin and Early Evolution of Birds: Proceedings of the International Symposium in Honor of John H. Ostrom*.
- Ricqlès, A. d., F. J. Meunier, J. Castanet, and H. Francillon-Vieillot. 1991. Comparative microstructure of bone; pp. 1-78 in B. K. Hall (ed.), *Bone: Bone Matrix and Bone Specific Products*. CRC Press, Boca Raton.
- Sander, P. M., and P. Andrásy. 2006. Lines of arrested growth and long bone histology in Pleistocene large mammals from Germany: what do they tell us about dinosaur physiology? *Palaeontographica A* 277:143-159.
- Scannella, J. B., and J. R. Horner. 2010. *Torosaurus* Marsh, 1891, is *Triceratops* Marsh, 1889 (Ceratopsidae: Chasmosaurinae): synonymy through ontogeny. *Journal of Vertebrate Paleontology* 30:1157-1168.
- Schweitzer, M. H., R. M. Elsey, C. G. Dacke, J. R. Horner, and E.-T. Lamm. 2007. Do egg-laying crocodilian (*Alligator mississippiensis*) archosaurs form medullary bone? *Bone* 40:1152-1158.
- Snoover, M. L., and A. A. Hohn. 2004. Validation and interpretation of annual skeletal marks in loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles. *Fishery Bulletin* 102:682-692.
- Starck, J. M., and A. Chinsamy. 2002. Bone microstructure and developmental plasticity in birds and other dinosaurs. *Journal of Morphology* 254:232-246.
- Tucker, A. D. 1997. Validation of skeletochronology to determine age of freshwater crocodiles (*Crocodylus johnstoni*). *Australian Journal of Marine and Freshwater Research* 48:343-351.
- Tumarkin-Deratzian, A. R. 2007. Fibrolamellar bone in wild adult *Alligator mississippiensis*. *Journal of Herpetology* 41:341-345.
- Turvey, S. T., O. R. Green, and R. Holdaway. 2005. Cortical growth marks reveal extended juvenile development in New Zealand moa. *Nature* 435:940-943.

Witmer, L. M. 1995. The extant phylogenetic bracket and the importance of reconstructing soft tissues in fossils; pp. 296 in J. Thomason (ed.), *Functional morphology in vertebrate paleontology*. Cambridge University Press.

CHAPTER THREE

OSTEOHISTOLOGICAL EVIDENCE FOR DETERMINATE GROWTH IN THE
AMERICAN ALLIGATOR

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: HollyNoelle W. Ballard

Contributions: Conceived the study, examined and photographed specimens, analyzed data, interpreted results, and wrote the manuscript.

Co-author: John R. Horner

Contributions: Provided funding and microphotograph equipment. Assisted with study design, discussed implications of results, and edited earlier drafts of the manuscript.

Co-author: James O. Farlow

Contributions: Allowed access to alligator thin sections used in the study by repositing them at the Museum of the Rockies, discussed the results, and edited earlier drafts of the manuscript.

Manuscript Information Page

Holly N. Woodward, John R. Horner, James O. Farlow

Journal Name: Journal of Herpetology

Status of Manuscript:

☐ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☒ Published in a peer-reviewed journal

Published by The Society for the Study of Amphibians and Reptiles

Issue 45(3): 339-342. 2011

The following chapter has been published in the Journal of Herpetology and appears in this dissertation with the journal's permission. The copyright is held by the Society for the Study of Amphibians and Reptiles.

Abstract

An external fundamental system (EFS) is a form of bone microstructure present in the outermost cortex of long bones in animals that have attained skeletal maturity. It indicates an effective cessation of any significant periosteal growth (i.e., growth in circumference or girth). Although an EFS has been noted in several reptile taxa, the idea that reptiles grow continually throughout their lives remains popular. Examination of femoral bone microstructure from captive American alligators (*Alligator mississippiensis*) reveals parallel-fibered tissue terminating periosteally in an EFS, thus confirming determinate growth in yet another reptile taxon. The results of this study have several important implications for both modern and fossil tetrapods: first, because many birds, non-avian dinosaurs, pterosaurs, and basal pseudosuchians all produce an EFS, it can be concluded that determinate growth is a shared characteristic of Archosauria; second, because the captive alligators were not senescent, an EFS should not be associated with “old age” when interpreting growth histories of extinct animals; third, if no EFS is present, this should not immediately suggest indeterminate growth but rather that skeletal maturity was not attained prior to death. In addition, this study highlights the need for more osteohistological studies to establish exactly how widespread determinate growth is within both extinct and extant members of Sauropsida, as this form of growth may be the rule rather than the exception.

Key Words: Alligator, Histology, External Fundamental Systems

Introduction

Although osteohistology has been applied extensively to extant non-avemetatarsalian sauropsids (i.e., “reptiles”) to gain insights into their growth history and ecophysiology, the importance of finding an external fundamental system (EFS) in reptile limb elements has not been significantly addressed, even though its presence challenges the popular idea that reptiles never reach skeletal maturity. An EFS is a form of bone microstructure sometimes observed in the outermost cortex within a transverse bone cross section, especially within long bones. It is sparsely vascularized and composed of slowly deposited parallel-fibered or lamellar tissue essentially producing a smooth, “finished” bone surface (e.g., Cormack, 1987; Starck and Chinsamy, 2002; Ponton et al., 2004). The presence of this feature is important because it signals an effective cessation of growth in the periosteal circumference of long bones and confirms attainment of skeletal maturity (although increases in mass are still possible). This structure has been positively identified in mammals (e.g., Cormack, 1987), birds (e.g., Ponton et al., 2004), non-avian dinosaurs (e.g., Erickson et al., 2004; Horner and Padian, 2004; Padian et al., 2004), and has been noted in some reptiles (e.g., Castanet et al., 1988; Buffrénil and Castanet, 2000; Ricqlès et al., 2003; Klein et al., 2009).

Numerous observational ecological studies suggest that some reptile taxa do become skeletally mature, such as garter snakes (Bronikowski and Arnold, 1999), green anoles (Lailvaux et al., 2004), Blanding's turtle (Congdon et al., 2001), and even *Alligator* (Chabreck and Joanen, 1979; Wilkinson and Rhodes, 1997; Wilkinson, 2008). In fact, some lepidosaurs are known to possess secondary centers of ossification in their

long bone epiphyses similar to those of mammals. These secondary centers fuse to the bone shaft at the metaphysis in mature individuals, effectively ceasing growth in length, although there is a range of individual maximum sizes for any particular taxon (Andrews, 1982). Secondary centers of ossification also develop in the tuatara (Andrews, 1982) after skeletal maturity is achieved within 15 to 25 years (Castanet et al., 1988).

Despite these lines of evidence, references to the indeterminate growth of reptiles can still be found in recent literature (e.g., Kozłowski, 1996; Olsson and Shine, 1996; Bronikowski and Arnold, 1999; Heino and Kaitala, 1999; Moore et al., 2000; Charnov et al., 2001; Chinsamy-Turan, 2005; Sparkman et al., 2007; Stamps, 2007; Tumarkin-Deratzian, 2007). The idea that reptiles have indeterminate growth may be perpetuated by the long lifespan of many taxa and the ability to adjust growth rates to changing environmental conditions, but it is important to establish which reptile groups are capable of attaining skeletal maturity as this affects how growth rates and other aspects of physiology are modeled. The current study reports on an external fundamental system within yet another reptile, the American Alligator (*Alligator mississippiensis*). The significance of finding an EFS in alligators as well as in other reptile taxa is discussed, as well as implications for interpreting the osteohistology of extinct sauropsids.

Materials and Methods

Eggs of *Alligator mississippiensis* were collected and artificially incubated from wild alligator breeding populations in 1972 and 1973 at the Rockefeller Wildlife Refuge in Grand Chenier, Louisiana as part of an alligator farming study (Joanen and McNease,

1987). The alligators were raised in captivity from hatching until their termination in 1998, at which point they were in their 26th or 27th year of life. Throughout their lives, food was provided on a weekly basis between March and October (alligators refused rations from November to February as ambient temperatures dropped).

After termination, mass and body length were recorded and femora were removed, their dimensions also recorded. These data have been used in several studies to date (Farlow and Britton, 2000; Farlow et al., 2005; Bonnan et al., 2008). Mid-diaphyseal thin sections of the femora were made prior to the current study. Thirty of those thin sections were utilized to assess crocodylian growth histories. The thin sections were observed using a Nikon Optiphot-Pol microscope and photographs were taken at 10x total magnification using a Nikon DS-Fi1 digital sight camera. The alligator individuals in this report represent the oldest completely captive-bred crocodylian population ever examined histologically.

Results

The inner femoral cortex microstructure of the specimens consists of loosely parallel-fibered tissue (i.e., collagen fibers with a parallel orientation but with some degree of disorganization) with radial, longitudinal, and reticular vascularization, interrupted periodically by lines of arrested growth (LAGs) (Fig. 3.1A). This microstructure is consistent with that described in an earlier ontogenetic study of alligator femoral histology (Lee, 2004). In all but three specimens there is an abrupt change after the final LAG to thin layers of highly organized parallel-fibered tissue separated by 3 to

11 closely spaced LAGs (Fig. 3.1B). This region is predominately avascular, but in some areas may contain several simple primary vascular canals. This tissue pattern strongly resembles the external fundamental systems described in fossils of non-avian dinosaurs and extinct pseudosuchians (Fig. 3.1C, 3.1D) (e.g., Chinsamy, 1990; Ricqlès et al., 2003; Erickson et al., 2004; Horner and Padian, 2004).

Discussion

The discovery of an EFS in alligators is predicted cladistically since an EFS was previously reported for several Triassic crocodylian-line pseudosuchian archosaurs (Ricqlès et al., 2003). As an EFS is also found in some pterosaurs and dinosaurs, it is likely this is a shared characteristic of Archosauria. For future research, pairing osteohistology with phylogeny may help to initially narrow the search for an EFS in sauropsids or suggest branches for exploration (Fig. 3.2).

Although there is increasing observational evidence for determinate growth in reptiles, it is often logistically impractical to carry out decades-long growth studies on long-lived reptiles to confirm initial results. Osteohistology can not only be used to validate observational growth history findings, but has the added advantage of taking very little time. Osteohistology requires only that the largest known individuals of a taxon are collected and examined for an EFS. Therefore, osteohistology should be used to quickly increase the number of reptile taxa examined for skeletal maturity and could aid in establishing how extensive determinate growth is within Sauropsida.

This would also help alleviate another feature contributing to the appearance of

indeterminate growth in reptiles: the large range of body sizes associated with the same age in many species of turtles, lizards, crocodylians, and perhaps some dinosaurs (e.g., Andrews, 1982; Gibson and Hamilton, 1984; Lovich et al., 1990; Congdon and van Loben Sels, 1993; Olsson and Shine, 1996; Horner and Padian, 2004; Sander and Klein, 2005). In fact, it has been demonstrated that year-old captive alligators often resemble wild alligators twice their age (Coulson et al., 1973). These studies suggest that body size is a poor way to diagnose indeterminate growth, and support the need for more osteohistological studies.

Prior the current study, an EFS had been noted in only one alligator (Klein et al., 2009). It could be argued that an EFS in a single alligator may be anomalous (especially given the life history of that particular animal), but the presence of an EFS in multiple captive individuals provides confirmation of determinate growth for *Alligator mississippiensis*. Three femora lacking EFS did have LAGs within the cortex and approaching the periosteal surface. Rather than demonstrating that determinate growth is inconsistent within *Alligator*, the three individuals without EFS most likely had not reached asymptotic size. As an asymptote represents the average age at which skeletal maturity is achieved, variation about this mean is expected in a population. Unfortunately, as LAG counts within the EFS are unreliable for aging (Horner et al., 1999), and the innermost-cortex LAGs are missing due to medullary expansion, individual ages at which skeletal maturity was obtained are impossible to determine. However, the large number of LAGs within the EFS in some of the individuals (up to 11) suggests that at least some of the alligators had reached skeletal maturity several years

prior to termination.

Based on results from this alligator population, the lack of an EFS in tetrapods with prolonged growth durations (whether extant or extinct) should not immediately suggest continued (i.e., indeterminate) growth, but rather that the animal did not attain skeletal maturity before death. The relative scarcity of an EFS in living and fossil reptiles testifies to the high degree of mortality before skeletal maturity is reached.

As most of the captive alligators had achieved skeletal maturity some years before termination, growth duration should not be confused with longevity (i.e., that the death of the animal was due to “old age”). The duration of skeletal maturity revealed by the presence of an EFS therefore has implications for extinct taxa. For example, the transition from LAGs in the cortex to an EFS in the oldest specimen of *Tyrannosaurus rex* figured to date (Erickson et al., 2004) occurred at 19-20 years of age. In this case, the presence of an EFS should not suggest the *Tyrannosaurus* had reached asymptotic age but only that this individual had attained skeletal maturity several years before its death. Thus, when interpreting growth histories of extinct animals, it must be kept in mind that they could potentially have lived for many years after the attainment of skeletal maturity.

Conclusions

Although many recent ecological studies suggest that the growth histories of reptiles are determinate, the idea that reptiles continue to grow throughout their lives remains popular. Osteohistology can be utilized to support mark-recapture results that suggest the growth of a particular taxon is determinate by providing evidence in the form

of an external fundamental system, the presence of which indicates a virtual cessation of growth in length. Osteohistology not only provides a way to validate observational studies, but can do so in very little time compared to the many years often required to obtain growth history data in the field and should therefore be used to provide a survey of EFS in extant sauropsids. In addition, the presence of an EFS in a bone from an extinct animal indicates it had obtained skeletal maturity before death. However, the results from the captive alligator study show that an EFS does not equate to longevity- a long-bone does not accurately record the remaining span of life after skeletal maturity is achieved and therefore an EFS does not indicate that an asymptotic age has also been obtained.

An EFS in crocodylians is comparatively rare, but the strong resemblance to features referred to as an EFS in extant mammals and birds, and particularly resembling those found in non-avian dinosaurs, supports their diagnosis here. In fact, an EFS in derived pseudosuchians should be expected as the presence of this structure was noted in several crocodylian-line Triassic pseudosuchians. Because determinate growth histories are found in some extinct pseudosuchians and repeatedly established for dinosaurs and pterosaurs, the confirmation of determinate growth in a long-term captive population of *Alligator mississippiensis* suggests that this is a basal trait of Archosauria. The discovery of an EFS in yet another reptile adds additional support to the hypothesis that determinate growth may be the rule rather than the exception for sauropsids and the results reported herein should encourage additional osteohistological studies to establish the extent to which determinate growth exists within Sauropsida.

Acknowledgments

We thank R. Elsey and the Louisiana Department of Wildlife and Fisheries for providing specimens for analysis and E.-T. Lamm (Museum of the Rockies) for thin section preparation. The Museum of the Rockies provided facilities for histological analyses and microphotograph digital imaging equipment. K. Padian contributed many helpful comments on an earlier draft, and J. Botha-Brink and A. Lee reviewed the submitted manuscript. The manuscript was strengthened further from suggestions by A. Lee. K. Padian and S. Werning supplied the thin section image of *Desmotosuchus*.

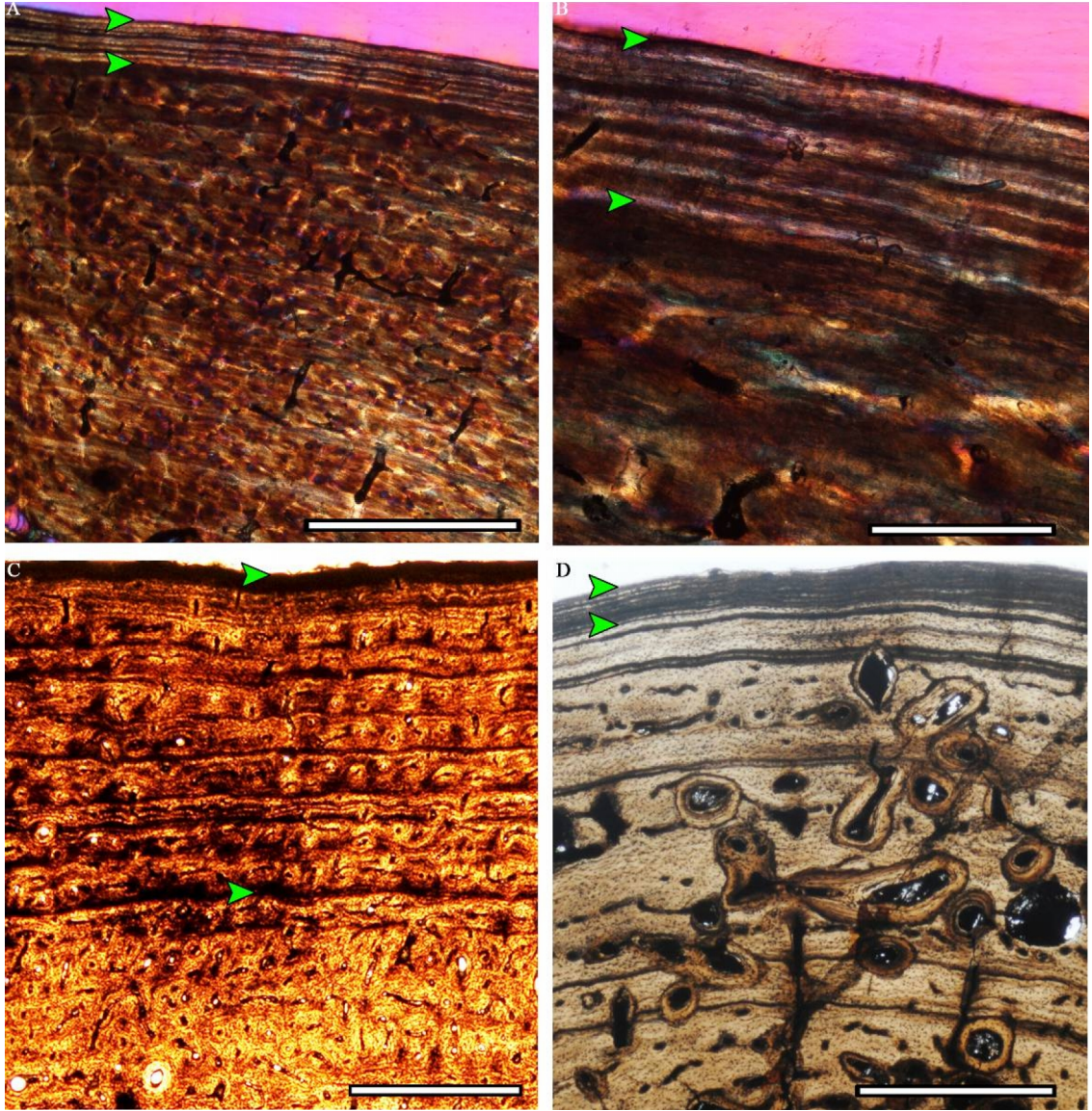


Figure 3.1. Bone histology of a captive *Alligator*, a *Tyrannosaurus*, and a *Desmotosuchus*. Arrows bound the region of an EFS. A) Captive *Alligator* femur diaphyseal section (Museum of the Rockies, RWR 29) spanning the inner cortex (bottom) to the periosteal surface (top). Tissue organization is parallel-fibered and interrupted periodically by LAGs. An EFS is visible near the bone surface. Scale bar, 2 mm. B) An enlargement of the EFS region from A) showing closely spaced LAGs separating annuli of parallel-fibered tissue. Scale bar, 500 μ m. C) Outer diaphyseal cortex from the fibula of a *Tyrannosaurus* (Museum of the Rockies, MOR 1152). An EFS is visible near the periosteal surface. Scale bar, 1 mm. D) Outer cortex from the humeral diaphysis of a *Desmotosuchus* (University of California Museum of Paleontology, UCMP 3218). An EFS is visible near the outermost cortex (top), made of closely spaced, avascular lamellar tissue containing multiple LAGs. Scale bar, 1 mm.

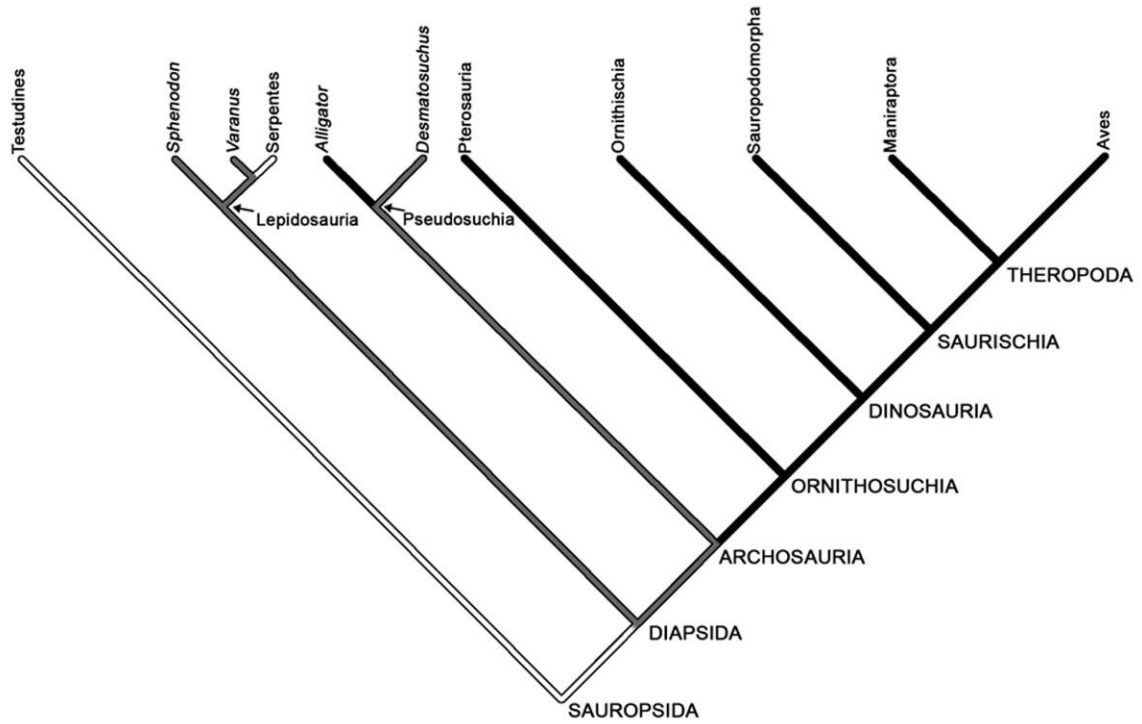


Figure 3.2. Generalized cladogram of Sauropsida (after Ricqlès et al., 2003) diagramming the presence of an EFS in various groups. Black shading indicates that an EFS is well established within a clade, while gray shading means an EFS has been reported in one to several specimens in that clade. An EFS has not been noted in taxa within white branches.

Literature Cited

- Andrews, R. M. 1982. Patterns of growth in reptiles; pp. 273-320 in C. Gans and F. H. Pough (eds.), *Biology of the Reptilia*. Academic Press, London, England.
- Bonnan, M. F., J. O. Farlow, and S. L. Masters. 2008. Using linear and geometric morphometrics to detect intraspecific variability and sexual dimorphism in femoral shape in *Alligator mississippiensis* and its implications for sexing fossil archosaurs. *Journal of Vertebrate Paleontology* 28:422-431.
- Bronikowski, A. M., and S. J. Arnold. 1999. The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. *Ecology* 80:2314-2325.
- Buffrénil, V. d., and J. Castanet. 2000. Age estimation by skeletochronology in the Nile monitor (*Varanus niloticus*), a highly exploited species. *Journal of Herpetology* 34:414-424.
- Castanet, J., D. G. Newman, and H. S. Giron. 1988. Skeletochronological data on the growth, age, and population structure of the tuatara, *Sphenodon punctatus*, on Stephens and Lady Alice Islands, New Zealand. *Herpetologica* 44:25-37.
- Chabreck, R. H., and T. Joanen. 1979. Growth rates of American alligators in Louisiana. *Herpetologica* 35:51-57.
- Charnov, E. L., T. F. Turner, and K. O. Winemiller. 2001. Reproductive constraints and the evolution of life histories with indeterminate growth. *Proceedings of the National Academy of Sciences, USA* 98:9460-9464.
- Chinsamy-Turan, A. 2005. *The Microstructure of Dinosaur Bone: Deciphering Biology with Fine-Scale Techniques*. The Johns Hopkins University Press, Baltimore.
- Chinsamy, A. 1990. Physiological implications of the bone histology of *Syntarsus rhodesiensis* (Saurischia: Theropoda). *Palaeontologia Africana* 27:77-82.
- Congdon, J. D., and R. C. van Loben Sels. 1993. Relationships of reproductive traits and body size with attainment of sexual maturity and age in Blanding's turtles (*Emydoidea blandingii*). *Journal of Evolutionary Biology* 6:547-557.
- Congdon, J. D., R. D. Nagle, O. M. Kinney, and R. C. van Loben Sels. 2001. Hypotheses of aging in a long-lived vertebrate, Blanding's turtle (*Emydoidea blandingii*). *Experimental Gerontology* 36:813-827.
- Cormack, D. 1987. *Ham's Histology*. Lippincott Williams & Wilkins, New York, 732 pp.

- Coulson, T. D., R. A. Coulson, and T. Hernandez. 1973. Some observations on the growth of captive alligators. *Zoologica*:47-52.
- Erickson, G. M., P. J. Makovicky, P. J. Currie, M. A. Norell, S. A. Yerby, and C. A. Brochu. 2004. Gigantism and comparative life-history parameters of tyrannosaurid dinosaurs. *Nature* 430:772-775.
- Farlow, J. O., and A. Britton. 2000. Size and body proportions in *Alligator mississippiensis*: implications for archosaurian ichnology. *Paleontological Society of Korea Special Publication* 4:189-206.
- Farlow, J. O., G. R. Hurlburt, R. M. Elsey, A. R. C. Britton, and W. Langston Jr. 2005. Femoral dimensions and body size of *Alligator mississippiensis*: estimating the size of extinct mesoeucrocodylians. *Journal of Vertebrate Paleontology* 25:354-369.
- Gibson, C. W. D., and J. Hamilton. 1984. Population processes in a large herbivorous reptile: the giant tortoise of Aldabra atoll. *Oecologia* 61:230-240.
- Heino, M., and V. Kaitala. 1999. Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. *Journal of Evolutionary Biology* 12:423-429.
- Horner, J. R., and K. Padian. 2004. Age and growth dynamics of *Tyrannosaurus rex*. *Proceedings of the Royal Society of London Series B* 271:1875-1880.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 1999. Variation in dinosaur skeletochronology indicators: implications for age assessment and physiology. *Paleobiology* 25:295-304.
- Joanen, T., and L. McNease. 1987. Alligator farming research in Louisiana, USA; pp. 329-340 in J. W. Grahame, S. Webb, C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.
- Klein, N., T. Scheyer, and T. Tütken. 2009. Skeletochronology and isotopic analysis of a captive individual of *Alligator mississippiensis* Daudin, 1802. *Fossil Record* 12:121-131.
- Kozłowski, J. 1996. Optimal allocation of resources explains interspecific life-history patterns in animals with indeterminate growth. *Proceedings of the Royal Society of London Series B* 263:559-566.
- Lailvaux, S. P., A. Herrel, B. VanHooydonck, J. J. Meyers, and D. J. Irschick. 2004.

- Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proceedings of the Royal Society of London Series B* 271:2501-2508.
- Lee, A. H. 2004. Histological organization and its relationship to function in the femur of *Alligator mississippiensis*. *Journal of Anatomy* 204:197-207.
- Lovich, J. E., C. H. Ernst, and J. F. McBreen. 1990. Growth, maturity, and sexual dimorphism in the wood turtle, *Clemmys insculpta*. *Canadian Journal of Zoology* 68:572-677.
- Moore, I. T., M. P. Lemaster, and R. T. Mason. 2000. Behavioural and hormonal responses to capture stress in the male red-sided garter snake, *Thamnophis sirtalis parietalis*. *Animal Behaviour* 59:529-534.
- Olsson, M., and R. Shine. 1996. Does reproductive success increase with age or with size in species with indeterminate growth? A case study using sand lizards (*Lacerta agilis*). *Oecologia* 105:175-178.
- Padian, K., J. R. Horner, and A. d. Ricqlès. 2004. Growth in small dinosaurs and pterosaurs: the evolution of archosaurian growth strategies. *Journal of Vertebrate Paleontology* 24:555-571.
- Ponton, F., A. Elzanowski, J. Castanet, A. Chinsamy, E. d. Margerie, A. d. Ricqlès, and J. Cubo. 2004. Variation of the outer circumferential layer in the limb bones of birds. *Acta Ornithologica* 39:21-24.
- Ricqlès, A. d., K. Padian, and J. R. Horner. 2003. On the bone histology of some Triassic pseudosuchian archosaurs and related taxa. *Annales de Paléontologie* 89:67-101.
- Sander, P. M., and N. Klein. 2005. Developmental plasticity in the life history of a prosauropod dinosaur. *Science* 310:1800-1802.
- Sparkman, A. M., S. J. Arnold, and A. M. Bronikowski. 2007. An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the garter snake *Thamnophis elegans*. *Proceedings of the Royal Society of London Series B* 274:943-950.
- Stamps, J. A. 2007. Growth-mortality tradeoffs and 'personality traits' in animals. *Ecology Letters* 10:355-363.
- Starck, J. M., and A. Chinsamy. 2002. Bone microstructure and developmental plasticity in birds and other dinosaurs. *Journal of Morphology* 254:232-246.

- Tumarkin-Deratzian, A. R. 2007. Fibrolamellar bone in wild adult *Alligator mississippiensis*. *Journal of Herpetology* 41:341-345.
- Wilkinson, P. M. 2008: American Alligator growth: determinate or indeterminate? Proceedings of the 19th Working Meeting of the IUCN-SSC Crocodile Specialist Group, Santa Cruz, Bolivia.
- Wilkinson, P. M., and W. E. Rhodes. 1997. Growth rates of American alligators in coastal South Carolina. *The Journal of Wildlife Management* 61:397-402.

CHAPTER FOUR

GROWTH DYNAMICS OF AUSTRALIA'S POLAR DINOSAURS

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

Author: HollyNoelle W. Ballard

Contributions: Conceived the study, processed specimens, analyzed data, interpreted results, and wrote the manuscript.

Co-author: Thomas H. Rich

Contributions: Provided access to specimens, laboratory materials and workspace, and suggested revisions on earlier drafts of the manuscript.

Co-author: Anusuya Chinsamy

Contributions: Supplied additional fossil material for analysis, suggested revisions on earlier drafts of the manuscript.

Co-author: Patricia Vickers-Rich

Contributions: Provided access to specimens, laboratory materials and workspace, and suggested revisions on earlier drafts of the manuscript

Manuscript Information Page

Holly N. Woodward, Thomas H. Rich, Anusuya Chinsamy, Patricia Vickers-Rich

Journal Name: Public Library of Science ONE

Status of Manuscript:

☐ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☒ Published in a peer-reviewed journal

Published by Public Library of Science

Issue 6(8): e23339. 2011

The following chapter has been published in the open-access journal PLoS ONE and appears in this dissertation under the terms of the Creative Commons Attribution License.

Abstract

Analysis of bone microstructure in ornithopod and theropod dinosaurs from Victoria, Australia, documents ontogenetic changes, providing insight into the dinosaurs' successful habitation of Cretaceous Antarctic environments. Woven-fibered bone tissue in the smallest specimens indicates rapid growth rates during early ontogeny. Later ontogeny is marked by parallel-fibered tissue, suggesting reduced growth rates approaching skeletal maturity. Bone microstructure similarities between the ornithopods and theropods, including the presence of LAGs in each group, suggest there is no osteohistologic evidence supporting the hypothesis that polar theropods hibernated seasonally. Results instead suggest high-latitude dinosaurs had growth trajectories similar to their lower-latitude relatives and thus, rapid early ontogenetic growth and the cyclical suspensions of growth inherent in the theropod and ornithopod lineages enabled them to successfully exploit polar regions.

Introduction

During the Early Cretaceous, the state of Victoria, Australia, lay within the Antarctic Circle between the paleolatitudes of 75°S and 80°S (Rich and Rich, 1989; Veevers et al., 1991). Although there is no firm consensus regarding the severity of environmental conditions (Wagstaff and McEwen-Mason, 1989; Rich and Vickers-Rich, 1999), this region certainly experienced prolonged periods of light and dark (Rich and Rich, 1989) with mean annual air temperatures ranging between -6° C and +10° C (Hallam, 1985; Gregory et al., 1989; Spicer and Corfield, 1992). Because of this, it has been hypothesized that dinosaurs inhabiting this unique environment were physiologically distinct from their counterparts at lower latitudes (Chinsamy et al., 1998; Chinsamy-Turan, 2005) in order to survive the environmental extremes. To date, no fully articulated dinosaur specimens have been recovered from Victoria. Often, specimens are quite fragmentary, making taxonomic assignment impossible. Interestingly, the majority of identifiable Early Cretaceous (Aptian-Albian) dinosaur material collected from Victoria is from small-bodied ornithopods (i.e., “hypsilophodontids”). As “hypsilophodontids” are considered a paraphyletic grouping (Horner et al., 2009) the use of the term here is informal and refers to small bodied ornithischians possessing basal ornithopod characteristics .

Because fossil bone histology provides insights into the growth dynamics of extinct animals (Chinsamy-Turan, 2005), histological analysis can be reliably used to assess hypotheses related to physiology. A previous osteohistologic study of femora from a Victorian polar theropod and hypsilophodontid suggested that these dinosaurs were

behaviorally different. Presence of zonal bone was interpreted to mean that the theropod likely hibernated during the severe winters, whereas the absence of zonal bone in the hypsilophodontid suggested that it remained active and continued to grow throughout the winter (Chinsamy et al., 1998). This hypothesis continues to influence interpretations of polar dinosaur behavior and physiology (e.g., (Horner et al., 1999; Tutken et al., 2004; Erickson, 2005; Martin, 2009; Trusler et al., 2010)).

Since that initial study, the number of hypsilophodontid fossils collected from Victoria has increased substantially, thus permitting a larger sample for osteohistologic examination. The current assessment includes nine femora and eight tibiae from hypsilophodontids as well as one theropod femur. Although the number of Early Cretaceous hypsilophodontid genera may be as high as six (Rich and Rich, 1989; Rich and Vickers-Rich, 1999), it has recently been argued that the diversity was much less (Agnolin et al., 2010) but this remains unresolved. Regardless, as only limb elements are used in this study the specimens are not identified beyond “hypsilophodontid”. The theropod femur is tentatively assigned to *Timimus* based on similarities to the type femur (Rich and Vickers-Rich, 1994), as well as to the femur examined by Chinsamy (Chinsamy et al., 1998). *Timimus* may either be an ornithomimid (Rich and Vickers-Rich, 1994), or a dromaeosaurid (Agnolin et al., 2010). Results of this study allow evaluation of ontogenetic changes in bone tissue, enable comparisons with the bone microstructure of lower-latitude hypsilophodontid and theropod relatives, and lead to a new hypothesis to explain the successful radiation of dinosaurs in high-latitude environments.

Results

Almost every hypsilophodontid long bone examined had cyclical growth marks in the form of lines of arrested growth (LAGs), or annuli followed by “bright lines” (which are probably equivalent to LAGs) (Table 1). Growth marks were fully traceable around the circumference of the thin sections except where truncated by secondary osteons, crushing, or poor preservation. The smallest hypsilophodontid femur and tibia studied displayed well-vascularized, woven bone tissue (Figure 4.1A) typical of rapidly growing animals (Francillon-Vieillot et al., 1990) and lacked growth marks. The largest hypsilophodontid specimens possessed parallel-fibered tissue in the outer cortex (Figure 4.1E; Figure 4.2) and at least seven LAGs (Table 1), although more may have been lost to remodeling. Included among the samples containing growth marks is the same hypsilophodontid femur (NMV 177935) examined by Chinsamy et al. (Chinsamy et al., 1998). Contrary to the original sections, the two new sections made for this report revealed interruptions in growth in the form of annuli followed by bright lines (Figure 4.2). As with LAGs, bright lines suggest hypermineralized tissue layers representing a brief hiatus in growth (Francillon-Vieillot et al., 1990).

The theropod femur (NMV 186317) sectioned by Chinsamy et al. (Chinsamy et al., 1998) was estimated at 44 cm in length and considered skeletally mature due to its microstructure. In contrast, the theropod femur (NMV 186323; Figure 4.3A) examined in this study is considerably smaller (19.3 cm in length; Table 1), and as in the young hypsilophodontids, the bone microstructure is well-vascularized with primary osteons in

woven tissue- typical of rapidly growing juvenile vertebrates (Francillon-Vieillot et al., 1990). There were no LAGs within the cortex of this specimen.

Discussion

Studies performed on extant taxa imply that growth mark periodicity is annual (e.g., (Buffrénil, 1980; Hemelaar and van Gelder, 1980; Hutton, 1986; Buffrénil and Castanet, 2000)), although the degree to which growth decreases is determined by the type of mark. Lines of arrested growth indicate periodic suspensions in bone mineral deposition and thus suspensions in long bone growth; annuli indicate periodic decreases in the rate at which mineral is deposited, hence only a periodic decrease in overall growth rate (Castanet et al., 1993). Among the hypsilophodontids, the occurrence of either LAGs (suspension of growth) or annuli followed by bright lines (dramatically slowed growth before suspension) in different specimens suggests some measure of inter-specific, intra-specific, or inter-elemental variation in the degree to which growth cyclically decreased.

As the smallest hypsilophodontid femur and tibia consist of woven-fibered tissue and lack growth marks (Figure 4.1A; Table 4.1), these bones may be from individuals less than one year old. In larger individuals, up to three growth marks interrupt the deposition of woven-fibered tissue (Figure 4.1C), indicating an initial 3 year period of rapid growth (Castanet et al., 1993). Thereafter, parallel-fibered tissue, reduced vascularity, and multiple LAGs (Figure 4.1E) reveal that growth slowed significantly but continued for several years until skeletal maturity was reached. Sexual maturity may be marked by the transition from rapid to more slowly formed tissue (Lee and Werning,

2008) at three years of age. The tissues and growth patterns observed in the polar hypsilophodontids are also noted in the bone microstructure of low-latitude basal ornithopods such as *Orodromeus*, *Dryosaurus*, and *Scutellosaurus* (Figure 4.1B, D, F; (Padian et al., 2004; Horner et al., 2009)).

The large theropod femur (NMV 186317) examined by Chinsamy et al. (Chinsamy et al., 1998) consisted of a vascularized woven-fibered inner-cortex that changed to poorly vascularized, parallel-fibered tissue after two LAGs. In all, nine LAGs were visible. This was interpreted as rapid growth for at least two years, and then likely the attainment of sexual maturity (Lee and Werning, 2008), after which growth slowed.

In contrast, the smaller theropod femur examined in this study (NMV 186323) had well vascularized, woven-fibered tissue throughout the cortex and lacked LAGs (Figure 4.3A). Although it is possible that LAGs were obliterated due to medullary expansion, the small size of the femur along with the bone tissue organization suggests that this dinosaur was growing quickly and that it was less than one year of age. The bone tissues observed in NMV 186317 and NMV 186323 strongly resemble the patterns of microstructural changes described in an ontogenetic study of the lower-latitude dromaeosaurid *Troodon formosus* (Figure 4.3B; (Varricchio, 1993)).

Despite the variability between LAGs and annuli within the hypsilophodontid samples, the microstructures and cyclical growth exhibited by both ornithopods and theropods from the high paleolatitude of southeastern Australia resembles patterns observed in dinosaurs from lower paleolatitudes, indicating similarities in growth dynamics and physiology. Although LAGs can form as a result of the slowed metabolic

processes experienced during hibernation, they are not structures exclusive to hibernators (Castanet et al., 1993). Therefore, contrary to the earlier hypothesis of Chinsamy et al. (Chinsamy et al., 1998), we suggest that the presence of growth marks alone cannot be used to support a hibernating behavior.

While it is entirely likely that polar dinosaurs had specialized behavioral and morphological adaptations to cope with their environment, only a few possibilities can be tested with fossil bone microanalyses. For instance, several microstructure studies have demonstrated that Triassic and Jurassic archosaurs (including dinosaurs) exhibited cyclical suspensions in growth as well as high growth rates early in life (Chinsamy, 1990; Ricqlès et al., 2003; Padian et al., 2004; Carrano et al., 2005; Ricqlès et al., 2008; Knoll et al., 2010). These characteristics, also observed in more derived archosaurs, are likely plesiomorphic for the archosauriform clade (Castanet et al., 1993; Ricqlès et al., 2008) and would have allowed dinosaurs to establish themselves in ecological extremes without requiring a change in physiology: early rapid growth reduced the time dinosaurs were highly sensitive to predation and environmental stresses, while periodic suspensions in bone deposition conserved resources during seasonal stresses. Therefore, on the basis of the bone tissues evident in the polar hypsilophodontids and theropods, we propose that the high initial growth rates observed in these taxa, along with cyclical suspensions of growth (possibly coinciding with the dark winter months), are exaptations that could explain their successful exploitation of a hostile high-latitude ecosystem.

Materials and Methods

Ethics Statement

This research was sponsored by the 2010 East Asia and Pacific Summer Institutes program, jointly organized by the National Science Foundation (proposal number OISE-1015130) and the Australian Academy of Sciences. Access to dinosaur fossil collections at Museum Victoria (Melbourne, Victoria, Australia) was provided via a letter of invitation from Dr. Thomas Rich (Senior Curator of Vertebrate Paleontology and Paleobotany) and Museum Victoria permit number VP 2010/07.

Procedure

Nine femora and eight tibiae from small bodied hypsilophodontid ornithopod dinosaurs and one femur from a theropod dinosaur were selected for histological analysis from the collections of Museum Victoria, Melbourne, Victoria, Australia (NMV). Isolated limb elements were collected from either the Wonthaggi Formation (Early Aptian) of the Strzelecki Group or the Eumeralla Formation (latest Aptian-Early Albian) of the Otway Group (Table S1) (Rich et al., 1989; Wagstaff and McEwen-Mason, 1989; Rich and Vickers-Rich, 1999). The small theropod femur examined here (NMV 186323) is tentatively assigned to *Timimus hermani*, based on morphological similarities to the theropod femur (NMV 186317) sampled by Chinsamy et al. (Chinsamy et al., 1998) as well as the type specimen (NMV P186303) (Rich and Vickers-Rich, 1994). However, the ornithopods are not identified beyond “hypsilophodontid”. The hypsilophodontid sample set includes the femur (NMV 177935) originally sampled by Chinsamy et al. (Chinsamy

et al., 1998), from which another section distal to the fourth trochanter was made. In addition, the three original thin sections made from NMV 177935 by Chinsamy et al. (Chinsamy et al., 1998) were provided for comparison, and we verified that no growth marks were visible in these sections.

Samples containing the mid diaphysis were removed from the shafts when possible and first processed using a procedure modified from Schweitzer et al. (Schweitzer et al., 2007). Each sample was next embedded with Specifix-40 (Struers) two-part epoxy resin and then prepared following Lamm (Lamm, 2007). Some thin section slices were mounted to frosted glass slides using two-part epoxy glue, while others were mounted to frosted plastic slides using cyanoacrylate glue. Within days of mounting the specimens onto glass slides the mounted surface developed a “cloudy” appearance suggesting poor adherence, while the plastic slides remained clear. Therefore, the exposed surfaces of the specimens mounted to glass slides were polished to a 600 grit finish and mounted to plastic slides with cyanoacrylate glue, “sandwiching” each specimen between a glass and plastic slide. The glass slide was then loaded onto glass slide chucks (Buehler Ltd.) and removed from the specimen using a 5-inch diameter diamond blade (Norton[®]) and an IsoMet 1000[®] Precision Saw (Buehler Ltd.). Some slides had already been polished to completion before the cloudiness occurred; these were soaked in xylene for clearing and coated with Polymount[®] (Polysciences Inc.) liquid cover slip medium, which penetrated underneath the specimen and restored the original visibility. Due to diagenetic staining of the fossils, many thin sections remained nearly opaque, even when polished very thin. In those cases, a mixture of IsoCut[®] (Buehler Ltd.)

fluid and mineral oil was applied to the finished surfaces, greatly enhancing microstructure visibility. Completed thin section slides were photographed with either 4x or 10x objectives using a Nikon Optiphot-Pol polarizing microscope. Photomicrographs were taken with a Nikon DS-Fi1 digital sight camera, and thin section images were compiled using the NIS-Elements BR 3.0 software.

Acknowledgements

Assistance and facilities were provided by the Australian Academy of Science, Museum Victoria, Monash University, and the Museum of the Rockies. D. Pickering, D. Henry, E. Fitzgerald, T. Holland, and D. Villiers provided access to specimens, equipment, and necessary data. J. Horner and E.-T. Lamm advised on histology procedure. E.-T. Lamm and R. Douglass assisted with specimen preparation. J. Horner, J. Scannella, T. Ballard, and D. Fowler commented on earlier drafts of the manuscript. External reviewers A. Farke, K. Padian, and H. Larsson provided helpful comments which greatly improved the manuscript.

Table 4.1. Data for Australian polar dinosaur fossils included in this study. Estimates of length indicated by asterisk. NMV: Museum Victoria, Melbourne, Victoria, Australia

Museum Number	Element	Element Length (cm)	Growth Marks Preserved	Locality Name	Formation	Locality Coordinates
NMV P186326	Femur	18.5	3 annuli	Dinosaur Cove East	Eumeralla	38° 46' 53±1" S, 143° 24' 14±1" E
NMV P177935	Femur	20.8*	2 annuli	Dinosaur Cove East	Eumeralla	38° 46' 53±1" S, 143° 24' 14±1" E
NMV P150054	Femur	15.5*	3 annuli	Eagle's Nest	Wonthaggi	38° 40.5' S, 145° 40.25' E
NMV P216768	Femur	4.7	0	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P208495	Femur	13*	1 LAG	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P199058	Femur	14.5*	3 LAGs	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P221151	Femur	16*	5 LAGs	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P180892	Femur	31.5*	7 LAGs	Rotten Point	Eumeralla	38° 46' 55±1" S, 143° 24' 3±1" E
NMV P186047	Femur	12.8	4 annuli	Slippery Rock	Eumeralla	38° 46' 54±1" S, 143° 24' 15±1" E
NMV P186334	Tibia	17.2	4 annuli	Dinosaur Cove East	Eumeralla	38° 46' 53±1" S, 143° 24' 14±1" E
NMV P210062	Tibia	11.5	0	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P208204	Tibia	14.4*	2 LAGs	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P208336	Tibia	19.4*	4 LAGs	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P199133	Tibia	16	3 LAGs	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P208189	Tibia	18.8	2 LAGs	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P228434	Tibia	21.8	7 LAGs	Flat Rocks	Wonthaggi	38° 39' 40±2" S, 145° 40' 52±3" E
NMV P228360	Tibia	20	6 LAGs	Point Lewis	Eumeralla	38° 50' 9 to 12" S, 143° 34' 53 to 57" E

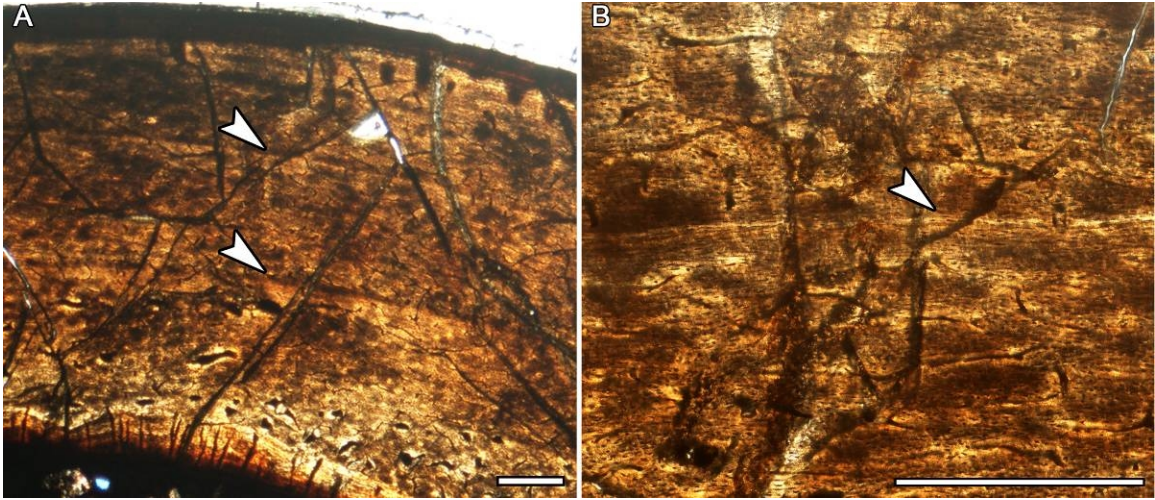


Figure 4.1. Bone microstructure of a mature polar hypsilophodontid. Arrows indicate annuli. Scale bars, 500 μm . A) Diaphyseal section of femur NMV 177935 consisting of parallel-fibered tissue with two growth marks within the cortex. B) Detail of inner growth mark showing a distinctive annulus terminating with a bright line.

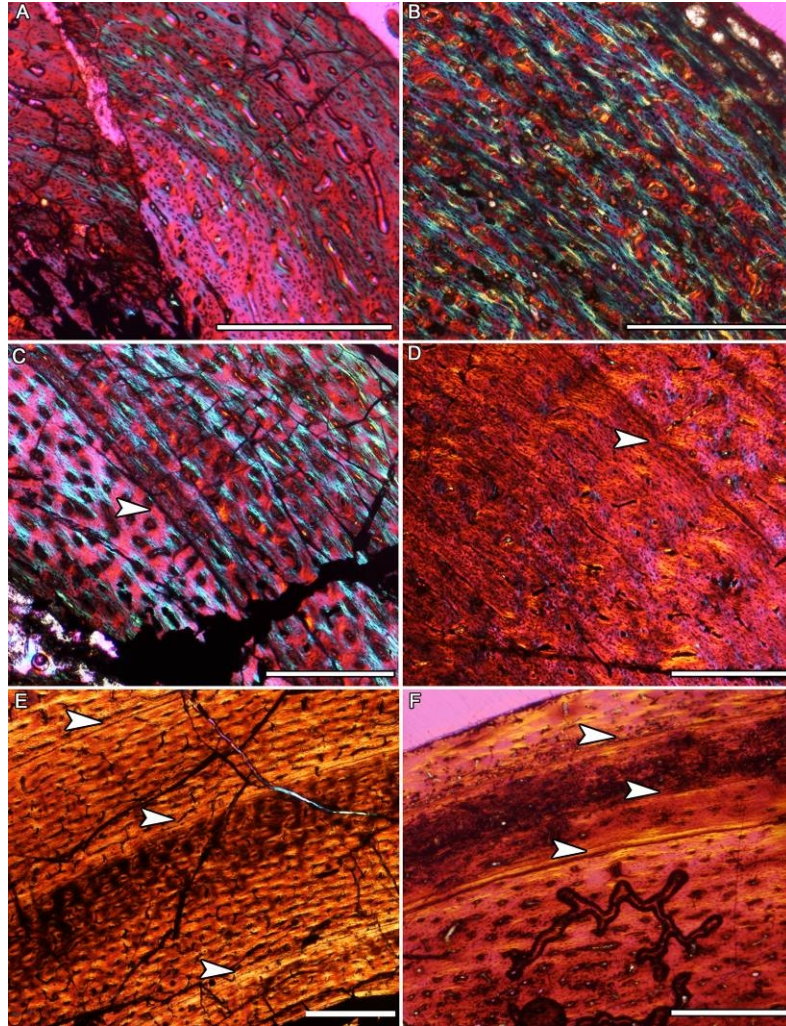


Figure 4.2. Hypsilophodontid ontogenetic bone microstructure. The bone microstructures observed in hypsilophodontids from Victoria suggest ontogenetic status, and strongly resemble microstructures observed in hypsilophodontids from lower latitudes (Museum of the Rockies- MOR; Brigham Young University- BYU). Arrows indicate growth marks (annuli and LAGs). Scale bars, 500 μ m. A) Femur (NMV 216768) diaphyseal section from an immature polar hypsilophodontid. Growth marks are absent and tissue consists of rapidly deposited, disorganized woven fibers. This resembles the femoral diaphyseal section in B), from an immature *Orodromeus* (MOR 407). C) Diaphyseal section of a femur (NMV 208495) from an immature hypsilophodontid showing one LAG within fast growing woven tissue. LAGs within woven tissue are also observed in D), an femur from an immature *Dryosaurus* (BYU 13312). E) Diaphyseal section of a polar hypsilophodontid tibia (NMV 228434) consisting of three well-defined LAGs within loosely parallel-fibered cortex, representing a more mature, slowly growing individual. A similar microstructural pattern is observed in F), an *Orodromeus* tibia (MOR 973), which contains LAGs within a parallel-fibered matrix.

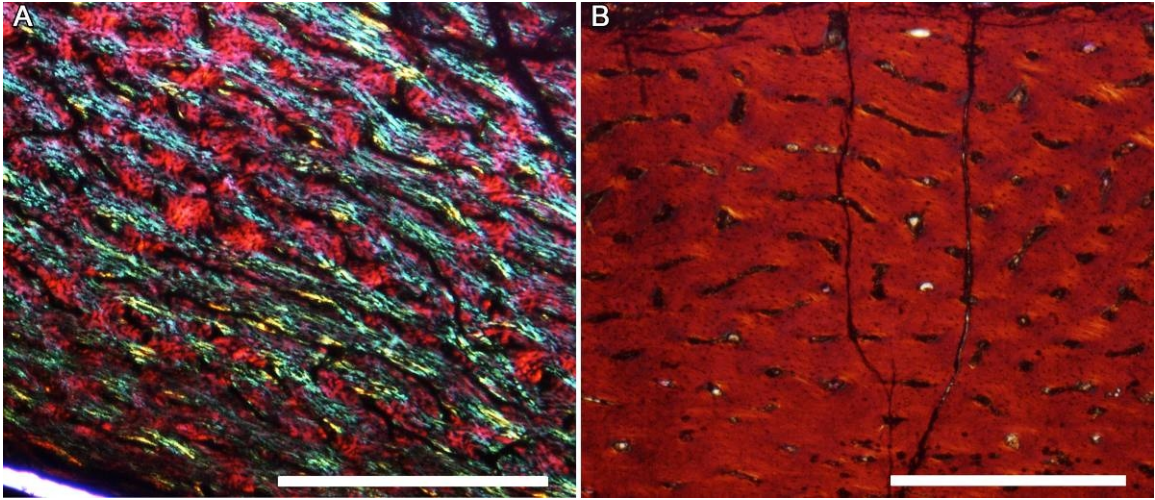


Figure 4.3. Theropod osteohistology. Diaphyseal sections of high latitude and lower latitude theropods. Scale bars, 500 μm . A) Theropod femur diaphyseal section from Victoria (NMV 186323). Note the well-vascularized cortex consisting of rapidly deposited tissue. B) *Troodon* femur (MOR 563) from Montana, USA, showing a similarly well-vascularized cortex and rapidly deposited tissue.

Literature Cited

- Agnolin, F. L., M. D. Ezcurra, D. F. Pais, and S. W. Salisbury. 2010. A reappraisal of the Cretaceous non-avian dinosaur faunas from Australia and New Zealand: evidence for their Gondwanan affinities. *Journal of Systematic Palaeontology* 8:257-300.
- Buffr  nil, V. d. 1980. Mise en evidence de l'incidence des conditions de milieu sur la croissance de *Crocodylus siamensis* (Schneider, 1801), et valeur des marques de croissance squelettiques pour l'evaluation de l'age individuel. *Archives de Zoologie Experimentale et Generale* 121:63-76.
- Buffr  nil, V. d., and J. Castanet. 2000. Age estimation by skeletochronology in the Nile monitor (*Varanus niloticus*), a highly exploited species. *Journal of Herpetology* 34:414-424.
- Carrano, M. T., J. R. Hutchinson, and S. D. Sampson. 2005. New information on *Segisaurus halli*, a small theropod dinosaur from the Early Jurassic of Arizona. *Journal of Vertebrate Paleontology* 25:835-849.
- Castanet, J., H. Francillon-Vieillot, P. J. Meunier, and A. d. Ricql  s. 1993. Bone and individual aging; pp. 245-283 in B. K. Hall (ed.), *Bone*. CRC Press, London.
- Chinsamy-Turan, A. 2005. The microstructure of dinosaur bone: deciphering biology with fine-scale techniques. Johns Hopkins University Press, Baltimore, 216 pp.
- Chinsamy, A. 1990. Physiological implications of the bone histology of *Syntarsus rhodesiensis* (Saurischia: Theropoda). *Palaeontologia Africana* 27:77-82.
- Chinsamy, A., T. Rich, and P. Vickers-Rich. 1998. Polar dinosaur bone histology. *Journal of Vertebrate Paleontology* 18:385-390.
- Erickson, G. M. 2005. Assessing dinosaur growth patterns: a microscopic revolution. *TRENDS in Ecology and Evolution* 20:677-684.
- Francillon-Vieillot, H., V. d. Buffr  nil, J. Castanet, J. Geraudie, F. J. Meunier, J. Y. Sire, L. Zylberberg, and A. d. Ricql  s. 1990. Microstructure and mineralization of vertebrate skeletal tissues; pp. 471-548 in J. G. Carter (ed.), *Skeletal biomineralization patterns, processes and evolutionary trends*. Van Nostrand Reinhold, New York.
- Gregory, R. T., C. B. Douthitt, I. R. Duddy, P. Vickers-Rich, and T. H. Rich. 1989. Oxygen isotopic composition of carbonate concretions from the lower Cretaceous of Victoria, Australia: implications for the evolution of meteoric waters on the

- Australian continent in a paleopolar environment. *Earth and Planetary Science Letters* 92:27-42.
- Hallam, A. 1985. A review of Mesozoic climates. *Journal of the Geological Society (London)* 142:433-445.
- Hemelaar, A. S. M., and J. J. van Gelder. 1980. Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. *Netherlands Journal of Zoology* 30:129-135.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 1999. Variation in dinosaur skeletochronology indicators: implications for age assessment and physiology. *Paleobiology* 25:295-304.
- Horner, J. R., A. d. Ricqlès, K. Padian, and R. D. Scheetz. 2009. Comparative long bone histology and growth of the "hypsilophodontid" dinosaurs *Orodromeus makelai*, *Dryosaurus altus*, and *Tenontosaurus tilletii* (Ornithischia: Euornithodpoda). *Journal of Vertebrate Paleontology* 29:734-747.
- Hutton, J. M. 1986. Age determination of living Nile crocodiles from the cortical stratification of bone. *Copeia* 2:332-341.
- Knoll, F., K. Padian, and A. d. Ricqlès. 2010. Ontogenetic change and adult body size of the early ornithischian dinosaur *Lesothosaurus diagnosticus*: implications for basal ornithischian taxonomy. *Gondwana Research* 17:171-179.
- Lamm, E.-T. 2007. Paleohistology widens the field of view in paleontology; pp. 50-51 in M. Marko, J. H. Scott, E. Vicenzi, S. Dekanich, J. Frafjord, P. Kotula, S. McKernan, and J. Shields (eds.), *Proceedings microscopy and microanalysis 2007*. Cambridge University Press, New York.
- Lee, A. H., and S. Werning. 2008. Sexual maturity in growing dinosaurs does not fit reptilian growth models. *Proceedings of the National Academy of Sciences, USA* 105:582-587.
- Martin, A. J. 2009. Dinosaur burrows in the Otway Group (Albian) of Victoria, Australia, and their relation to Cretaceous polar environments. *Cretaceous Research* 30:1223-1237.
- Padian, K., J. R. Horner, and A. d. Ricqlès. 2004. Growth in small dinosaurs and pterosaurs: the evolution of archosaurian growth strategies. *Journal of Vertebrate Paleontology* 24:555-571.

- Rich, T. H., and P. V. Rich. 1989. Polar dinosaurs and biotas of the Early Cretaceous of southeastern Australia. *National Geographic Research* 5:15-53.
- Rich, T. H., and P. Vickers-Rich. 1994. Neoceratopsians and ornithomimosaurs: dinosaurs of Gondwana origin? *National Geographic Research and Exploration* 10:129-131.
- Rich, T. H., and P. Vickers-Rich. 1999. The Hypsilophodontidae from Southeastern Australia; pp. 167-180 in Y. Tomida, T. H. Rich, and P. Vickers-Rich (eds.), *Proceedings of the Second Gondwanan Dinosaur Symposium*. National Science Museum Monographs, Tokyo.
- Rich, T. H., P. V. Rich, B. Wagstaff, J. McEwen-Mason, C. B. Douthitt, and R. T. Gregory. 1989. Early Cretaceous biota from the northern side of the Australo-Antarctic rift valley. *Geological Society Special Publications* 47:121-130.
- Ricqlès, A. d., K. Padian, and J. R. Horner. 2003. On the bone histology of some Triassic pseudosuchian archosaurs and related taxa. *Annales de Paléontologie* 89:67-101.
- Ricqlès, A. d., K. Padian, F. Knoll, and J. R. Horner. 2008. On the origin of high growth rates in archosaurs and their ancient relatives: complementary histological studies on Triassic archosauriforms and the problem of a "phylogenetic signal" in bone histology. *Annales de Paléontologie* 94:57-76.
- Schweitzer, M. H., R. M. Elsey, C. G. Dacke, J. R. Horner, and E.-T. Lamm. 2007. Do egg-laying crocodilian (*Alligator mississippiensis*) archosaurs form medullary bone? *Bone* 40:1152-1158.
- Spicer, R. A., and R. M. Corfield. 1992. A review of terrestrial and marine climates in the Cretaceous with implications for modelling the 'Greenhouse Earth'. *Geological Magazine* 129:169-180.
- Trusler, P., P. Vickers-Rich, and T. H. Rich. 2010. *The artist and the scientists: bringing prehistory to life*. Cambridge University Press, Melbourne, 308 pp.
- Tutken, T., H.-U. Pfretzschner, T. W. Vennemann, G. Sun, and Y. D. Wang. 2004. Paleobiology and skeletochronology of Jurassic dinosaurs: implications from the histology and oxygen isotope compositions of bone. *Palaeogeography, Palaeoclimatology, Palaeoecology* 206:217-238.
- Varricchio, D. J. 1993. Bone microstructure of the Upper Cretaceous theropod dinosaur *Trodon formosus*. *Journal of Vertebrate Paleontology* 13:99-104.

- Veevers, J. J., C. M. Powell, and S. B. Roots. 1991. Review of seafloor spreading around Australia, I. Synthesis of the patterns of spreading. *Australian Journal of Earth Science* 38:373-389.
- Wagstaff, W. E., and J. McEwen-Mason. 1989. Palynological dating of Lower Cretaceous coastal vertebrate localities, Victoria, Australia. *National Geographic Research* 5:54-63.

CHAPTER FIVE

REANALYSIS OF “*RAPTOREX KRIEGSTEINI*”: A JUVENILE TYRANNOSAURID
DINOSAUR FROM MONGOLIA

Contribution of Authors and Co-Authors

Manuscript in Chapter 5

Author: Denver W. Fowler

Contributions: Conceived and designed the study, performed experiments, analyzed data, and wrote and compiled the manuscript.

Co-author: HollyNoelle W. Ballard

Contributions: Conceived and designed the study, performed experiments, analyzed data, and wrote a section of the manuscript.

Co-author: Elizabeth A. Freedman

Contributions: Conceived and designed the study, performed experiments, analyzed data, and wrote a section of the manuscript.

Co-author: Peter L. Larson

Contributions: Conceived and designed the study, performed experiments, analyzed data, and contributed to sections of the manuscript.

Co-author: John R. Horner

Contributions: Analyzed data, contributed materials and analytical tools.

Manuscript Information Page

Denver W. Fowler, Holly N. Woodward, Elizabeth A. Freedman, Peter L. Larson, John R. Horner

Journal Name: Public Library of Science ONE

Status of Manuscript:

☐ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☒ Published in a peer-reviewed journal

Published by Public Library of Science

Issue 6(6): e31376. 2011.

The following chapter has been published in the open-access journal PLoS ONE and appears in this dissertation under the terms of the Creative Commons Attribution License.

Abstract

The carnivorous Tyrannosauridae are among the most iconic dinosaurs: typified by large body size, tiny forelimbs, and massive robust skulls with laterally thickened teeth. The recently described small-bodied tyrannosaurid *Raptorex kreigsteini* is exceptional as its discovery proposes that many of the distinctive anatomical traits of derived tyrannosaurids were acquired in the Early Cretaceous, before the evolution of large body size. This inference depends on two core interpretations: that the holotype (LH PV18) derives from the Lower Cretaceous of China, and that despite its small size, it is a subadult or young adult. Here we show that the published data is equivocal regarding stratigraphic position and that ontogenetic reanalysis shows there is no reason to conclude that LH PV18 has reached this level of maturity. The probable juvenile status of LH PV18 makes its use as a holotype unreliable, since diagnostic features of *Raptorex* may be symptomatic of its immature status, rather than its actual phylogenetic position. These findings are consistent with the original sale description of LH PV18 as a juvenile *Tarbosaurus* from the Upper Cretaceous of Mongolia. Consequently, we suggest that there is currently no evidence to support the conclusion that tyrannosaurid skeletal design first evolved in the Early Cretaceous at small body size.

Key Words: Dinosauria; Theropoda; Tyrannosauridae; *Raptorex*; *Tarbosaurus*; Lycopteridae; histology; ontogeny; growth; stratigraphy; Mongolia; China.

Introduction

The Tyrannosauridae are probably the most famous of all dinosaurs. They are characterized by Upper Cretaceous taxa such as the iconic *Tyrannosaurus rex* from North America (Osborn, 1905) and *Tarbosaurus bataar* from Mongolia (Maleev, 1955; Rozhdestvensky, 1965): stocky carnivores of enormous size, with massive heads, huge laterally thickened teeth, and diminutive forelimbs. These and many other distinctive features are instantly recognizable and largely set tyrannosaurids apart from other theropods (Holtz Jr., 2004). Finds of relatively small, basal tyrannosauroids in the Upper Jurassic and Lower Cretaceous of North America (Madsen, 1974; Carpenter et al., 2005), Europe (Hutt et al., 2001; Rauhut, 2003; Benson, 2008), Asia (Xu et al., 2004; Xu et al., 2006), and possibly Australia ((Benson et al., 2010); although see (Herne et al., 2010)) indicated that these characteristics were gradually acquired through time, along with an increase in body size.

An intriguing twist to the evolutionary history of Tyrannosauridae was proposed in 2009 when Sereno et al. (2009) described *Raptorex kreigsteini* as a small-bodied tyrannosaurid from the Lower Cretaceous of China. The beautifully preserved holotype skeleton (LH PV18; Long Hao Institute of Geology and Paleontology, Hohhot, Nei Mongol, China) first surfaced at the Tucson Gem Mineral and Fossil Show several years ago where it was purchased by Dr. Henry Kriegstein, an American ophthalmologist and private fossil collector (Clark, 2009). Dr. Kriegstein showed the specimen to Paul Sereno, who recognized its scientific value and arranged to coauthor its description on the condition that it should be repatriated to China, from which it was assumed that it had

been illegally removed (Sereno et al., 2009). LH PV18 was important as it seemed to indicate that tyrannosaurid skeletal design first evolved at small body size in the Early Cretaceous. This inference depends on two core interpretations, that LH PV18 derives from the Lower Cretaceous, and that despite its small size, it is a subadult or young adult.

In the original description, LH PV18 is referred to as having been “discovered in the Lujiatun Beds (Hauterivian-Barremian, ca. 130 Ma) of the Lower Cretaceous Jehol Group” (Sereno et al., 2009; p. 419). However, uncertainty is indicated in the Supporting Online Material, and news articles (Achenbach, 2009; Boyle, 2009; Mullen, 2009) including the Chicago Tribune (Mullen, 2009) where Sereno is quoted: “From sediments, fossil fish bones, turtles, clam shells and other fauna we recovered from the rock matrix alongside the *Raptorex* fossil, we could generally pinpoint where it had been dug up in an area along the border with Inner Mongolia”. Contrary to this statement (Mullen, 2009), published associated fossils (Sereno et al., 2009; Supporting Online Material) consist only of a crushed pelecypod and a fish centrum. As the crushed pelecypod is unidentified, the age of LH PV18 is based primarily on the fish centrum which is identified as “cf. *Lycoptera*” (Sereno et al., 2009; Supporting Online Material). This is used to suggest LH PV18 derives from the Lower Cretaceous, since the known stratigraphic range of *Lycopteridae* is ~122-135 Ma (Li et al., 1994). LH PV18 is suggested to have been collected from the Lujiatun Beds of the Yixian Formation, China, as this unit commonly yields articulated dinosaurs along with fossils of pelecypods and *Lycoptera*, as opposed to other potential units in China (e.g. the Iren Dabasu Formation).

LH PV18 was diagnosed as a subadult or young adult based on osteological analysis and a histological section of the right femur (Serenó et al., 2009). Osteological evidence cited as indicative of maturity includes complete obliteration of the internasal suture, partial coossification of several cranial sutures, coossification of some vertebral neural arches to centra, and partial fusion of the pelvis. In their histological analysis, spacing of Line(s) of Arrested Growth (LAGs) led the authors to suggest that LH PV18 had undergone an exponential growth period, and that growth was possibly slowing at death. From reconstructed LAG history it was suggested that LH PV18 died at an age of 5-6 years, as a subadult or young adult.

Herein, we show that the stratigraphic evidence provided (Serenó et al., 2009; Supporting Online Material) is uninformative and cannot be used to infer provenance. We demonstrate that the fish centrum is not of *Lycoperidae* and therefore the proposed stratigraphic position is not supported. Using histological and osteological analysis, we show there is no reason to believe that LH PV18 has reached subadult or young adult ontogenetic status. Further, we reveal that the specimen was originally sold as a juvenile *Tarbosaurus* from Mongolia, a diagnosis consistent with its morphology and the results of our independent analysis.

Materials and Methods

Identification of associated fossil material and histological analysis was conducted by reference to photographs provided in the Sereno et al. (2009) Supporting Online Material and comparison to adult and subadult tyrannosaurid specimens held at

the Museum of the Rockies and the Black Hills Institute. Consultation with Hollis Butts (pers. comm. to PL), who sold LH PV18 to Henry Kriegstein, was conducted at Tuscon Mineral Fair, Arizona, January 2011.

Here we follow Brochu (1996; p. 51-52) in his definition of vertebral neural arch fusion. A “closed” vertebra is defined as “one in which the neurocentral suture is no longer visible” (i.e. the suture is obliterated). An open vertebra is defined as “completely visible from all aspects, and the centrum and neural arch can be separated easily”. A partially closed vertebra is “one in which the suture has started closing, but is still discernible”.

Results and Discussion

Stratigraphy

The stratigraphic evidence (fish vertebra; rarity of articulated skeletons in the Iren Dabasu Formation; (Serenio et al., 2009; Supporting Online Material)) is uninformative and cannot be used to reliably infer provenance. The LH PV18 fish centrum was assigned to *Lycoptera* without comment on how this diagnosis was made (Serenio et al., 2009; Supporting Online Material). Because isolated *Lycoptera* centra have never been described in detail, such positive identification is difficult. The only published account of *Lycoptera* vertebrae is Zhang 2002 (not cited by Serenio et al. (2009)), whose morphological description is so different from the LH PV18 fish vertebra that this specimen cannot be a *Lycoptera*. Several features of the LH PV18 fish centrum that strongly differ from those of *Lycoptera* and related Osteoglossiformes, are instead more

consistent with centra of Ellimmichthyiformes (double-armored herrings; Figure 5.1) or Hiodontiformes. *Lycoptera* centra are highly unusual in being thin-walled and tubular with a large notochordal foramen (Zhang, 2002), whereas the LH PV18 fish centrum (Serenio et al., 2009), Ellimmichthyiformes, and Hiodontiformes (Brinkman and Neuman, 2002; Newbrey et al., 2010) are deeply amphicoelous, with thicker walls relative to the notochordal foramen. The LH PV18 fish centrum and Ellimmichthyiformes possess mid-dorsal and lateral-dorsal fossae posterior to the neural pits (Brinkman and Neuman, 2002; Newbrey et al., 2010); *Lycoptera* lacks these fossae (M. G. Newbrey pers. comm. 2010). A full description of *Lycoptera* vertebrae and comparison to Ellimmichthyiformes and Hiodontiformes is in preparation (Newbrey et al., in review).

In addition, the LH PV18 fish centrum is far larger than any centra found in an articulated *Lycoptera* specimen. *Lycoptera* are small fish, generally ranging in size from 7 to 13 cm, and their centra are typically <1 mm to 2 mm long (Ma, 1987; Zhang, 2002). By contrast, the LH PV18 centrum (Serenio et al., 2009) is 4 mm in diameter and approximately 4 mm in length. This would be anomalously large for a *Lycoptera* centrum, but is fully within the size range expected for ellimmichthyiform centra, which are commonly up to 7 mm in diameter (Newbrey et al., 2010; Figure 5.1).

Although the LH PV18 fish centrum cannot be more precisely identified without further comprehensive comparisons, its morphology and size exclude the possibility of it being *Lycoptera*, but are consistent with centra of Ellimmichthyiformes. As Ellimmichthyiformes in China range from the Early Cretaceous to the Eocene (Zaragüeta, 2004; Newbrey et al., 2010), the LH PV18 fish centrum cannot be used as definitive

evidence of an Early Cretaceous age for the specimen. Therefore there is no biostratigraphic evidence for placement of the specimen in the Lower Cretaceous.

The presence of pelecypod and *Lycoptera* fish fossils in the Jehol Biota is used by Sereno et al. (Sereno et al., 2009) as supporting evidence for assignment of LH PV18 to the Lujiatun beds of the Yixian Fm. We have shown that the fish vertebra does not pertain to *Lycopteridae*, so this argument is reduced to the presence of pelecypods and fish, which is typical for many if not most Mesozoic terrestrial formations and cannot be used as supporting evidence. Further, taphonomic data cannot exclude the possibility of a Late Cretaceous age, since articulated and partly articulated dinosaur remains are known from the Iren Dabasu Formation (Currie and Eberth, 1993).

We suggest that other methods of chronostratigraphic analysis might provide independent age assessment of LH PV18. Analysis of palynomorphs or detrital zircons, extracted from the enclosing “tuffaceous sandstone” matrix (which might be expected to have potential for such analyses) could be used to assess stratigraphic position. While these methods provide only rough ages, often variably accurate within a range of ~5 million years, this would still be adequate considering the ~60my disparity between potential Early and Late Cretaceous ages. Conceivably, analyzing Rare Earth Element content of fossil bone might also help elucidate provenance (Trueman and Benton, 1997; Trueman et al., 2006).

Independent testimony states that the specimen was collected in Mongolia, not China. Before its final sale in the United States, LH PV18 was purchased from a Mongolian fossil collector by one of the brokers, an American businessman residing in

Tokyo, Japan: at no time had the specimen been represented as coming from China (seller Hollis Butts pers. comm. to PL). Dr. Kriegstein confirmed (pers. comm. to PL) that he discussed the origin of LH PV18 with Mr. Butts and that upon purchase it was tentatively identified as a juvenile *Tarbosaurus*, a tyrannosaurid known only from the Late Cretaceous (Campanian and/or Maastrichtian; (Holtz Jr., 2004)). Variability in personal communications regarding the specimen makes it difficult to ascertain exact details that were known at the time of purchase, highlighting the problems of dealing with commercial specimens that have been illegally collected. It is unclear how the specimen came to be considered as having originated from the Lower Cretaceous of China, but a Mongolian (Upper Cretaceous) origin is consistent with other aspects of our stratigraphic reanalysis. Regardless of independent testimony, published stratigraphic data are uninformative. LH PV18 is of currently unknown provenance.

Ontogeny

Ontogeny has an enormous influence on the skeletal morphology of dinosaurs, most obviously affecting both proportions and ornament of the skull (Brown and Schlaikjer, 1940; Rozhdestvensky, 1965; Russell, 1970; Dodson, 1975, 1976; Maryanska and Osmolska, 1979; Horner, 1983; Dodson and Currie, 1988; Horner and Currie, 1994; Sampson, 1995; Sampson et al., 1997; Carr, 1999; Ryan et al., 2001; Currie, 2003a; Carr and Williamson, 2004; Evans et al., 2005; Horner and Goodwin, 2006; Evans et al., 2007; Currie et al., 2008; Horner and Goodwin, 2008; Brown et al., 2009; Horner and Goodwin, 2009; Knoll et al., 2010; Scannella and Horner, 2010; Whitlock et al., 2010; Tsuihiji et al., 2011), but also postcrania (Russell, 1970; Dilkes, 2001; Wedel, 2003; Reisz et al.,

2005; Tsuihiji et al., 2011). While it has long been recognized that immature individuals can appear morphologically more basal than adults of the same species (Rozhdestvensky, 1965), only relatively recently has this begun to be acknowledged in taxonomic studies (Rozhdestvensky, 1965; Russell, 1970; Dodson, 1975; Sampson et al., 1997; Carr, 1999; Currie, 2003a; Horner and Goodwin, 2009; Knoll et al., 2010; Scannella and Horner, 2010; Tsuihiji et al., 2011). Historically, many specimens of immature dinosaurs have been described as unique taxa, only later being recognized as juveniles and subsequently synonymized with a different taxon (Russell, 1970; Dodson, 1975; Dodson and Currie, 1988; Sampson et al., 1997; Carr, 1999; Currie and Dong, 2001; Currie, 2003a; Carr and Williamson, 2004; Horner and Goodwin, 2009; Knoll et al., 2010; Scannella and Horner, 2010; Margottini, 2011). The emerging view of dinosaur ontogeny is that fully derived cranial and postcranial characters are typically only gained near to and/or upon reaching maturity (potentially beyond skeletal maturity), hence juvenile dinosaurs exhibit morphological characters that might make them appear more basal than they really are (Brown and Schlaikjer, 1940; Rozhdestvensky, 1965; Russell, 1970; Dodson, 1975, 1976; Maryanska and Osmolska, 1979; Horner, 1983; Dodson and Currie, 1988; Horner and Currie, 1994; Sampson, 1995; Sampson et al., 1997; Carr, 1999; Ryan et al., 2001; Currie, 2003a; Carr and Williamson, 2004; Evans et al., 2005; Horner and Goodwin, 2006; Evans et al., 2007; Currie et al., 2008; Horner and Goodwin, 2008; Brown et al., 2009; Horner and Goodwin, 2009; Knoll et al., 2010; Scannella and Horner, 2010; Whitlock et al., 2010; Margottini, 2011; Tsuihiji et al., 2011). This is illustrated in Tyrannosauridae by MPC-D 107/7 (Mongolian Paleontological Center, Ulaan Bataar,

Mongolia), a recently described (Tsuihiji et al., 2011) juvenile *Tarbosaurus bataar* (Nemegt Formation, Mongolia), nearly identical in size to LH PV18 (skull length 290 mm and 300 mm, respectively) and extremely similar in morphology. Cladistic analysis recovers MPC-D 107/7 as the sister taxon to Tyrannosauridae (with LH PV18 as the next more basal node) rather than the sister taxon to *Tarbosaurus*. Consequently, if LH PV18 is shown to be non-mature, then it is possible that purported basal characteristics do not reflect a true phylogenetic position, but rather its immature status. Assessing whether LH PV18 is a juvenile *Tarbosaurus* (as stated at its original sale) requires reanalysis of ontogenetic data, as the specimen was originally described as a subadult or young adult (Serenó et al., 2009).

Osteological Indications of Maturity

Reanalysis of the published osteological data demonstrates that the specimen should not be considered as close to mature. Partial fusion of the internasal suture of LH PV18 (Serenó et al., 2009) is not indicative of maturity as the nasal suture begins to fuse very early in the ontogeny of tyrannosaurids (Carr and Williamson, 2004; Tsuihiji et al., 2011).

“Closed and coossified” neurocentral sutures in the cervical, anterior dorsal, and sacral vertebrae were used to infer a near mature condition for LH PV18 (Serenó et al., 2009), but this diagnosis is at odds with personal examination of the specimen by one of the present authors (PL), where (using the definition of Brochu (1996)) neurocentral sutures of anterior dorsal and cervical vertebrae were observed to be only partially closed. Moreover, neurocentral sutures remain open in the anterior caudal and posterior dorsal

vertebrae, some of which have become slightly disarticulated. The ontogenetic pattern for neurocentral closure has not yet been fully studied for tyrannosaurid dinosaurs (Carr et al., 2005), but the initially observed pattern does not match that described for crocodylians, where the caudal series can be closed before hatching, and fusion proceeds cranially (Brochu, 1996). Moreover, neurocentral fusion is an inconsistently observed character in tyrannosaurids suggesting that it is unreliable in determining maturity. The very large *Tyrannosaurus* FMNH PR2081 (“Sue”; (Brochu, 2003); Field Museum of Natural History, Chicago) exhibits unobliterated neurocentral sutures through the presacral and sacral series, as well as the first 15 caudal vertebrae. The juvenile *Tarbosaurus* IVPP V4878, (= *Shanshanosaurus huoyanshanensis*; Subashi Formation, Upper Cretaceous, Xinjiang, China; (Dong, 1977; Currie and Dong, 2001); Institute of Vertebrate Paleontology and Paleoanthropology, Beijing, China) is of near-identical size to LH PV18 and exhibits variable states of fusion in preserved cervical and dorsal vertebrae with some apparently closed, and others with visible sutures (Currie and Dong, 2001). Neurocentral sutures also appear closed in the cervical vertebrae of the juvenile tyrannosaurid IGM 100/1844 (Institute of Geology, Ulaan Baatar, Mongolia), described as the holotype of *Alioramus altai* (Brusatte et al., 2010). This demonstrates that neurocentral closure is known in tyrannosaurids that are not close to being mature, making it difficult to use this feature to support an adult status for LH PV18. Finally, in the recently described dromaeosaurid theropod *Balaur* (Csiki et al., 2010), extensive postcranial fusion is noted in both the holotype and paratype specimens, yet the paratype pertains to an individual 45% larger than the holotype. Csiki et al. (2010) were not able to

conduct independent histological analysis of ontogenetic status, but acknowledge that one hypothesis to account for this observation is that “growth continued after fusion of numerous regions of the skeleton”. Partial neurocentral fusion is therefore an unreliable indicator of maturity in theropods, at least without further study.

The narrow maxillary tooth crowns of LH PV18 are suggested to be different from “mature tyrannosaurids” (Sereno et al., 2009). Narrow crowns are typical of both immature Late Cretaceous tyrannosaurids (Carr, 1999; Currie, 2003a) and basal tyrannosauroids (Hutt et al., 2001; Xu et al., 2004; Xu et al., 2006). Since narrow crowns would be expected in both immature and basal tyrannosaurids then we suggest this feature is of equivocal use for determining ontogenetic status.

Histology

Histological sectioning of limb bones remains the only reliable assessment of ontogenetic status. Sereno et al. (2009) conclude that LH PV18 is a nearly mature subadult based on a histological section of the right femur. However, the section exhibits typically juvenile histological features. If LH PV18 was nearly mature then bone remodeling in the form of secondary osteons should be widespread in the histologic section (Francillon-Vieillot et al., 1990; Horner et al., 2001; Klein and Sander, 2008). However, secondary osteons are only seen in two very small areas, both associated with migration of adductor muscle attachment (Figure 5.2). Furthermore, the femur is composed entirely of plexiform fibro-lamellar bone indicating rapid growth and consistent with an immature status.

Sereno et al.'s (2009) suggestion that the distance between LAG 2 and the surface records a transition to exponential growth compared to the distance between LAGs 1 and 2 is questionable as the spacing between LAG 1 and the missing LAGs was just as large if not larger. The amount of growth represented by the distance between LAG 1 and 2 is anomalous as it is bound on either side by bone thicknesses representing larger amounts of growth. In addition, if the distance between LAG 2 and the surface represents the beginning of an exponential growth phase, then this individual should be considered immature because a subadult would be characterized by a reducing, not increasing, growth rate. There is no indication from tissue organization or LAG spacing that suggests growth is slowing down or that adult or subadult size has been approached. Because a fibro-lamellar tissue complex is consistent throughout, even up to the periosteal surface, growth was still proceeding rapidly at time of death.

The outer layer described as a possible third LAG is difficult to fully trace around the circumference in the images available, and may be an artifact of diagenetic staining. The close-up image using transmitted light (Sereno et al., 2009; Fig. S7b) supports this, as the image shows that the cortex near the periosteal surface is less clearly preserved and there is no "well-defined" third LAG. Sereno et al. interpret this possible LAG, so close to the periosteal surface, to indicate a decrease in bone deposition associated with approaching skeletal maturity. For this to be true, a corresponding and permanent change from plexiform tissue to slowly deposited parallel-fibered or lamellar bone tissue is necessary. If there is indeed a third LAG near the surface, the few lamellae deposited after it remain plexiform like the rest of the section. In this case, a single LAG so close to

the surface means only that this animal died shortly after its deposition, not that growth was slowing down.

It has been suggested that the long bone histology of small dinosaurs (i.e. those of small adult size) and basal birds is different from that of large dinosaurs (Case, 1978; Padian et al., 2001; Ricqlès et al., 2003; Horner and Padian, 2004; Padian et al., 2004). Padian et al. (2001; p. 405) state: “In contrast to large dinosaurs and pterosaurs, small ones apparently grew more slowly. Their long bone cortices were less well vascularized, the vessels were primarily longitudinal, and the bones may show more closely spaced growth lines”. If LH PV18 was a near-mature individual of a small tyrannosaur species we would expect to observe the aforementioned histological features in the section. However, LH PV18 exhibits histology more consistent with that of an immature large dinosaur, rather than a mature (or even immature) small dinosaur.

Histology also indicates that the original age estimate of 5-6 years (Serenó et al., 2009) is an overestimate. We suggest that an age of 3-6 years is more accurate, with strong suspicion of a true age closer to 3 years. The annual periodicity of cortical growth marks (LAGs) has been demonstrated in extant archosaurs (e.g. Hutton, 1986; Tucker, 1997), thus allowing their use in estimating age at death for dinosaurs. Sereno et al. determined the age of LH PV18 by counting the LAGs present within the cortex, and adding to that the number of LAGs estimated to be missing due to bone resorption during medullary cavity expansion. Because the distance between two LAGs reveals the amount of cortical growth in one year, Sereno et al. (2009) use the spacing (or growth “zone”) between LAG 1 and 2 to assess the number of missing LAGs by determining how many

of those zones would fit within the medullary cavity. However, this method is problematic. The deep cortex shown on the left side of the section is less affected by medullary expansion than the right side, affording a more complete record of cortical growth. Here the distance from the edge of the medullary cavity to LAG 1 is greater than the distance between LAG 1 and 2 (Figure 5.2), and equivalent to the spacing between LAG 2 and the surface. Therefore the amount of cortical growth in the year separating LAG 1 and LAG 2 is actually less than the amount of cortical growth from the previous year (edge of medullary cavity to LAG 1), as well as less than the cortical growth from the year after (the zone between LAG 2 and the surface). Therefore, the distance between LAG 1 and LAG 2 should not be used to estimate the number of missing LAGs. Despite being incomplete due to resorption, the innermost growth zone is approximately twice as large as the distance between LAG 1 and LAG 2, and would be a better estimate of the width of the missing growth zones. It is also equally plausible that the bone tissue destroyed by medullary expansion was free of additional LAGs. Ontogenetic histology studies of large dinosaurs demonstrate a rapid increase in body size prior to the deposition of the first LAG (Horner et al., 2000; Erickson et al., 2004; Bybee et al., 2006). Following this model of growth, missing LAGs destroyed by medullary expansion would not be expected in LH PV18 and the actual age at death was 2-3 years. This is supported by the ontogenetic analysis of the near-identically sized juvenile *Tarbosaurus* MPC-D 107/7. Histological analysis of the fibula and tibia from MPC-D 107/7 revealed an age of 2-3 years with no possibility of missing LAGs (Tsuihiji et al., 2011). Therefore, given the presence of a rapidly deposited bone tissue typical of an immature animal, and

because in general LAG spacing decreases throughout ontogeny as growth rate slows, we consider an age estimate of 2-3 years most likely for LH PV18.

Taxonomy

It is not the main purpose of this study to reassess the taxonomic status and possible affinities of LH PV18; however, some comment is appropriate. In their description of a juvenile *Tarbosaurus bataar* (MPC-D 107/7), Tsuihiji et al. (2011) discuss morphology of juvenile tyrannosaurs, including characters considered diagnostic of “*Raptorex*” which are also seen in MPC-D 107/7. They conclude that it can be challenging to discriminate juvenile characters from plesiomorphic characters, with which we concur. Tsuihiji et al. (2011) tentatively suggest that LH PV18 is not assignable to *T. bataar* for two reasons. Firstly, LH PV18 has “reportedly very different stratigraphic age and provenance”, which we show here to be based on incorrect interpretation by Sereno et al. (2009). Secondly, there are some morphological differences between LH PV18 and MPC-D 107/7, including cranial characters (dorsoventral extension of the rostroventral lamina of the ventral ramus of the lacrimal, and size of caudal surangular foramen) and significantly, that LH PV18 is reported as lacking a vertical crest on the ilium (a character seen in all other tyrannosauroids (Holtz Jr., 2001, 2004)). However, personal observation of LH PV18 by one of us (PL) found that a subtle crest is visible on the ilium (as expected). Thus, the only definable differences between a juvenile *Tarbosaurus bataar* and LH PV18 are two minor skull characters, which Tsuihiji et al. (2011) suggest could be intraspecific variation (although intraspecific variation in tyrannosaurids (Carr, 1999; Currie, 2003a, b; Hurum and Sabath, 2003), especially

juveniles, requires further study). Morphological and histological consistency between the two specimens supports our conclusion that LH PV18 is a juvenile of a much larger tyrannosaurid species, and may indicate affinity with *Tarbosaurus*, although not necessarily being assignable to *T. bataar*. Comparison to the tyrannosaurid record of North America (Holtz Jr., 2004) over the equivalent time frame (Late Cretaceous; Campanian - Maastrichtian; ~83 - 66 Ma; (Weishampel et al., 2008; Hone et al., 2011)) suggests that some diversity might be expected in Asian taxa. North American dinosaur taxa often form lineages of non-overlapping chronospecies, even within the same formation (Holmes et al., 2001; Currie and Russell, 2005; Ryan and Evans, 2005; Ryan and Russell, 2005; Scannella and Fowler, 2009). Small changes seen among Asian tyrannosaurids may similarly be the result of slight stratigraphic variation. However, as demonstrated with *Triceratops* (Scannella and Fowler, 2009; Scannella and Horner, 2010), making sense of multiple morphologies first requires knowledge of ontogenetic change as well as relative stratigraphic position of specimens, emphasizing the necessity for such data in specimen descriptions.

The lack of provenance data and juvenile status of LH PV18 make its use as a holotype problematic. When sample sizes are extremely limited, all specimens may appear to be useful records of morphology, even if they lack essential locality data. However, specimens without stratigraphic data can be extremely difficult to compare properly with newly discovered material for which stratigraphic position is known. Thus, many historically collected specimens (even holotypes) will eventually become morphologies without context and mostly useless for any detailed scientific investigation

(something we have personally encountered while studying historical collections of *Triceratops*). This can only be countered by relocation (where possible) of the original collection quarries (Tanke, 2005; Scannella and Fowler, 2009; Everhart, 2011). Similarly, definition of taxa from juvenile specimens may not be useful because they are notoriously difficult to associate with adult morphologies (without intermediate forms for reference), which can result in their eventual designation as nomina dubia (e.g. *Brachyceratops montanensis*; *Monoclonius crassus*; (Sampson et al., 1997)). Tsuihiji et al (2011) had great difficulty assigning the juvenile MPC-D 107/7 to *Tarbosaurus bataar*, finding only three characters shared by both juvenile and adult (including number of tooth positions which varies even between left and right sides of a single individual (Tsuihiji et al., 2011), and is thought to change through ontogeny; (Carr, 1999)). Given that MPC-D 107/7 is morphologically dissimilar to an adult *T. bataar*, and that it is of almost identical ontogenetic status as LH PV18, we would not expect the adult morphology of “*Raptorex*” to look overly similar to LH PV18. Thus, even if “*Raptorex*” is a genuine taxon, it cannot be reliably compared to other species that are diagnosed based on adult specimens.

Conclusions

LH PV18 cannot be demonstrated to be from the Lower Cretaceous, therefore the conclusion that derived features of tyrannosaurids evolved before the Late Cretaceous cannot be supported. As histology demonstrates that LH PV18 is immature then the conclusion that typical tyrannosaurid features evolved at a small size also cannot be

supported, since the small size of LH PV18 is more likely the result of its immature status. Furthermore, the probable juvenile status of LH PV18 makes its use as a holotype unreliable, since diagnostic features of *Raptorex* may be symptomatic of its immature status, rather than its actual phylogenetic position. Unless stronger evidence is presented, *Raptorex* should be considered a *nomen dubium*. LH PV18 more likely represents the juvenile of a larger tyrannosaurid from the Late Cretaceous of Mongolia, such as *Tarbosaurus* (as per its original sale description), although testing of this hypothesis awaits description of new specimens of immature tyrannosaurids.

Misidentification of immature dinosaur specimens as new taxa is a persistent and increasingly pervasive problem that can be detected and diagnosed only by thorough and proper histological analysis. Combined ontogenetic and stratigraphic analyses have great potential to reveal new information on the mode and tempo of dinosaur evolution, but as this reanalysis exemplifies, such studies must be based upon solid and replicable data.

Acknowledgements

Our gratitude is extended to Michael Newbrey (Tyrell Museum of Paleontology, TMP) for detailed consultation on fish vertebrae, and comments on the manuscript. Donald Brinkman (TMP) provided helpful comments on an early draft of the manuscript. Ellen Lamm (MOR) prepared histology slides used for comparison. Hollis Butts and Dr. Henry Kriegstein are thanked for their communications regarding the LH PV18 specimen. Thanks to John Scannella for discussion and help with references. Special thanks to Paul Sereno for unimpeded access to LH PV18.

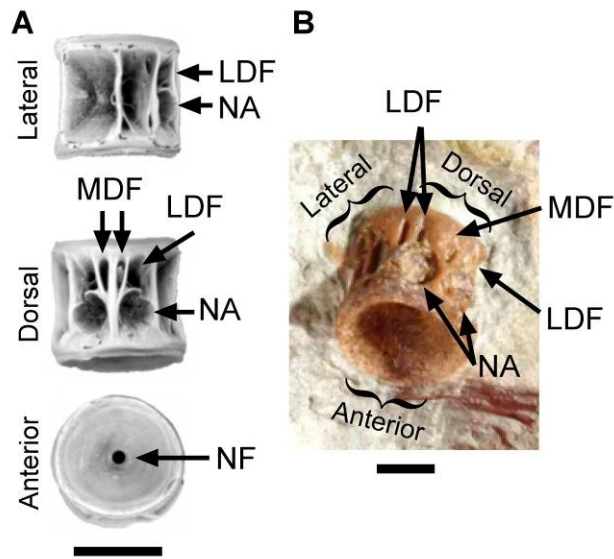


Figure 5.1 Comparison of the LH PV18 fish centrum (D) to an ellimmichthyiform centrum (A-C) (Horseshoeichthyidae) from the Dinosaur Park Formation (Campanian) of Alberta. The notochordal foramen (NF) in the LH PV18 fish centrum (D) is so small that it cannot be seen from the angle of the photograph. The exact number and positions of mid-dorsal fossae (MDF) and laterodorsal fossae (LDF) are variable within the same taxon (Brinkman and Neuman, 2002). Photos (A-C) have been rotated to align with the general orientation of the obliquely figured LH PV18 fish centrum. Picture (A) was also flipped horizontally to simulate the right lateral view. Additional abbreviations: NA: neural arch articular pit. (A-C) adapted from Newbrey et al. (2010; Figure 9h). (D) adapted from Sereno et al. (2009; Figure S8B).

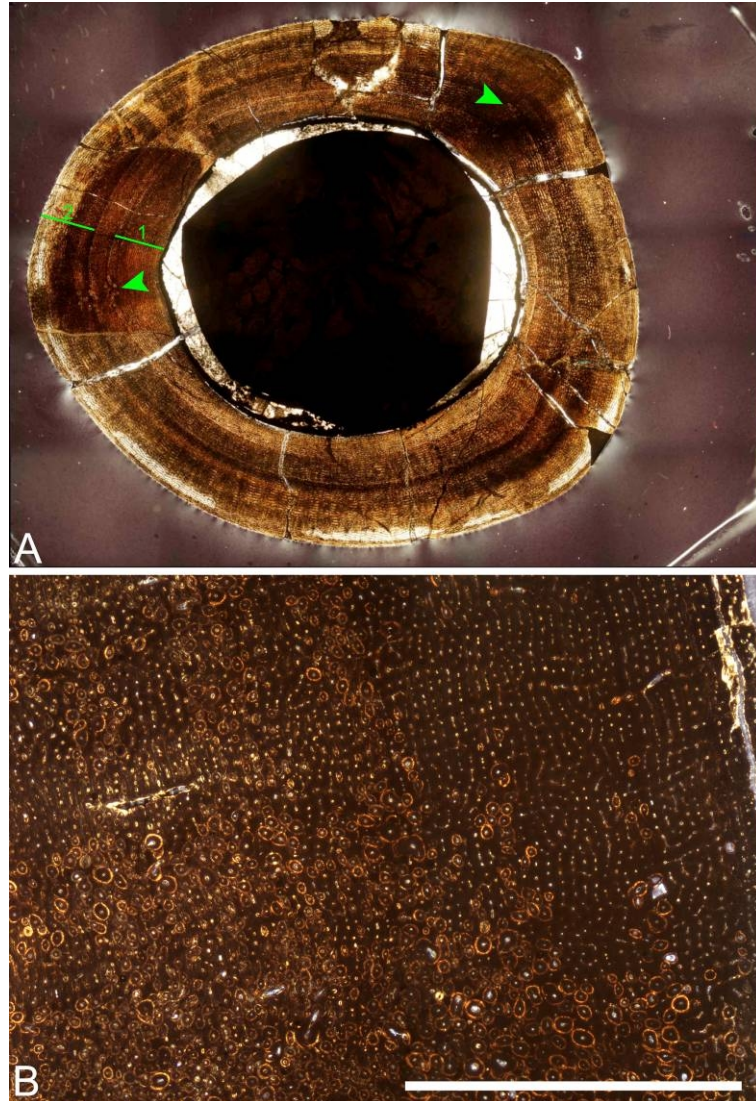


Figure 5.2. Osteohistological features of LH PV18 and a near adult *Tyrannosaurus rex* for comparison. A) Femur cross section of LH PV18 modified from Sereno et al. (2009; Figure S7A). Only two small clusters of secondary osteons (arrows) are visible, associated with adductor muscle attachment sites. The width of cortical bone from the edge of the medullary cavity to LAG 1 (green bar labeled 1) is nearly the same as the width of cortical bone from LAG 2 to the outer surface (green bar labeled 3). B) Mid to outer cortex of a femur cross section from a *Tyrannosaurus rex* (MOR 1198; Museum of the Rockies, Bozeman, Montana) described (Horner and Padian, 2004) as approaching adult length. The bone surface is located to the right of the picture. Several generations of secondary osteons nearly obliterate primary tissue within the deeper cortex and become scattered close to the surface. This pattern is typical of dinosaurs approaching asymptotic size. Scale bars, 5 mm.

Literature Cited

- Achenbach, J. Unveiled: The Surprisingly Small Precursor of T. Rex. Washington Post 18th September 2009. <http://www.washingtonpost.com/wp-dyn/content/article/2009/09/17/AR2009091702573.html?hpid=topnews&sid=ST2009091800725>. Retrieved 2011 March 8.
- Benson, R. B. J. 2008. New information on *Stokesosaurus*, a tyrannosauroid (Dinosauria: Theropoda) from North America and the United Kingdom. *Journal of Vertebrate Paleontology* 28:732-750.
- Benson, R. B. J., P. M. Barrett, T. H. Rich, and P. Vickers-Rich. 2010. A Southern Tyrant Reptile. *Science* 327:1613.
- Boyle, A. Tiny T. rex? Big surprise! MSNBC News. <http://cosmiclog.msnbc.msn.com/archive/2009/09/17/2072603.aspx>. Retrieved 2011 March 8.
- Brinkman, D. B., and A. G. Neuman. 2002. Teleost centra from uppermost Judith River Group (Dinosaur Park Formation, Campanian) of Alberta. *Canadian Journal of Paleontology* 76:138-155.
- Brochu, C. A. 1996. Closure of neurocentral sutures during crocodilian ontogeny: implications for maturity assessment in fossil archosaurs. *Journal of Vertebrate Paleontology* 16:49-62.
- Brochu, C. A. 2003. Osteology of *Tyrannosaurus rex*: Insights from a nearly complete skeleton and high-resolution computed tomographic analysis of the skull. *Journal of Vertebrate Paleontology* Memoir 7:1-138.
- Brown, B., and E. Schlaikjer. 1940. The structure and relationships of *Protoceratops*. *Annals of the New York Academy of Sciences* 40:133-266.
- Brown, C. M., A. P. Russell, and M. J. Ryan. 2009. Pattern and transition of surficial bone texture of the Centrosaurine frill and their ontogenetic and taxonomic implications. *Journal of Vertebrate Paleontology* 29:132-141.
- Brusatte, S. L., T. D. Carr, G. M. Erickson, G. S. Bever, and M. A. Norell. 2010. A long-snouted, multihorned tyrannosaurid from the Late Cretaceous of Mongolia. *Proceedings of the National Academy of Science* 106:17261-17266.

- Bybee, P. J., A. H. Lee, and E. Lamm. 2006. Sizing the Jurassic theropod dinosaur *Allosaurus*: assessing growth strategy and evolution of ontogenetic scaling of limbs. *Journal of Morphology* 267:347-359.
- Carpenter, K., C. Miles, and K. Cloward. 2005. New small theropod from the Upper Jurassic Morrison Formation of Wyoming; pp. 23-48 in K. Carpenter (ed.), *The Carnivorous Dinosaurs*. Indiana University Press, Bloomington.
- Carr, T. D. 1999. Craniofacial ontogeny in Tyrannosauridae (Dinosauria, Coelurosauria). *Journal of Vertebrate Paleontology* 19:497-520.
- Carr, T. D., and T. E. Williamson. 2004. Diversity of Late Maastrichtian Tyrannosauridae (Dinosauria: Theropoda) from western North America. *Zoological Journal of the Linnean Society-London* 142:419-523.
- Carr, T. D., T. E. Williamson, and D. E. Schwimmer. 2005. A new genus and species of tyrannosaurid from the Late Cretaceous (Middle Campanian) Demopolis Formation of Alabama. *Journal of Vertebrate Paleontology* 25:119-143.
- Case, T. J. 1978. Speculation on the growth rate and reproduction of some dinosaurs. *Paleobiology* 4:320-328.
- Clark, J. 2009. Becoming *T. rex*. *Science* 326:373-374.
- Csiki, Z., M. Vremir, S. L. Brusatte, and M. A. Norell. 2010. An aberrant island-dwelling theropod dinosaur from the Late Cretaceous of Romania. *Proceedings of the National Academy of Science* 107:15357-15361.
- Currie, P. J. 2003a. Allometric growth in tyrannosaurids (Dinosauria: Theropoda) from the Upper Cretaceous of North America and Asia. *Canadian Journal of Earth Sciences* 40:651-665.
- Currie, P. J. 2003b. Cranial anatomy of tyrannosaurid dinosaurs from the Late Cretaceous of Alberta, Canada. *Acta Palaeontologica Polonica* 48:191-226.
- Currie, P. J., and D. A. Eberth. 1993. Palaeontology, sedimentology, and palaeoecology of the Iren Dabasu Formation (Upper Cretaceous), Inner Mongolia, People's Republic of China. *Cretaceous Research* 14:127-144.
- Currie, P. J., and Z. Dong. 2001. New information on *Shanshanosaurus huoyanshanensis*, a juvenile tyrannosaurid (Theropoda, Dinosauria) from the Late Cretaceous of China. *Canadian Journal of Earth Sciences* 38:1729-1737.

- Currie, P. J., and D. A. Russell. 2005. The geographic distribution of articulated and associated dinosaur remains; pp. 537-569 in P. J. Currie and E. B. Koppelhaus (eds.), *Dinosaur Provincial Park*. Indiana University Press, Bloomington.
- Currie, P. J., W. Langston, and D. H. Tanke. 2008. A new species of *Pachyrhinosaurus* (Dinosauria, Ceratopsidae) from the Upper Cretaceous of Alberta, Canada; pp. 1-108 in P. J. Currie, W. Langston, and D. H. Tanke (eds.), *A New Horned Dinosaur from an Upper Cretaceous Bone Bed in Alberta*. NRC Research Press, Ottawa.
- Dilkes, D. W. 2001. An ontogenetic perspective on locomotion in the Late Cretaceous dinosaur *Maiasaura peeblesorum* (Ornithischia: Hadrosauridae). *Canadian Journal of Earth Sciences* 38:1205-1227.
- Dodson, P. 1975. Taxonomic implications of relative growth in Lambeosaurine hadrosaurs. *Systematic Zoology* 24:37-54.
- Dodson, P. 1976. Quantitative aspects of relative growth and sexual dimorphism in *Protoceratops*.
- Dodson, P., and P. J. Currie. 1988. The smallest ceratopsid skull - Judith River Formation of Alberta. *Canadian Journal of Earth Sciences* 25:926-930.
- Dong, Z. 1977. On the dinosaurian remains from Turpan, Xinjiang. *Vertebrata Palasiatic* 15:59-66.
- Erickson, G. M., P. J. Makovicky, P. J. Currie, M. A. Norell, S. A. Yerby, and C. A. Brochu. 2004. Gigantism and comparative life-history parameters of tyrannosaurid dinosaurs. *Nature* 430:772-775.
- Evans, D. C., C. A. Forster, and R. R. Reisz. 2005. The type specimen of *Tetragonosaurus erectofrons* (Ornithischia: Hadrosauridae) and the identification of juvenile Lambeosaurines; pp. 349-366 in P. J. Currie and E. B. Koppelhaus (eds.), *Dinosaur Provincial Park*. Indiana University Press, Bloomington.
- Evans, D. C., R. R. Reisz, and K. Dupuis. 2007. A juvenile *Parasaurolophus* (Ornithischia: Hadrosauridae) braincase from Dinosaur Provincial Park, Alberta, with comments on crest ontogeny in the genus. *Journal of Vertebrate Paleontology* 27:642-650.
- Everhart, M. J. 2011. Rediscovery of the *Hesperornis regalis* Marsh 1871 holotype locality indicates an earlier stratigraphic occurrence. *Transactions of the Kansas Academy of Science* 114:59-68.

- Francillon-Vieillot, H., V. d. Buffrenil, J. Castanet, J. Geraudie, F. J. Meunier, J. Y. Sire, L. Zylberberg, and A. d. Ricqlès. 1990. Microstructure and mineralization of vertebrate skeletal tissues; pp. 471-548 in J. G. Carter (ed.), *Skeletal biomineralization patterns, processes and evolutionary trends*. Van Nostrand Reinhold, New York.
- Herne, M. C., J. P. Nair, and S. W. Salisbury. 2010. Comment on "A Southern Tyrant Reptile". *Science* 329:1013.
- Holmes, R. B., C. A. Forster, M. Ryan, and K. M. Shepherd. 2001. A new species of *Chasmosaurus* from the Dinosaur Park Formation of Southern Alberta. *Canadian Journal of Earth Sciences* 38:1423-1438.
- Holtz Jr., T. R. 2001. The phylogeny and taxonomy of the Tyrannosauridae; pp. 64-83 in D. H. Tanke and K. Carpenter (eds.), *Mesozoic Vertebrate Life*. Indiana University Press, Bloomington.
- Holtz Jr., T. R. 2004. Tyrannosauroidae; pp. 111-136 in D. Weishampel, P. Dodson, and H. Osmolska (eds.), *The Dinosauria*. University of California Press, Berkeley.
- Hone, D. W., K. Wang, C. Sullivan, X. Zhao, S. Chen, D. Li, S. Ji, Q. Ji, and X. Xu. 2011. A new tyrannosaurine theropod, *Zhuchengtyrannus magnus* is named based on a maxilla and dentary. *Cretaceous Research* doi: 10.1016/j.cretres.2011.03.005.
- Horner, J. R. 1983. Cranial osteology and morphology of the type specimen of *Maiasaura peeblesorum* (Ornithischia: Hadrosauridae), with discussion of its phylogenetic position. *Journal of Vertebrate Paleontology* 3:29-38.
- Horner, J. R., and P. J. Currie. 1994. Embryonic and neonatal morphology and ontogeny of a new species of *Hypacrosaurus* (Ornithischia, Lambeosauridae) from Montana and Alberta; pp. in K. Carpenter, K. F. Hirsch, and J. R. Horner (eds.), *Dinosaur Eggs and Babies*. Cambridge University Press, New York.
- Horner, J. R., and K. Padian. 2004. Age and growth dynamics of *Tyrannosaurus rex*. *Proceedings of the Royal Society of London Series B* 271:1875-1880.
- Horner, J. R., and M. B. Goodwin. 2006. Major cranial changes during *Triceratops* ontogeny. *Proceedings of the Royal Society of London Series B* 273:2757-2761.
- Horner, J. R., and M. B. Goodwin. 2008. Ontogeny of cranial epi-ossifications in *Triceratops*. *Journal of Vertebrate Paleontology* 28:134-144.

- Horner, J. R., and M. B. Goodwin. 2009. Extreme cranial ontogeny in the Upper Cretaceous dinosaur *Pachycephalosaurus*. Public Library of Science ONE 4:e7626.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 2000. Long bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. Journal of Vertebrate Paleontology 20:115-129.
- Horner, J. R., K. Padian, and A. d. Ricqlès. 2001. Comparative osteohistology of some embryonic and perinatal archosaurs: developmental and behavioral implications for dinosaurs. Paleobiology 27:39-58.
- Hurum, J. H., and K. Sabath. 2003. Giant theropod dinosaurs from Asia and North America: skulls of *Tarbosaurus bataar* and *Tyrannosaurus rex* compared. Acta Palaeontologica Polonica 48:161-190.
- Hutt, S., D. W. Naish, D. M. Martill, M. J. Barker, and P. Newberry. 2001. A preliminary account of a new tyrannosauroid theropod from the Wessex Formation (Early Cretaceous) of southern England. Cretaceous Research 22:227-242.
- Hutton, J. M. 1986. Age determination of living Nile crocodiles from the cortical stratification of bone. Copeia 2:332-341.
- Klein, N., and P. M. Sander. 2008. Ontogenetic stages in the long bone histology of sauropod dinosaurs. Paleobiology 34:248-264.
- Knoll, F., K. Padian, and A. d. Ricqlès. 2010. Ontogenetic change and adult body size of the early ornithischian dinosaur *Lesothosaurus diagnosticus*: implications for basal ornithischian taxonomy. Gondwana Research 17:171-179.
- Li, P.-X., D.-Y. Su, Y.-G. Li, and J.-X. Yu. 1994. Age assignment of the *Lycoptera*-bearing bed. Acta Geologica Sinica-English Edition 7:329-349.
- Ma, F. 1987. Review of *Lycoptera davidi*. Vertebrata Palasiatic 25:8-19.
- Madsen, J. H. 1974. A new theropod dinosaur from the Upper Jurassic of Utah. Journal of Paleontology 48:27-31.
- Maleev, E. A. 1955. Giant carnivorous dinosaurs of Mongolia. Doklady Akademii Nauka SSSR+ 104:634-637.
- Margottini, L. 2011. Is it time to declutter the dinosaur roster? Science 332:782.

- Maryanska, T., and H. Osmolska. 1979. Aspects of hadrosaurian cranial anatomy. *Lethaia* 12:265-273.
- Mullen, W. Fossil identified as mini-T.rex. Chicago Tribune, 18th September 2009. http://articles.chicagotribune.com/2009-09-18/news/0909170802_1_fossil-bones-tyrannosaurus-rex-chicago-paleontologist-paul-sereno. Retrieved 2011 March 8.
- Newbrey, M. G., A. M. Murray, D. B. Brinkman, M. V. H. Wilson, and A. G. Neuman. 2010. A new articulated freshwater fish (Clupeomorpha, Ellimmichthyiformes) from the Horseshoe Canyon Formation, Maastrichtian, of Alberta, Canada. *Canadian Journal of Earth Sciences* 47:1183-1196.
- Osborn, H. F. 1905. *Tyrannosaurus* and other Cretaceous carnivorous dinosaurs. *Bulletin of the American Museum of Natural History* 21:259-265.
- Padian, K., A. d. Ricqlès, and J. R. Horner. 2001. Dinosaurian growth rates and bird origins. *Nature* 412:405-408.
- Padian, K., J. R. Horner, and A. d. Ricqlès. 2004. Growth in small dinosaurs and pterosaurs: the evolution of archosaurian growth strategies. *Journal of Vertebrate Paleontology* 24:555-571.
- Rauhut, O. W. M. 2003. A tyrannosauroid dinosaur from the Upper Jurassic of Portugal. *Palaeontology* 46:903-910.
- Reisz, R. R., D. Scott, H.-D. Sues, D. C. Evans, and M. A. Raath. 2005. Embryos of an early Jurassic prosauropod dinosaur and their evolutionary significance. *Science* 309:761-764.
- Ricqlès, A. d., K. Padian, and J. R. Horner. 2003. On the bone histology of some Triassic pseudosuchian archosaurs and related taxa. *Annales de Paléontologie* 89:67-101.
- Rozhdestvensky, A. K. 1965. Growth changes in Asian dinosaurs and some problems of their taxonomy. *Paleontologicheskii Zhurnal* 3:95-109.
- Russell, D. A. 1970. Tyrannosaurs from the Late Cretaceous of western Canada. *National Museums of Canada Publications in Paleontology* 1:1-34.
- Ryan, M. J., and D. C. Evans. 2005. Ornithischian dinosaurs; pp. 312-348 in P. J. Currie and E. B. Koppelhaus (eds.), *Dinosaur Provincial Park*. Indiana University Press, Bloomington.

- Ryan, M. J., and A. P. Russell. 2005. A new centrosaurine ceratopsid from the Oldman Formation of Alberta and its implications for centrosaurine taxonomy and systematics. *Canadian Journal of Earth Sciences* 42:1369-1387.
- Ryan, M. J., A. P. Russell, D. A. Eberth, and P. J. Currie. 2001. The taphonomy of a *Centrosaurus* (Ornithischia: Ceratopsidae) bone bed from the Dinosaur Park Formation (Upper Campanian), Alberta, Canada, with comments on cranial ontogeny. *Palaios* 16:482-506.
- Sampson, S. D. 1995. Two new horned dinosaurs from the Upper Cretaceous Two Medicine Formation of Montana; with a phylogenetic analysis of Centrosaurinae (Ornithischia: Ceratopsidae). *Journal of Vertebrate Paleontology* 15:743-760.
- Sampson, S. D., M. J. Ryan, and D. Tanke. 1997. Craniofacial ontogeny in centrosaurine dinosaurs (Ornithischia: Ceratopsidae): taxonomic and behavioral implications. *Zoological Journal of the Linnean Society* 121:293-337.
- Scannella, J. B., and D. W. Fowler. 2009. Anagenesis in *Triceratops*: evidence from a newly resolved stratigraphic framework for the Hell Creek Formation. *Cincinnati Museum Center Science Contributions*.
- Scannella, J. B., and J. R. Horner. 2010. *Torosaurus* Marsh, 1891, is *Triceratops* Marsh, 1889 (Ceratopsidae: Chasmosaurinae): synonymy through ontogeny. *Journal of Vertebrate Paleontology* 30:1157-1168.
- Sereno, P. C., L. Tan, S. L. Brusatte, H. J. Kriegstein, X. Zhao, and K. Cloward. 2009. Tyrannosaurid skeletal design first evolved at small body size. *Science* 326:418-422.
- Tanke, D. H. 2005. Identifying lost quarries; pp. 34-53 in P. J. Currie and E. B. Koppelhaus (eds.), *Dinosaur Provincial Park*. Indiana University Press, Bloomington.
- Trueman, C. N., and M. J. Benton. 1997. A geochemical method to trace the taphonomic history of reworked bones in sedimentary settings. *Geology* 25:263-266.
- Trueman, C. N., A. K. Behrensmeyer, R. Potts, and N. Tuross. 2006. High-resolution records of location and stratigraphic provenance from the rare earth element composition of fossil bones. *Geochimica et Cosmochimica Acta* 70:4343-4355.
- Tsuihiji, T., M. Watabe, K. Tsogtbaatar, R. Barsbold, S. Suzuki, A. H. Lee, R. C. Ridgely, Y. Kawahara, and L. M. Witmer. 2011. Cranial osteology of a juvenile specimen of *Tarbosaurus bataar* from the Nemegt Formation (Upper Cretaceous) of Bugin Tsav, Mongolia. *Journal of Vertebrate Paleontology* 31:497-517.

- Tucker, A. D. 1997. Validation of skeletochronology to determine age of freshwater crocodiles (*Crocodylus johnstoni*). Australian Journal of Marine and Freshwater Research 48:343-351.
- Wedel, M. J. 2003. The evolution of vertebral pneumaticity in sauropod dinosaurs. Journal of Vertebrate Paleontology 23:344-357.
- Weishampel, D. B., D. E. Fastovsky, M. Watabe, D. Varricchio, F. Jackson, K. Tsogtbaatar, and R. Barsbold. 2008. New oviraptorid embryos from Bugin-Tsav, Nemegt Formation (Upper Cretaceous), Mongolia, with insights into their habitat and growth. Journal of Vertebrate Paleontology 28:1110-1119.
- Whitlock, J. A., J. A. Wilson, and M. C. Lamanna. 2010. Description of a nearly complete juvenile skull of *Diplodocus* (Sauropoda: Diplodocoidea) from the Late Jurassic of North America. Journal of Vertebrate Paleontology 30:442-457.
- Xu, X., M. A. Norell, X. Kuang, X. Wang, Q. Zhao, and C. Jia. 2004. Basal tyrannosauroids from China and evidence for protofeathers in tyrannosauroids. Nature 431:680-684.
- Xu, X., C. A. Forster, M. A. Norell, G. M. Erickson, D. A. Eberth, C. Jia, and Q. Zhao. 2006. A basal tyrannosauroid dinosaur from the Late Jurassic of China. Nature 439:715-718.
- Zaragüeta, B. 2004. Basal clupeomorphs and ellimmichthyiform phylogeny; pp. 391-404 in G. Arratia and A. Tintori (eds.), Mesozoic Fishes 3 - Systematics, Paleoenvironments, and Biodiversity. Verlag Dr. Friedrich Pfeil, Munich.
- Zhang, J.-Y. 2002. A new species of *Lycoptera* from Liaoning, China. Vertebrata Palasiatic 40:257-266.

CHAPTER SIX

A POPULATION HISTO-VARIABILITY ANALYSIS OF THE HADROSAURID
DINOSAUR *MAIASAURA PEBBLESORUM*

Contribution of Authors and Co-Authors

Manuscript in Chapter 6

Author: HollyNoelle W. Ballard

Contributions: Conceived the study, obtained and processed specimens, analyzed data, interpreted results, and wrote the manuscript.

Co-author: John R. Horner

Contributions: Provided funding, specimen processing facilities and equipment, microscope and microphotograph equipment. Assisted with study design, discussed implications of results, and edited earlier drafts of the manuscript.

Co-author: Elizabeth A. Freedman

Contributions: Assisted with statistical analyses and interpretations.

Manuscript Information Page

Holly N. Woodward, John R. Horner, Elizabeth A. Freedman

Status of Manuscript:

☒ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☐ Published in a peer-reviewed journal

Abstract

Forty eight tibiae of the hadrosaurid dinosaur *Maiasaura peeblesorum* were collected from a single bone bed and used in an ontogenetic histologic analysis. This represents the largest single-element, monospecific dinosaur histology study to date, the purpose of which is to establish the amount of individual variation present within a taxon both ontogenetically as well as amongst similarly sized samples. Robust regression equations ($R^2 > 0.9$) were produced, showing a strong relationship between tibia length and variables such as diaphyseal perimeter, cortical area, and bone wall thickness. Detailed histologic analyses, determination of annual apposition rates, and both ontogenetic body length and body mass curves support the findings of a previous study on *Maiasaura*. This hadrosaur genus possessed woven tissue throughout ontogeny, indicating growth was rapid until skeletal maturity was attained. Adult size, indicated by the presence of an external fundamental system, was achieved by three individuals in the sample, ranging between nine and ten years of age. Approximately half of the 7 m adult body length was attained within the first year of growth, but mortality rate was also the highest during this first year. Additionally, there is considerable variation in regard to body size each year, and this strongly cautions against generalizations about growth or the construction of growth curves based upon scanty sample material.

Introduction

Vertebrate bone tissue microstructures reveal details about ontogenetic status, annual cyclicity, and ranges of daily bone apposition rates in extant taxa (e.g., Margerie

et al., 2004; Montes et al., 2010). Because of this, bone microstructure can be used to infer growth rates, age at death, and to produce ontogenetic growth curves from bones of extinct vertebrates such as non-avian dinosaurs (e.g., Cormack, 1987; Francillon-Vieillot et al., 1990; Games, 1990).

In extant relatives of non-avian dinosaurs (crocodylians and birds), bone tissue organization varies somewhat across individuals within a taxon (Castanet et al., 2000; Woodward et al., *In prep.*). These differences in tissue organization indicate individual variation in growth rates, revealing the importance of assessing histovariability before hypothesizing about growth. Unfortunately, dinosaur histovariability is often overlooked due to the small sample sizes used in analyses. This leads to inaccuracies when generalizing about developmental trends and estimating growth trajectories of dinosaur taxa.

To assess the general potential for histological variation in dinosaurs and increase what is known about the ontogeny of at least one taxon, forty-seven tibiae from the hadrosaurid *Maiasaura peeblesorum* were histologically examined. This ontogenetic sample corresponds to individuals with body lengths between 2.5 m and 7 m. A sample of this size allows a more robust assessment of tibia histovariability both within an ontogenetic context as well as among tibiae of similar size, and represents the largest single-element, monospecific dinosaur osteohistology study to date.

This research builds upon a previous ontogenetic study of *Maiasaura*, making this genus one of the most ontogenetically well-understood. To address the question of intraskeletal variability, Horner et al. (2000) examined over fifty skeletal elements from

an ontogenetic series of *Maiasaura*. Elements sampled included vertebrae, ribs, limb girdles, and long bones. From this data six growth stages were identified based on tissue structures observed. In general, the smaller and presumably younger long bones consisted of rapidly deposited woven-fibered tissue, and secondary remodeling developed while the animals were still growing. Growth marks in the form of lines of arrested growth (“LAGs”) became apparent at the sub-adult stage. LAGs are important features in dinosaur osteohistology because they represent annual pauses in bone apposition in extant vertebrates (Peabody, 1961; Frylestam and von Schantz, 1977; Hemelaar and van Gelder, 1980; Ricqlès, 1980; Hutton, 1986; Tucker, 1997; Castanet et al., 2004; Bourdon et al., 2009). Therefore, their presence in *Maiasaura* enables determination of age at death as well as yearly rates of growth.

Horner et al. (2000) found that woven fibered tissue was present in most of the cortex of the largest *Maiasaura* long bone specimen sampled, but near the periosteal surface a lamellar external fundamental system (“EFS”) was present, which indicates that skeletal maturity was attained (Cormack, 1987; Castanet et al., 1988; Ponton et al., 2004; Klein et al., 2009; Woodward et al., 2011). The authors also noted that there was considerable histological variability between long bones and non weight-bearing elements. The latter appeared to grow more slowly, and in the adults elements such as ribs and ischia consisted of dense Haversian tissue except for a thin layer of slow growing lamellar primary bone at the periosteal surface.

Despite the large sample size, Horner et al. (2000) estimated growth rates from just six femora. Based on estimated bone apposition rates and retrocalculation of missing

growth marks, the authors determined *Maiasaura* achieved skeletal maturity within six to eight years.

The present study reexamines the results of Horner et al. (2000) by providing a more detailed analysis of how the bone tissue microstructure of a single element varies within individuals of similar size, as well as ontogenetically. Finally, several regressions and growth curves are produced for *Maiasaura* based on this relatively large sample set, providing some of the most well-constrained dinosaur growth data to date.

Materials and Methods

Background

Maiasaura specimens used in this study were collected from the Two Medicine Formation of Montana. This formation was deposited between 82-71 Ma (Lorenz and Gavin, 1984) and is approximately 600 m thick at its westernmost exposure in the Disturbed Belt, thinning eastward and pinching out at the western limb of the Sweetgrass Arch (Lorenz and Gavin, 1984; Rogers, 1990; Trexler, 2001). Along the eastern edge of the Rocky Mountains this formation extends from southern Alberta to central Montana (Trexler, 2001) and is the stratigraphic equivalent of the more eastward coastal plain deposits of the Judith River Formation (Lorenz and Gavin, 1984). The presence of red beds, caliche horizons, and grey to red mudstones suggests that overall, the Two Medicine Formation was deposited during semi-arid conditions that included a long dry season and warm temperatures (Lorenz and Gavin, 1984).

The original discovery of *Maiasaura* reported on tiny, immature hadrosaurs within a bowl-shaped, nest-like depression and a large individual in close proximity (Horner and Makela, 1979). Crushed eggshell fragments within the nest, as well as poorly formed epiphyses on the long bones of the hatchlings, provided strong evidence that *Maiasaura* hatchlings were altricial and fed by parents (Horner and Makela, 1979; Barreto, 1997; Horner et al., 2001a).

Overlying the *Maiasaura* nesting horizons described by Horner and Makela is an extensive monospecific bone bed consisting of the disarticulated remains of numerous *Maiasaura* individuals spanning an area of approximately two square kilometers (Horner et al., 2001b; Horner, 2002). The matrix consists of a black, silty, micritic carbonate with a high ash content intermixed with cobbles and boulders, located just below the lowermost lacustrine carbonate (Trueman, 1999; Horner et al., 2001b). The bone bed was most likely the result of a sediment gravity flow due to its lateral extent, random bone orientation, a disconformity at the base, and the uneven thickness of the deposit (Trueman, 1999). Rare earth analysis indicates that the site is a restricted, low time-averaged sample, most likely incorporating relatively fresh bones (Trueman, 1999).

Maiasaura fossils collected from this site include skull fragments, vertebrae, ribs, sacral elements, scapulae, humeri, radii, ulnae, femora, tibiae, fibulae, and manual and pedal elements. Because none of the fossils are articulated or even associated, it is probable that the bones were transported and then buried after a period of decomposition. There is likely some time-averaging in the sample as evinced from the disarticulation and transport. But because no elements exhibit weathering or splintering/crushing associated

with prolonged surface exposure this bone bed is considered to represent a population of *Maiasaura* that experienced similar seasonal environmental stresses over a brief period of time. Therefore differences in bone microstructures should be influenced largely by individual variability and less so by the environment.

Procedure

The diaphyses of forty-seven *Maiasaura peeblesorum* tibiae (MOR 005; Museum of the Rockies, Bozeman, Montana) collected from a single bone bed were selected for histological examination, representing a minimum of thirty one individuals. Tibiae within this project were numbered T-1 through T-47. Out of convention, the femur is often preferred for histological analyses. But as with all hadrosaurids, the fourth trochanter in the femur of *Maiasaura* extends to nearly half of the length of the bone. This process is frequently remodeled during growth, thus affecting the visibility of primary tissue along most of the diaphyseal length. Therefore the tibia was chosen for analyses because it lacks such a process and was therefore less subject to intense remodeling. Although there is variation in the bone apposition rates of different elements within a skeleton (Horner et al., 2000; Woodward et al., *In prep.*), this study samples only tibiae in order to directly compare the variation present in a single element across multiple individuals. In addition, the tibia is likely amongst the fastest growing of the long bones (Woodward et al., *In prep.*), allowing reasonable estimates of maximum growth rates.

Each *Maiasaura* tibia was photographed and hand traced in lateral view prior to thin sectioning. The straight line distance from the anterior edge of the cnemial crest to

the distal tip of the lateral malleolus was recorded. Conservative length estimates were made in several cases where either epiphysis was incomplete.

A section of the diaphysis containing the least circumference for each tibia was removed using a tile saw fitted with a diamond blade, and then prepared following Lamm (2007). Two transverse cuts, approximately 0.5 cm in thickness, were made to either side of the line of minimum diaphyseal circumference. In most cases, only one of the two slices was completely processed for analysis while the second was held as a duplicate.

Completed slides were examined with either 4 X or 10 X objectives using a Nikon Optiphot-Pol polarizing microscope. Photomicrographs of the entire sections were taken using a Nikon DS-Fi1 digital sight camera, and the resulting images were compiled using the NIS-Elements BR 3.0 software. To create a single image, this software combined together single photos of the entire section taken incrementally, essentially “stitching” together a mosaic of photographs. Therefore, these composite images often have a pixilated appearance due to multiple single images merged together as one. Transverse section surface perimeters as well perimeters of medullary cavities and LAGs were traced in Adobe Photoshop CS3. Perimeters, and the areas enclosed by the perimeters, were quantified using NIH ImageJ (Table 6.1).

Average daily bone apposition rates for each year of growth were determined for complete, uncrushed transverse diaphyseal sections (Table 6.2). The distances along major and minor axes from the geometric centroid to the first LAG were measured and averaged to obtain a cortical thickness. This procedure was repeated for each consecutive LAG so that annual growth could be determined. Annual cortical thickness was then

converted to a daily apposition rate ($\mu\text{m}/\text{day}$) assuming a 370 day year during the Campanian. The apposition rates of cortical thicknesses between the outermost LAG and the periosteal surface were not calculated as this thickness does not represent an entire year of growth.

Regressions were determined (Fig. 6.1-6.2) from the relationship between tibia length and: minimum diaphyseal perimeter, transverse section area, medullary cavity area, medullary cavity perimeter, and bone wall thickness (BWT; the area of the transverse section excluding the medullary cavity). In many cases the resulting R^2 values were high enough to enable the prediction of tibia length from a given measurement, or vice versa. Out of the forty-seven tibiae, only forty were used to determine the transverse area and diaphyseal perimeter regressions because the periosteal surface was largely missing in some specimens or the diaphyses were too crushed to obtain accurate measurements. Bone wall thickness, medullary perimeter, and medullary cavity area regressions were determined from only twenty nine specimens from which both diaphyseal area and medullary cavity measurements could be obtained.

Growth curves were also produced. For a *Maiasaura* with a body length of 7 m, Horner et al. (2000) estimated a corresponding femur length of 100 cm. Because the present study examines tibiae rather than femora, to construct ontogenetic growth curves for *Maiasaura* individuals the proportional relationship between femur and tibia length had to be determined. This was obtained from a large, partially articulated *Maiasaura* specimen (ROM 44770; Royal Ontario Museum, Toronto, Ontario, Canada) with a femur length of 102 cm, a fibula length of 98.5 cm, and by using the equation of Anderson et al.

(1985), a body mass of 3,500 kg. Although the specimen lacks a tibia, fibula length provides a reasonable proxy. Therefore, based on the femur/tibia length ratio of ROM 44770, and the femur/body length estimate from Horner et al. (2000), body length could be determined from tibia length (Table 6.3). Thus, a 7 m long *Maiasaura* with a 100 cm femur had a tibia length of 96.5 cm.

Additionally, the regression formula obtained from plotting tibia length against minimum diaphyseal perimeter ($R^2 = 0.95$) allows the prediction of tibia length for each year of growth (represented by LAGs) (Table 6.3). Finally, by using the femur/tibia length ratio obtained from ROM 44770 and the regression formula, ontogenetic growth curves of body length over time were produced for nine *Maiasaura* individuals (Fig. 6.3A).

The femur/tibia length ratio also allows the prediction of femur lengths from tibia lengths recorded in this sample set (Table 6.3). These data can then be used to construct ontogenetic mass curves (Table 6.3, Figure 6.3B) by applying the developmental mass extrapolation equation, which determines body mass from femur length (Erickson and Tumanova, 2000). However, this widely used mass equation is in turn based on the equation of Anderson et al. (1985). Packard et al. (2009) recently demonstrated that the mass equation of Anderson et al. (1985) results in overestimates. Thus, the ontogenetic mass estimates of *Maiasaura* individuals and the resulting regression equation should be considered extremely suspect. Despite these caveats, the growth history of an animal is best illustrated in three dimensions. Ontogenetic mass curves are therefore included (Fig.

6.3B) to supplement the linear curves and to illustrate the possible volumetric growth trajectory of *Maiasaura*.

The values represented by the periosteal surface (i.e., body length and body mass at death) are omitted from curves representing growing individuals as the time interval between the outermost growth mark and the periosteal surface is less than a year and therefore cannot be determined. The opposite problem is present in skeletally mature *Maiasaura* individuals, as the EFS can represent multiple years (Woodward et al., 2011). For individuals with an EFS, the value represented by the periosteal surface is displayed as a dashed line on corresponding growth curves. To supply data for time zero on growth curves, the minimum diaphyseal perimeter of a hatchling tibia (YPM-PU 22432; Princeton University collections now curated at the Yale Peabody Museum, New Haven, Connecticut, USA) was measured and a body length of 0.5 m (Horner et al., 2000) was used.

Results

Although ontogeny is a continuum, for descriptive purposes the *Maiasaura* tibiae are divided into categories based on length. General tissue organizational trends for each length category are described, as well as features specific to individuals that are unusual or lacking in other similarly sized tibiae. As noted by Horner et al. (2000), tissue organization remains woven until skeletal maturity is approached. However, vascular organization changes frequently throughout ontogeny.

Tibia Lengths 33 cm – 40 cm (n = 7)

The smallest tibiae in the sample represent individuals between 2.5 and 2.9 m in body length (Fig. 6.4). The lack of a complete endosteal layer and the presence of scattered resorption cavities from the inner to mid cortex suggest medullary expansion was occurring. Secondary osteons are sparse within the cortex but when present are located around the medullary cavity. In two tibiae (T-1 and T-9), there are remnants of very immature-looking tissue bordering the medullary cavity (Figure 6.5). These crescents of bone have large vascular canals, on the order of erosion cavities in size, representing incompletely filled primary osteons. The overall vascular pattern of this immature tissue is “mesh-like”.

In almost all specimens the innermost cortex is laminar, except in T-4 where it is reticular. In general, the vascular arrangement becomes largely reticular by the mid cortex and then changes to laminar prior to the periosteal surface, although there is considerable individual variability in vascular arrangement (Fig. 6.6). For instance, in T-1, the anterior side remains reticular from mid cortex to the surface but is laminar elsewhere. The mid to outer cortex on the medial and posterior sides of T-7 is laminar, but reticular elsewhere. The mid cortex of T-4 is laminar, but this changes to reticular in the outer cortex and back to laminar just prior to the periosteal surface. Radial anastomoses are present throughout the cortex of T-9, on the anterior and posterior sides of T-8, on the anterior, posterior, and lateral sides of T-7, and on the posterior and lateral sides of T-5.

Several tibiae (T-1, T-3, T-9, T-10) preserve an unusual banding pattern within the mid cortex (Fig. 6.7), which was also observed in long bones of this size by Horner et al. (2000). These bands are the result of several factors: minute changes in the average diameter of primary osteons, a change in vascular arrangement, and a color change in the diagenetic staining of the fossil bone. They become increasingly difficult to identify at higher magnifications as the differences between the bands and surrounding regions become less distinct.

The outer cortex on the lateral side of T-9 is unusual because the reticular and laminar vascularity abruptly changes to radial (Figure 6.8A-B). A thin band of more longitudinally arranged canals is observed in the middle of this radially vascularized tissue.

Tibia Lengths 40 – 50 cm (n = 22)

As with the smaller tibiae, there is considerable individual variability in vascular canal orientation from inner to outer cortex in this size category, which represents individuals between 3 m and 3.5 m in body length (Fig. 6.9). Vascularity is reticular or laminar in the innermost cortex and may either alternate between the two organizational patterns throughout the cortex, or remain as one or the other to the periosteal surface (Fig. 6.10A-B). One specimen (T-26) even has somewhat plexiform vascularity at the periosteal surface on the medial and antero-medial sides. Radial anastomoses are also frequent but are not present in all specimens. They are especially prevalent on the lateral side of T-20, the posterior side of T-19, and both the anterior and posterior sides of T-21.

Secondary osteons within the inner cortex are more frequent than in the previous size group (Fig. 6.10C-D). They are particularly concentrated on the anterior side of the medullary cavity in T-13 and T-29, and on the lateral side of the medullary cavity in T-16 and T-24.

Along with secondary osteons, resorption cavities are more common in this size category than in smaller tibiae and are sometimes concentrated in certain regions. In T-2, they are most frequent on the antero-medial and postero-medial sides; on the anterior side in T-13 and T-29; on the medial and anterior sides of T-11; and largely on the medial side in T-14, T-23, and T-21. Some tibiae also exhibit resorption cavities arranged in circular rings within the cortex (Fig. 6.11). In T-19, there are three rings of resorption cavities within the inner and mid cortex, while there is a single circular ring of resorption cavities in the mid cortex of T-16. The resorption cavity ring in T-6 is incomplete, forming a crescent within the mid cortex that does not complete the circle on the medial side. The beginnings of a resorption ring may be seen in T-25 and T-24, where there is a string of resorption cavities within the mid cortex on the antero-lateral side and posterior sides, respectively.

As observed in the previous size group, bands of tissue resulting from slight changes in the diameters of laminar vascular canals (Fig. 6.7) are obvious within the cortex at low magnifications in almost half (T-12, T-20, T-11, T-16, T-17, T-6, T-28, T-26, T-27, T-25, T-22, T-29) of the specimens in this size range. However, the number of bands observed in each tibia differs.

Some tibiae also preserve a column of tissue with radial vascularity extending from the inner to outer cortex (Fig. 6.10E-F) located on the lateral (T-20), antero-lateral (T-18), or anterior (T-10, T-19, T-28, T-25, T-22) sides (the “plugs” of Hübner (2012)). When present, this column is always associated with the anterior border of the tibia, a prominent ridge at the periosteal surface.

Additionally, there is a crescent of bone at the periosteal surface on the anterior side of T-20 that is largely radial (Fig. 6.8C-D). This resembles the structure observed in T-9 somewhat but it is not as extensively developed.

Tibia Lengths 50 cm – 60 cm (n = 1)

There is only one tibia in this length category (T-30), corresponding to an individual 4.1 m in body length (Fig. 6.12). This individual was likely within its third year of life, as there are two LAGs within the cortex. Large resorption cavities surround the medulla and vascularity of the inner cortex is largely reticular but becomes more laminar approaching the first LAG. Vascularity is laminar after the first LAG but changes to reticular between the first and second LAG, except on the posterior side where it is completely laminar within this zone. Immediately after the second LAG the tissue is largely of longitudinal vascularity before becoming a mix of laminar and reticular vascularity closer to the surface (Fig. 6.13B).

Tibia Lengths 60 – 70 cm (n = 3)

There are only three tibiae in this size category (Fig. 6.12), and like T-30 they both possess two LAGs. The individuals represented by these tibiae were between 4.6 m

and 5 m in body length. In T-31, vascularity is largely reticular within the inner cortex and becomes more laminar prior to the first growth mark. Secondary osteons are scattered but more frequent on the posterior side. After the first LAG vascularity is somewhat laminar but becomes a mixture of reticular and laminar vascularity for most of the zone. However, the anterior and antero-medial sides are more strongly reticular. Immediately after the second LAG vascularity is more strongly longitudinal before becoming either plexiform or laminar.

In T-32, secondary osteons are dense within the innermost cortex but particularly concentrated on the postero-medial side of the medullary cavity. Here they spread halfway between the medullary cavity and the first LAG. Resorption cavities are sparsely scattered throughout the inner and mid cortex (Fig. 6.13C). Where primary tissue is visible prior to the first LAG, it is either laminar or longitudinal in vascularity. Between the first and second LAG vascularity is reticular but becomes more strongly laminar immediately prior to the second LAG. Between the second LAG and the surface, the tissue is predominately reticular, although immediately after the second LAG vascularity is largely longitudinal. There is a column of radially vascularized bone from the inner to outer cortex on the anterior side, corresponding to the anterior border at the periosteal surface (Fig. 6.13D).

Large resorption cavities surround the medullary cavity in T-47. Some are lined with lamellar tissue, indicating conversion of the inner cortex to cancellous tissue. Secondary osteons are scattered within this region as well. The remaining primary tissue of the inner cortex is a mixture of reticular and laminar vascularity. Prior to the first LAG

vascularity remains a combination of reticular and laminar except on the lateral side where it is a mixture of laminar and plexiform. Between the first and second LAG, vascularity is largely reticular. The cortex on the lateral side between the second LAG and the surface is largely plexiform, but a mixture of laminar and plexiform elsewhere. Vascular canals are smaller in diameter and more longitudinal immediately after the second LAG.

Tibia Lengths 70 – 80 cm (n = 3)

Tibiae in this category (Fig. 6.14) exhibit considerable medullary cavity expansion (Fig. 6.15A, C) and between five and seven LAGs. Corresponding body lengths range from 5.4 m to 5.7 m. In the zone of cortex between the medullary cavity and the first LAG, resorption cavities are frequent and secondary osteons are densely clustered. Secondary osteons and resorption cavities are also scattered throughout the cortex. In addition, a thick column of reticular primary tissue and secondary osteons stretches from the inner to outer cortex on the anterior side and is associated with the anterior border at the periosteal surface (Fig. 6.15D). Radial anastomoses are frequent in the cortex on both anterior and posterior sides.

Vascularity before the first LAG appears laminar where visible, and vascularity between the first and second LAGs is a mixture of laminar and reticular. After the second LAG vascularity is generally laminar to plexiform. The tissue immediately after the second LAG consists of mostly longitudinal or reticular vascular canals, which changes to laminar or plexiform vascularity for the majority of the zone thickness (Fig. 6.15B). This change in vascular pattern is then repeated within the consecutive zones.

Tibia Lengths 80 – 90 cm (n = 6)

The tibiae in this category (Fig. 6.16) possess between six and nine LAGs and individuals would have been between 6 m and 6.4 m in body length. The LAG spacing in the outer cortex (Fig. 6.17) suggests growth has slowed considerably and these individuals would have soon attained skeletal maturity. In fact, an external fundamental system is present in T-40 (Fig. 6.17C) after just nine LAGs, indicating this individual achieved skeletal maturity in nine years.

The inner cortex between the first LAG and the medullary cavity contains numerous resorption cavities and secondary osteons. In places the secondary osteons completely obscure primary tissue, except in T-36 and T-43 where secondary remodeling is not as prevalent. Where visible the tissue in this first zone appears laminar, except in T-40 where it is largely reticular.

After the first LAG vascularity is a combination of longitudinal, laminar, and reticular. A more pronounced vascular pattern is observed after the second LAG (Fig. 6.17B), where vascularity is either longitudinal or reticular but then becomes laminar to plexiform for the majority of the zone. This vascular pattern is repeated in the zones between other LAGs as well and seems to be more prominent on the posterior side in most specimens. In three individuals there is a column of secondary osteons from the inner to outer cortex, associated with the anterior border (Fig. 6.17E). However, this column is absent in T-40, T-43, T-44, and T-36.

Tibia Lengths 90 – 100 cm (n = 5)

Tibiae in this category (Fig. 6.18) correspond to individuals between 6.5 m and 7.2 m in body length. LAG counts range from four to ten (Fig. 6.19E), but the lower LAG counts are likely due to missing outer cortex in those specimens, as tibiae with a complete outer cortex have nine or ten LAGs. Although still visible, the innermost (and presumably first year) LAG is often partially destroyed by medullary expansion and secondary reconstruction (Fig. 6.19E). The remaining cortex between the medullary cavity and the first LAG is largely invaded by resorption cavities or secondary osteons, but interstitial primary tissue appears laminar. Resorption cavities and secondary osteons are scattered throughout the cortex, but are not as dense as within the first zone.

Between the first and second LAG the tissue is reticular to laminar in vascularity. After the second LAG, vascularity is initially longitudinal or reticular but vascular canals soon become laminar or plexiform. This pattern repeats within consecutive zones and is best observed on the posterior side, although it is not as evident in T-42 or T-41. Radial anastomoses are also frequent on the posterior side. As in the previous size groupings, there is a column of secondary tissue from the inner to outer cortex corresponding to the anterior border.

After ten LAGs, an EFS is present in T-46 (Fig. 6.19D). An EFS is also found in T-45 (Fig. 6.19B), but the exact LAG count cannot be determined due to a combination of extensive secondary remodeling within the inner cortex as well as diagenetic altering so great that structures are difficult to discern (Fig. 6.19A).

In T-42, there is an unusual crescent of bone on the postero-lateral side of the tibia made of highly vascularized radial tissue (Fig. 6.8E, F). At the periosteal surface of this crescent the vascular diameters decrease and the tissue becomes slightly more reticular.

Discussion

Ontogenetic Histology Analysis

The results of this study support the original findings of Horner et al. (2000) with regards to general ontogenetic changes in tissue organization, but the results also provide a more detailed examination of ontogenetic variability within a population. The vascularity of the smallest tibiae (33 cm – 50 cm) varied between laminar and reticular, alternating between the two arrangements both from inner to outer cortex, as well as circumferentially about the transverse sections. Additionally, radial anastomoses were largely absent in some specimens but fairly common in others.

Changes in vascular orientation within a woven-fibered matrix may indicate different apposition rates (Margerie et al., 2004), but there can also be considerable overlap in the ranges of growth rates for different vascular arrangements (Castanet et al., 1996; Castanet et al., 2000; Margerie, 2002; Starck and Chinsamy, 2002; Margerie et al., 2004). The exception is radial vascularity, which is associated with extremely high growth rates- between 109 $\mu\text{m}/\text{day}$ and 171 $\mu\text{m}/\text{day}$ in king penguin chicks, for example (Margerie et al., 2004). The variation in vascular arrangement (especially that of radial vascularity) observed in the smallest *Maiasaura* size categories therefore suggests a degree of variability in apposition rates during early ontogeny not observed in older

individuals. In addition, the “mesh like” woven tissue with large primary Haversian canals observed in the inner cortex of the two smallest tibiae (Fig. 6.5B, D) may be remnants of an earlier, even faster growing period, perhaps corresponding to nestling sizes.

Tibiae between 33 cm and 50 cm in length also exhibited an unusual circular banding pattern within the cortex (Fig. 6.7), which was previously noted by Horner et al. (2000) in similarly sized elements. Because the number or even presence of bands varies across individuals, and because they are the result of slight changes in vascularity, the bands do not represent pauses in growth. Instead, these marks likely indicate brief, periodic fluctuations in the bone apposition rates of rapidly growing individuals. Additionally, this kind of vascular banding is only observed in tibiae representing individuals less than or nearing one year of age (i.e., lacking LAGs).

An interesting feature observed in some but not all tibiae between 40 cm and 50 cm is the presence of concentric resorption cavity “rings” (Fig. 6.11). This arrangement is absent in any other size category. A similar pattern of concentric resorption cavity rings was reported in an immature humerus from the sauropod *Alamosaurus* (Woodward and Lehman, 2009). Thus, it may be that this pattern of bone resorption is a characteristic of immature and therefore fast growing dinosaur long bones, but only during a particular ontogenetic period. In both larger and smaller *Maiasaura* size classes, resorption begins within the inner cortex and progresses outward. Perhaps during the stage of rapid growth represented by the 40 cm - 50 cm size class this resorption pattern allowed medullary expansion to keep pace with the high apposition rates of the cortex. Due to the brief

ontogenetic period in which it appears, coupled with the smaller sample sizes of most other dinosaur ontogenetic studies, it is unsurprising that this concentric resorption pattern has gone largely unreported.

Lines of arrested growth, which indicate annual pauses in apposition, were not found in tibiae less than 50 cm in length. However, every specimen between 50 cm and 70 cm had two LAGs within the cortex (Fig. 6.13), indicating that the entire sample is missing individuals between one and two years of age. This confirms the same observations made by Horner et al. (2000). However, when the innermost LAG perimeter in larger tibiae (>50 cm) is measured and converted to a tibia length estimate, the values reveal tibia lengths between 39 cm and 51 cm. It is therefore likely that specimens within the 40 cm – 50 cm category were essentially yearlings (Fig. 6.20) that had not yet formed a growth mark, as previously suggested by Horner et al. (2000). If true, a range of 39 cm to 51 cm for yearlings corresponds to body lengths between 2.9 m and 3.5 m, further illustrating the variability in growth rates present even at this young age.

Body lengths of two year old individuals vary almost as much as one year olds, with the second LAG perimeters corresponding to individuals between 3.7 m and 4.2 m. Vascularity continues to vary both within zones and circumferentially across a section, with plexiform vascularity becoming more common between the second LAG and the periosteal surface. Secondary osteons are scattered within the innermost cortex but are dense on the posterior side.

Specimens greater than 70 cm in length have between five and ten growth marks. At this size, continued medullary expansion and frequent secondary osteons threaten to

destroy the first LAG. However, secondary reconstruction as well as diagenetic alteration is pervasive throughout the cortex only in T45 (Fig. 6.19A). This is the second largest tibia specimen, and primary tissue is only visible interstitially. Secondary osteons extend to the periosteal surface in places, making an exact LAG count impossible.

For tibiae in this size group, spacing between the growth marks decreases considerably after the second LAG but then remains fairly constant for several years (Fig. 6.19E). Near the periosteal surface in the largest tibiae (80 cm – 100 cm), spacing between the outermost LAGs resumes its decrease, suggesting individuals in this size range were approaching skeletal maturity. Based on the presence of EFS, skeletal maturity was attained in three specimens. These individuals would have had body lengths of between 6.3 m and 7.2 m, again demonstrating the size variability inherent in this genus. However, even in the closely spaced LAGs just prior to the EFS, vascularity remains high and tissue organization is still woven.

Beginning with tibiae in the 40 cm – 50 cm category, some specimens preserve a narrow column of reticular, radial, or secondary tissue spanning from the inner to the outer cortex, corresponding to the anterior border. This column was not observed in tibiae between 33 cm and 40 cm in length. In extant animals, such features in skeletal elements correspond to areas of tendon insertion. The location of this insertion on the tibiae suggests it was related to dorsal flexion of the pes. Thus, perhaps the appearance of a strong tendon insertion in individuals nearing the end of their first year corresponds to a change in body posture occurring at this time. This may support the hypothesis proposed by Dilkes (2001), that young *Maiasaura* were bipedal but there was eventually a shift to

quadrupedality. However, more research into this hypothesis is necessary as the presence of this column of radial or secondary tissue, as well as the presence of a prominent anterior border, is variable in every size class, suggesting the degree of muscle development varied across individuals.

Within the entire sample of forty eight tibiae, three tibiae exhibited unusual cuffs of radial bone at the periosteal surface (Fig. 6.8). These were found in two individuals less than one year of age (T-9, T-20) and another at least five years of age (T-42). This unusual growth bears a striking resemblance to the limb bone histology of extant chickens infected with avian petrosis (Fig. 6.8G). Unlike mammalian petrosis, which is sex-linked, avian petrosis is a virus affecting RNA (Campbell, 1965). It presents as fusiform swellings beginning first in the metatarsals and eventually spreading to all long bones, and has also been known to induce kidney and muscle tumors (Campbell, 1965). New periosteal and endosteal bone is deposited radially in layers, and the new endosteal tissue eventually destroys the medullary cavity (Campbell, 1965). All three *Maiasaura* tibiae show this radial layering at the periosteal surface. The largest affected tibia, T-42, is also the most extensively affected, with evidence of endosteal invasion in addition to periosteal growth. T-42 is also the only one of the three specimens to exhibit an obvious swelling on the diaphysis.

Several other unusual patterns of vascularity were also observed in the entire sample set, but these do not appear to be pathologic. As previously discussed, an unusual vascular banding pattern was observed in many tibiae less than a year of age. But in somewhat older individuals there is another repeating vascularization pattern:

immediately after the second LAG, vascular canals are small and predominately longitudinal but then become reticular, laminar, or plexiform for the majority of the zone (e.g., Fig. 6.15B). This pattern continues between each LAG until the EFS is formed, but becomes less obvious in the more closely spaced zones prior to the EFS. This pattern is consistently observed on the posterior side, but may not be present at other locations around the section.

Although histologic examination of extant large-bodied vertebrates is needed for comparison, it is possible these annually repeated changes in vascular organization correspond to seasonal or biological changes. Perhaps after emerging from the growth hiatus represented by a LAG, *Maiasaura* growth resumed at a different rate for a short period, possibly triggered by temporarily more abundant food resources or perhaps in preparation for mating/nesting.

After the second LAG, zone spacing becomes noticeably reduced, corresponding to a reduction in annual body mass increase. Lee and Werning (2008) correlated the decrease in the rate of body mass addition to the attainment of sexual maturity in several dinosaur taxa. If this is the case for *Maiasaura* as well, this decrease in body mass addition suggests that *Maiasaura* achieved sexual maturity in its third year of growth (i.e., after the second LAG).

Regressions and Growth Curves

When tibia lengths were plotted against various measurements obtained from the diaphysis, the reliability of predicting one measurement from the other was determined (Fig. 6.1, 6.2). There is a very strong linear relationship between tibia length and

diaphyseal perimeter ($R^2 = 0.95$ for both), and very strong exponential relationships between tibia length and total cortical area ($R^2 = 0.96$) as well as tibia length and diaphyseal bone wall thickness ($R^2 = 0.97$). However, predicting medullary cavity perimeter or area from tibia length (or vice versa) is much less reliable ($R^2 = 0.79$ for both), indicating considerable variability in medullary cavity values associated with similar tibia lengths.

Nevertheless, the strong relationship between diaphyseal perimeter and tibia length allows the prediction of tibia length at each LAG, which can then be used to determine body length and mass for each year of growth. As previously discussed, medullary cavity expansion had not yet destroyed the LAG representing the close of the first year of growth in large individuals. Thus, a complete ontogenetic record from yearling to skeletal maturity is preserved within the sample. Of the nineteen tibiae possessing more than one LAG, only nine tibiae were preserved well enough to include in growth curves.

For tibiae lacking an EFS, the cortical thickness between the outermost LAG and the periosteal surface represents less than a year of growth. In these cases, the outermost zone is not plotted on body length and body mass curves. Additionally, some tibiae preserve more LAGs than are plotted on the curves because the outer LAGs were either incomplete or impossible to accurately trace due to crushing or missing cortex.

Although three tibiae had an EFS, growth mark perimeters could only be accurately traced in one of them (T-46). For this individual the thickness of the cortex

within the EFS is portrayed on the growth curves as a dashed line. This is done because after attainment of skeletal maturity the marks within the EFS are not necessarily annual.

Although error is introduced when estimating body length or mass from tibia lengths, these curves are included to provide a better visualization of *Maiasaura* ontogeny. A plot of body length over time (Fig. 6.3A) reveals variability in growth in the form of overlap in body sizes, but generally *Maiasaura* achieved half of its body length (~ 3.5 m) in the first year of growth. A body length of 3.5 m is also what was estimated by Horner et al. (2000) for yearlings. After the first year, growth begins to slow and eventually begins to plateau during the sixth to ninth year of life. The skeletally mature individual (T-46) included in the growth curves (Fig. 6.3) achieved its adult size in ten years. However, plotting multiple individuals demonstrates the variability in growth rates. Both the timing of skeletal maturity and the body size at skeletal maturity varies as well. For instance, in another individual (T-40) LAGs could be counted but not accurately measured. This tibia had nine growth marks prior to the EFS but was considerably smaller in body length, at 6.3 m. Still another tibia (T-38) not included in the curves had ten growth lines and no EFS, but was only 6.5 m in length. Regardless, a range of nine to ten years for skeletal maturity is similar to the original range of six to eight years as suggested by Horner et al. (2000).

When body mass is plotted over time (Fig. 6.3B), a very weak s-curve is produced. There appears to be a growth plateau in body length for the skeletally mature individual (T-46), but its corresponding mass curve does not reflect this plateau. If the mass curve was the only curve produced from this data, the results could be interpreted as

evidence for indeterminate growth. However, the presence of the EFS signifies only that no appreciable increase in the length of the diaphysis is possible; an animal may still continue to increase in mass after growth in length has ceased. Therefore, the most complete ontogenetic picture is provided by supplying both body length and body mass curves, as including only one or the other can lead to misinterpreting the growth of individuals. Additionally, the variability in both body length and body mass at each year of growth is substantial enough to caution against using a single individual to construct growth curves, and suggests that previously published growth curves using small sample sets are highly suspect.

In addition to growth curves, average annual apposition rates were determined for all “yearlings” and tibiae with more than one LAG (6.2). Not every specimen was used, as often not every yearly apposition rate could be determined due to tibiae being too crushed or incomplete to obtain cortical thickness averages. Apposition rates were highest during the first year of growth, between 54 $\mu\text{m/day}$ and 71 $\mu\text{m/day}$, which is reflected in the body length curve indicating that on average half adult body length was reached after just one year. Apposition rate decreases to between 15 $\mu\text{m/day}$ and 32 $\mu\text{m/day}$ for the second year of life and falls again during the third year of life to between 10 $\mu\text{m/day}$ and 16 $\mu\text{m/day}$. From the fourth year of growth until skeletal maturity, growth rate stays relatively constant- between 0.71 $\mu\text{m/day}$ and 9 $\mu\text{m/day}$. These apposition rate estimates agree with those used by Horner et al. (2000) to determine the timing of skeletal maturity.

Since the bone fiber orientation remains woven throughout growth, these ranges in apposition rate illustrate observations made by previous studies regarding the overlapping rates of various kinds of tissue and vascularization. However, the duration of annual arrested growth is often not taken into account when determining apposition rates. For instance, alligators in Louisiana have a growing season of 214 days per year (Joanen and McNease, 1987), so their annual apposition rate would be determined by dividing average cortical thickness by 214 days, not 365 days. The duration of the growth hiatus in *Maiasaura* is unknown, so dividing by the number of days in a Campanian year (370) is likely unrealistic and results in underestimates of annual apposition rate. Additionally, as in all vertebrates, the spacing between LAGs decreases as skeletal maturity is approached. This may be due to an overall decrease in yearly growth rate, but it could also be the result of prolonging the annual growth hiatus later in life. However, this hypothesis has never been tested in extant vertebrates.

Frequency Graph

Interestingly, the majority of this sample set is made of tibiae less than 50 cm in length, corresponding to individuals a year or less of age (Fig. 6.20). Although nothing can be said about the mortality rate of hatchlings and nestlings, the mortality rate of individuals prior to reaching the close of their first year of growth was extremely high. Extant alligators from Louisiana will cease or slow growth during the winter, when environmental stresses are higher- the weather is cooler and resources are less plentiful (Joanen and McNease, 1987). Perhaps the growth hiatus of *Maiasaura* also corresponded to a seasonally stressful period, during which time there was high juvenile mortality. If

immature *Maiasaura* perished during the period of a growth hiatus, this would explain the absence of LAGs in individuals that are within the appropriate yearling size range as LAGs only become evident once growth again resumes.

Tibiae greater than 50 cm in length are less common in the sample set and there is a considerable lack of tibiae between 50 cm and 80 cm in length (individuals between three and five years of age). This suggests that the mortality rate was extremely high for the very young and also moderately high for individuals close to, or having attained, skeletal maturity. Assuming that the sample set represents a population, and assuming that *Maiasaura* nested only once a year, the frequency distribution implies that the smallest tibiae (33 cm – 40 cm in length) were from the most recent nesting season, and the yearlings (40 cm – 50 cm tibia lengths) were therefore from the previous nesting season.

Conclusions

This detailed population histovariability analysis contributes to the already considerably well-studied hadrosaur *Maiasaura*, making this taxon's ontogenetic history one of the best understood of all non-avian dinosaurs. The age-size frequency distribution of tibiae suggests a high mortality rate for yearlings and those individuals approaching or having achieved skeletal maturity. Additionally, robust relationships between tibia length and other variables including diaphyseal perimeter, cortical area, and bone wall thickness enable regression equations predicting the value of one variable given the other.

Histological examination of forty-seven tibiae, representing a minimum of thirty one individuals, allows for the quantification of individual growth rate variation within a population. The perimeter of the first LAG in larger tibiae corresponds to diaphyseal perimeters in the smaller tibiae, revealing that an entire ontogenetic record of growth from yearlings to skeletal maturity is preserved in this sample set.

In general, the observations of Horner et al. (2000) are supported by this research. Their estimate of six to eight years to achieve skeletal maturity, based on comparative apposition rates and retrocalculation, was fairly accurate; at least two *Maiasaura* individuals in this study attained skeletal maturity between nine and ten years. Results of this study confirm that *Maiasaura* growth was rapid throughout ontogeny, and that individuals achieved approximately half of their adult length in the first year of growth. Still, this new study reveals that there is considerable variation in vascularity throughout ontogeny as well as within the diaphyseal section which corresponds to considerable body length and mass variability for each year of age within the population. These are often lurking variables within an ontogenetic study on dinosaur taxa as sample sizes are frequently inadequate to observe the presence of any variability.

Acknowledgements

Funding for this research was provided to the senior author by Gerry Ohrstrom and the Museum of the Rockies, and grants were awarded by the Jurassic Foundation and the Geological Society of America. E.-T. Lamm, B. Baziak, E. Morschhauser, and J. Kern assisted with *Maiasaura* thin section preparation. D. Evans and N. Campione

(Royal Ontario Museum) provided measurements from a partially articulated *Maiasaura* that were used in the regressions and growth curves. The Museum of the Rockies provided additional funding for slide processing, as well as facilities for histological analyses including microphotograph digital imaging equipment.

Table 6.1. Data collected from forty eight histologically sectioned *Maiasaura* tibiae.

Tibia Code	Orientation	Perimeter of Thin Section (cm)	Perimeter of Medullary Cavity (cm)	Tibia Length (cm)	Medullary Cavity Area (cm ²)	Transverse Section Area (cm ²)	Bone Wall Thickness (cm ²)
YPM-PU 22432	-	2.5	-	-	-	0.5	-
T01	L	13.03	4.57	35.0	1	11.41	10.41
T02	L	14.33	Crushed	41.5	Crushed	11.59	-
T03	L	14.39	3.3	33.8	0.5	13.25	12.75
T04	R	14.38	6.02	39.7	2.2	14.15	11.95
T05	L	14.9	3.7	39.4	0.64	14.12	13.48
T06	L	14.98	3.15	46.0	0.63	14.6	13.97
T07	L	14.43	4.06	37.2	1.087	14.23	13.143
T08	R	15.1	Crushed	36.2	Crushed	13.43	-
T09	R	13.62	5.52	38.4	0.89	12.57	11.68
T10	L	14.86	7.86	42.1	1.9	14.69	12.79
T11	R	15.22	Crushed	43.5	Crushed	12.94	-
T12	R	15.53	Crushed	41.2	Crushed	15.74	-
T13	R	16.24	5.71	43.1	1.58	14.91	13.33
T14	R	15.72	6.49	44.3	2.55	16.79	14.24
T15	R	16.65	4.78	46.4	1.32	17.59	16.27
T16	L	15.36	4.41	44.0	0.98	15.97	14.99
T17	L	14.93	Crushed	45.2	Crushed	13.29	-
T18	R	17.22	5.77	43.3	1.75	18.13	16.38
T19	R	16.99	Crushed	43.8	Crushed	15.54	-
T20	R	15.39	6.09	43.4	2.27	16.08	13.81
T21	R	16.37	Crushed	46.4	Crushed	14.57	-
T22	R	15.86	5.04	48.5	1.53	17.56	16.03
T23	R	17.23	Crushed	45.4	Crushed	16.98	-
T24	L	18.27	Crushed	48.8	Crushed	17.24	-
T25	R	16.67	5.8	48.1	1.72	17.99	16.27
T26	R	16.82	Crushed	47.4	Crushed	17.46	-
T27	R	16.62	6.27	48.0	2.16	19	16.84
T28	R	16.57	Crushed	47.2	Crushed	16.36	-
T29	R	17.02	Crushed	48.5	Crushed	15.57	-
T30	R	21.35	7.77	56.0	4.17	32.04	27.87
T31	R	21.43	11.22	63.0	3.66	31.65	27.99
T32	R	21.34	4.88	69.0	1.27	29.06	27.79
T33	R	27.95	9.49	78.0	5.42	54.47	49.05
T34	L	Cortex missing	12.98	75.0	10.59	57.02	46.43
T35	L	Cortex missing	Crushed	75.0	Crushed	54.72	-
T36	L	30.3	11.59	88.0	7	60.33	53.33
T37	L	Crushed	Crushed	83.0	Crushed	55.81	-
T38	R	Crushed	Crushed	90.0	Crushed	79.04	-
T39	L	31.6	11.39	83.0	7.04	62.2	55.16
T40	R	32.9	10.17	87.5	6.57	67.73	61.16
T41	R	Cortex missing	13.96	95.0	13.27	72.58	59.31
T42	R	Cortex missing	Crushed	90.5	Crushed	70.58	-
T43	R	32.92	10.94	88.0	6.22	76.31	70.09
T44	L	38.24	Crushed	87.5	Crushed	90.24	-
T45	R	35.27	13.25	99.7	10.56	86.84	76.28
T46	L	33.16	16.25	93.0	17.38	77.03	59.65
T47	R	26.52	-	69.5	-	-	-
T48	R	23.32	9.13	-	5.68	38.22	32.54

Table 6.2. Average daily apposition rates for each year of growth, assuming 370 days in the Campanian year. Only tibiae and the growth zones that were preserved well enough to determine an average zone thickness were included. The smaller tibiae lacked growth marks but were assumed to be nearly a year in age.

Tibia Code	Growth Mark	Average Zone Thickness (mm)	Average Apposition Rate (um/day)
T4	-	21.65	58.51
T5	-	21.06	56.93
T6	-	22.08	59.66
T10	-	22.61	61.09
T13	-	23.08	62.36
T14	-	23.41	63.26
T15	-	24.32	65.72
T16	-	22.54	60.92
T18	-	24.62	66.53
T20	-	23.45	63.36
T22	-	23.98	64.82
T25	-	24.06	65.03
T28	-	24.06	65.03
T30	1	21.75	58.77
	2	6.93	18.72
T31	1	20.23	54.68
	2	5.02	13.57
T32	1	20.37	55.04
	2	5.79	15.65
T33	1	26.41	71.39
	2	7.21	19.48
	3	4.64	12.54
	4	1.09	2.95
	5	1.00	2.70
T34	1	25.81	69.77
	2	6.89	18.61
	3	6.10	16.49
T36	1	21.55	58.24
	2	9.72	26.26
	3	5.06	13.67
	4	3.41	9.22
T43	1	23.35	63.10
	2	10.50	28.39
	3	5.39	14.55
	4	3.61	9.75
	5	3.01	8.14
	6	0.26	0.71
	7	2.12	5.73
T46	1	22.81	61.65
	2	12.05	32.56
	3	3.82	10.32
	4	2.02	5.46
	5	2.62	7.08
	6	1.80	4.86
	7	2.08	5.61
	8	0.88	2.39
	9	0.96	2.58

Table 6.3. Estimates of *Maiasaura* femur length, body length, and body mass based on regression formulae and mass equations.

Tibia Code	Growth Mark	Femur Length (cm)	Body Length (m)	Body Mass (kg)
YPM-PU 22432	-	7.80	7.50	2.00
T01	-	36.24	2.54	157.02
T02	-	42.97	3.01	261.76
T03	-	35.00	2.45	141.42
T04	-	41.11	2.88	229.16
T05	-	40.80	2.86	224.00
T06	-	47.63	3.34	356.48
T07	-	38.52	2.70	188.53
T08	-	37.49	2.63	173.73
T09	-	39.76	2.79	207.37
T10	-	43.60	3.05	273.28
T11	-	45.05	3.16	301.46
T12	-	42.66	2.99	256.12
T13	-	44.63	3.13	293.22
T14	-	45.87	3.21	318.40
T15	-	48.05	3.37	365.86
T16	-	45.56	3.19	311.97
T17	-	46.81	3.28	338.20
T18	-	44.84	3.14	297.32
T19	-	45.36	3.18	307.74
T20	-	44.94	3.15	299.38
T21	-	48.05	3.37	365.86
T22	-	50.22	3.52	417.82
T23	-	47.01	3.29	342.71
T24	-	50.53	3.54	425.62
T25	-	49.81	3.49	407.56
T26	-	49.08	3.44	390.03
T27	-	49.71	3.48	405.03
T28	-	48.88	3.42	385.11
T29	-	50.22	3.52	417.82
T30	0	7.77	0.54	1.55
	1	42.95	3.01	261.38
	2	55.26	3.87	556.62
	surface	57.99	4.06	643.17
T31	0	7.77	0.54	1.55
	1	40.57	2.84	220.16
	2	53.32	3.74	500.06
	surface	65.24	4.57	915.76
T32	0	7.77	0.54	1.55
	1	45.34	3.18	307.46
	2	55.15	3.86	553.23
	surface	71.45	5.01	1203.11
T33	0	7.77	0.54	1.55
	1	50.65	3.55	428.65
	2	63.02	4.41	825.41
	3	71.36	5.00	1198.70
	4	73.42	5.14	1305.08
	5	75.44	5.28	1415.98
	surface	80.77	5.66	1737.97
T34	0	7.77	0.54	1.55

Table 6.3 continued.

	1	52.99	3.71	490.63
	2	64.82	4.54	898.11
	3	73.92	5.18	1332.24
	4	76.62	5.37	1483.48
	5	-	-	-
	6	-	-	-
	7	-	-	-
	surface	-	-	-
T35	-	77.66	5.44	1545.05
T36	0	7.77	0.54	1.55
	1	48.55	3.40	377.34
	2	66.98	4.69	991.09
	3	75.44	5.28	1415.98
	4	82.13	5.75	1826.96
	5	-	-	-
	6	-	-	-
	surface	91.13	6.38	2495.79
T37	-	85.95	6.02	2094.08
T38	-	93.20	6.53	2669.85
T39	-	85.95	6.02	2094.08
T40	-	90.61	6.35	2453.49
T41	-	98.38	6.89	3140.00
T42	-	93.72	6.56	2714.60
T43	0	7.77	0.54	1.55
	1	49.05	3.44	389.26
	2	66.73	4.67	979.91
	3	75.86	5.31	1439.85
	4	82.44	5.77	1847.66
	5	87.33	6.12	2196.33
	6	89.69	6.28	2379.29
	7	91.88	6.44	2558.04
	8	-	-	-
	9	-	-	-
	surface	91.13	6.38	2495.79
T44	-	90.61	6.35	2453.49
T45	-	103.24	7.23	3629.48
T46	0	7.77	0.54	1.55
	1	47.56	3.33	354.87
	2	68.05	4.77	1039.25
	3	74.93	5.25	1387.69
	4	78.59	5.50	1600.70
	5	83.42	5.84	1914.59
	6	86.74	6.08	2152.10
	7	90.53	6.34	2447.02
	8	92.38	6.47	2600.52
	9	-	-	-
	surface	96.30	6.75	2945.83
T47	-	71.97	5.04	1229.46
T48	0	7.77	0.54	1.55
	1	48.38	3.39	373.42
	2	59.51	4.17	694.93
	surface	66.28	4.64	960.23

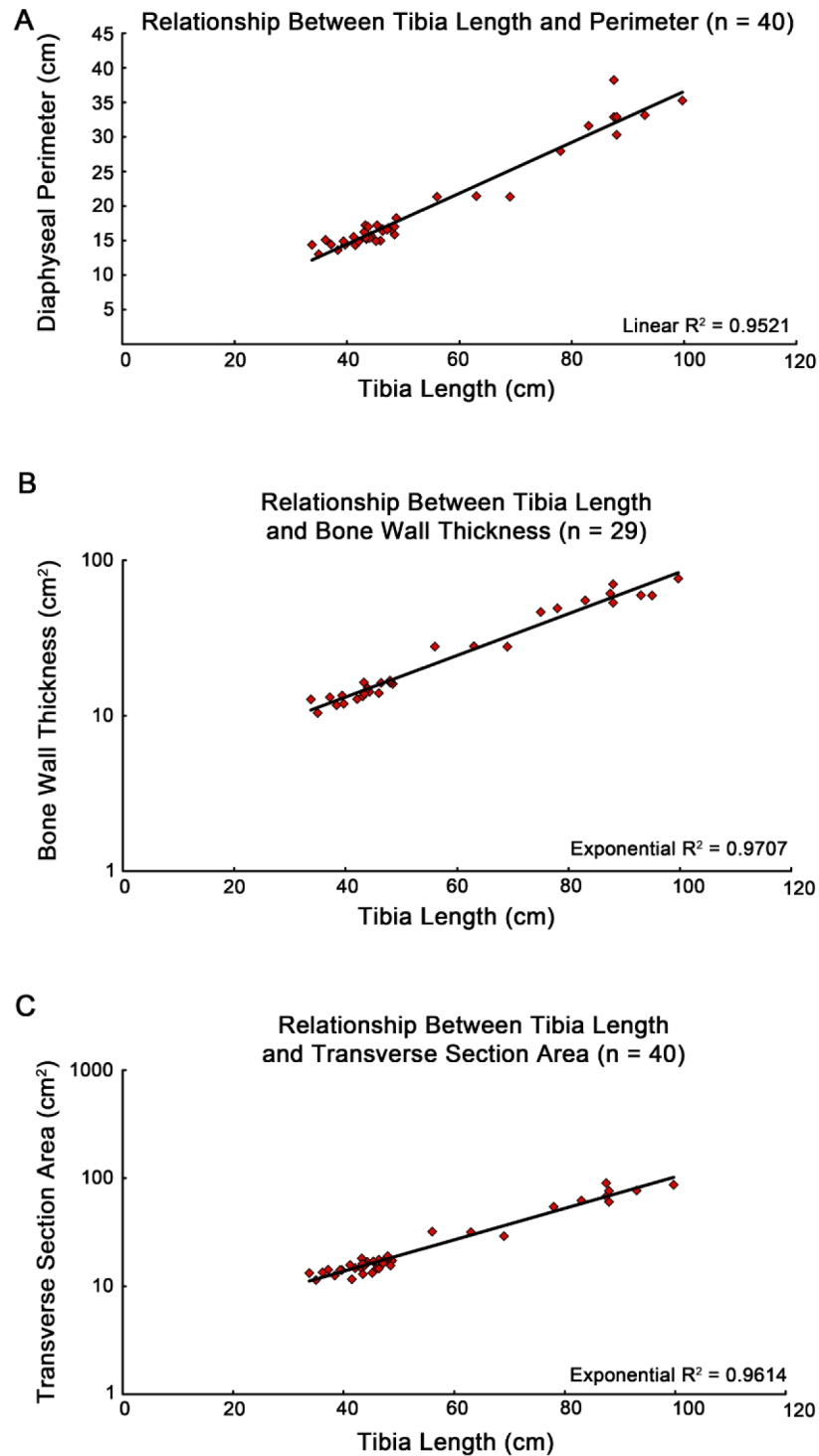


Figure 6.1. Tibia length plotted against A) diaphyseal perimeter, B) bone wall thickness, and C) transverse section area. The high R^2 value (> 0.9) in all cases indicates a strong relationship between tibia length and the tested variables, allowing reasonable predictions of one value if provided the other.

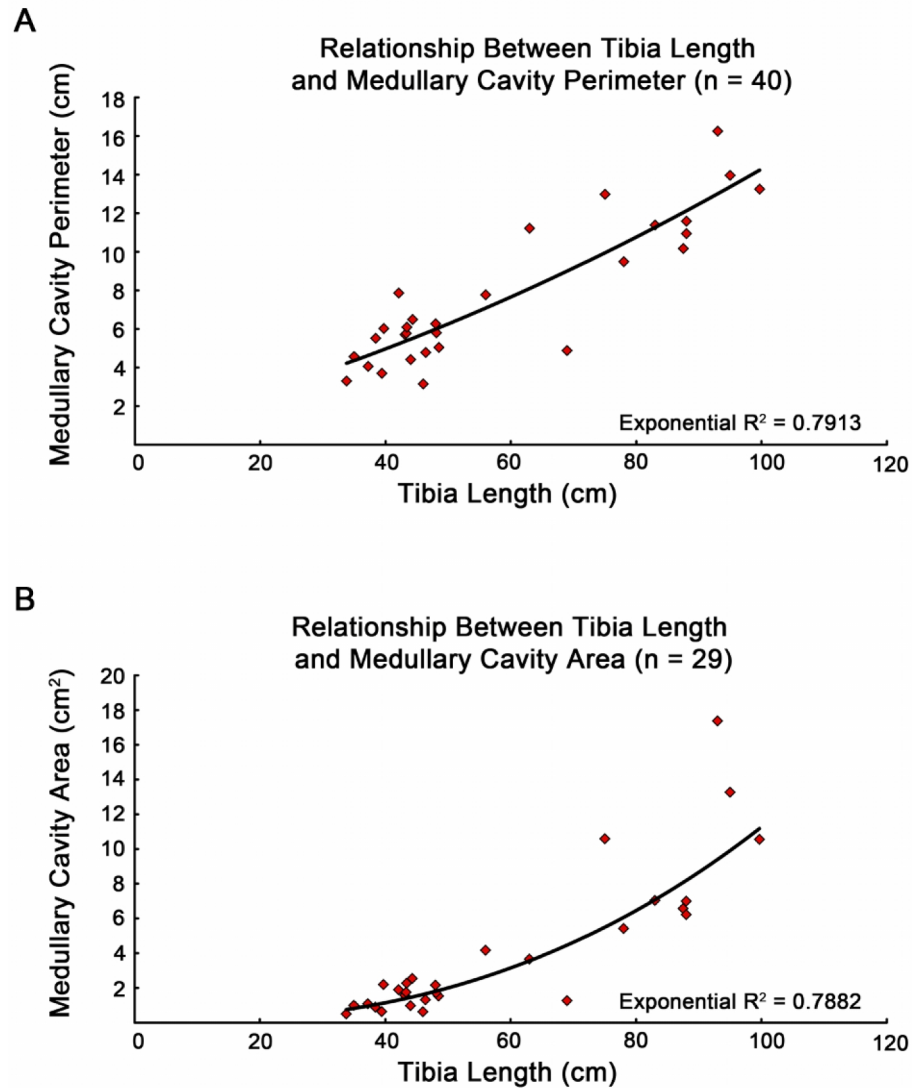


Figure 6.2. Tibia length plotted against A) medullary cavity perimeter and B) medullary cavity area. The low R^2 values (<0.8) indicates a weak relationship between tibia length and medullary cavity measurements. This suggests there is considerable variability in medullary cavity dimensions for tibiae of similar size.

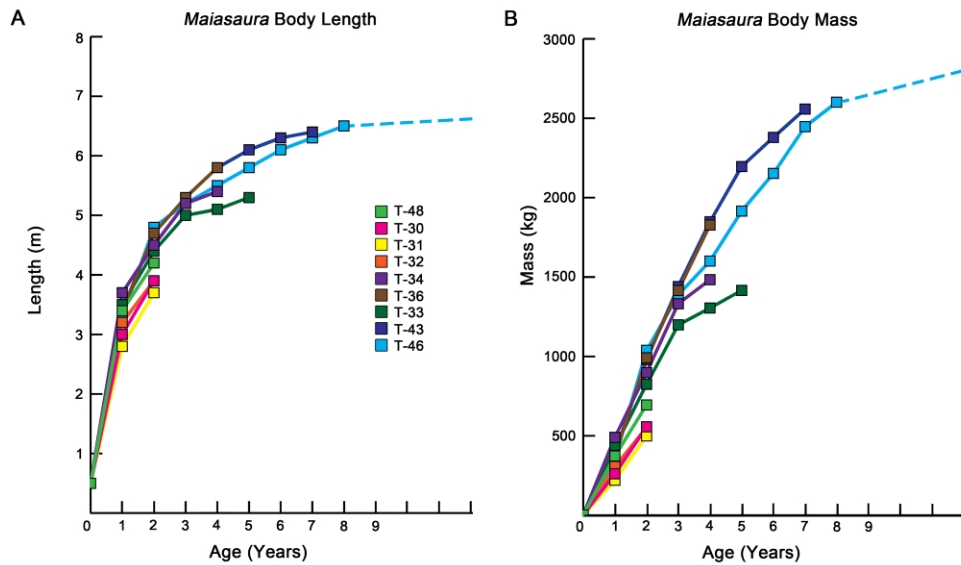


Figure 6.3. Ontogenetic growth curves for nine *Maiasaura* individuals. A) When body length is plotted over time, individual variability becomes evident. Despite this, after just one year of growth *Maiasaura* had achieved approximately half of its estimated 7 m adult length. B) The individual variability observed in the body length curves is more pronounced by the body mass curves. The dashed lines for T-46 indicates the unknown time interval represented by the EFS.



Figure 6.4. Transverse sections from the seven *Maiasaura* tibiae between 33 cm and 40 cm in length. Parallel streaks are artifacts of imaging the sections on a flatbed scanner. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Right tibia transverse sections were inverted for easier comparison. Scale bar, 5 cm.

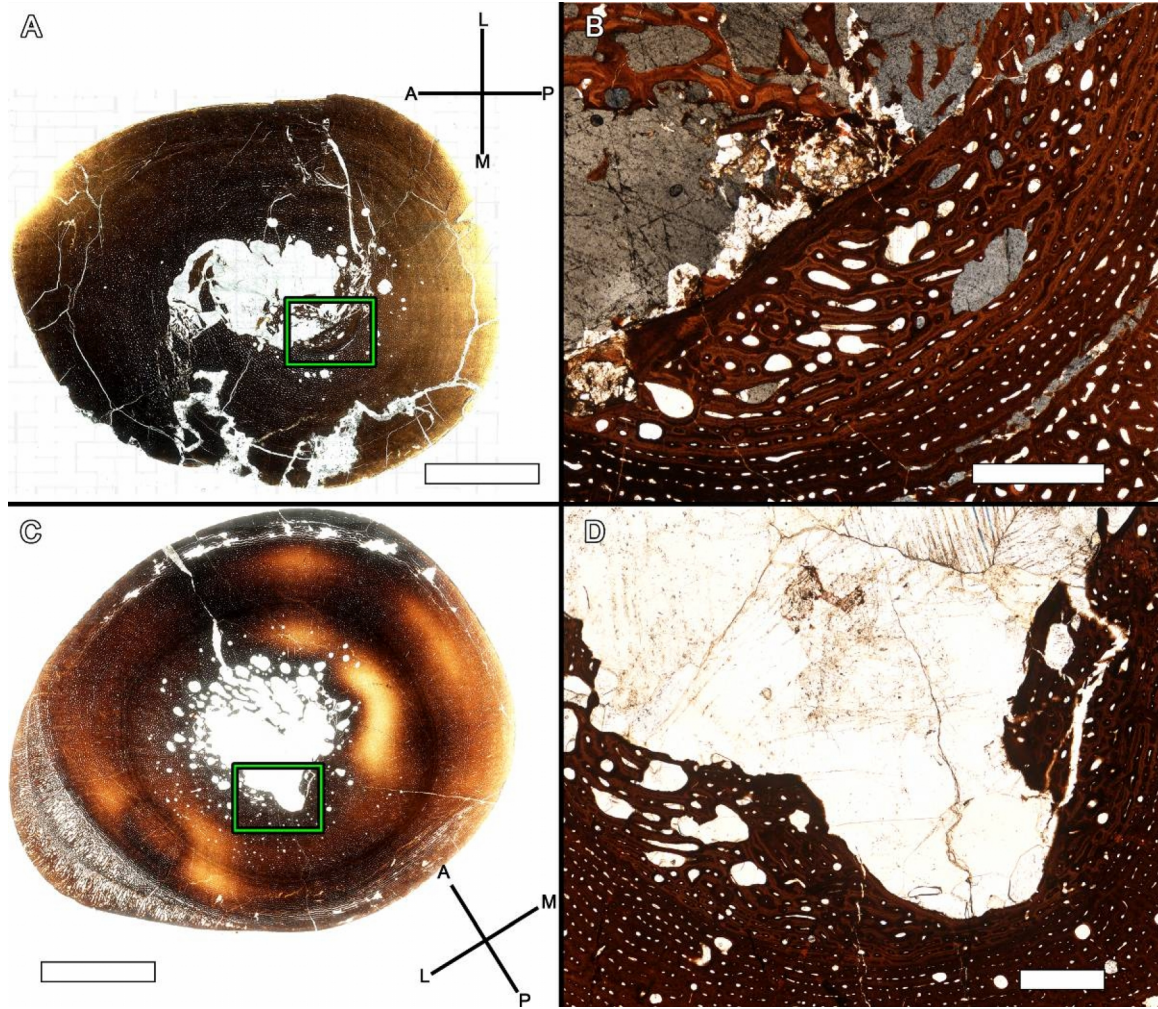


Figure 6.5. Possible nestling bone preserved within tibiae lengths between 33 and 40 cm. A) The tibia T-1 possesses B) woven tissue with large vascular canals bordering the medullary cavity on the postero-medial side. C) The tibia T-9 possess a similar structure D) on the posterior side of the medullary cavity. In both tibiae, this “mesh-like” woven primary tissue is nearly destroyed by medullary cavity expansion. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Green boxes in A) and C) indicate enlarged areas shown in B) and D). Scale bars, 1 cm (A and C); 1 mm (B and D).

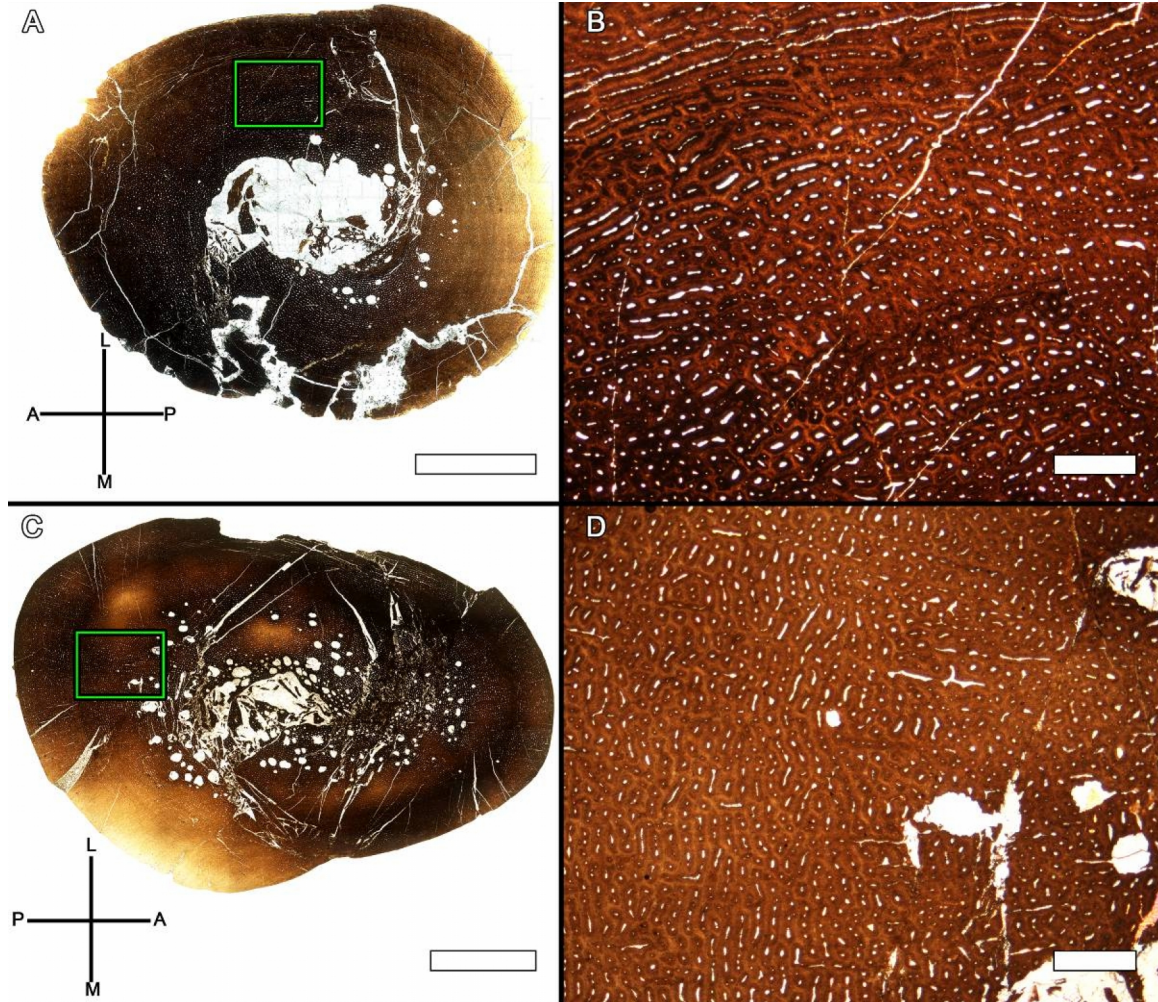


Figure 6.6. Reticular to laminar vascular organization observed in two tibiae (A, T-1; B, T-8) between 33 cm and 40 cm in length. Vascularity is variable between individuals, as well as within a single sample depending on the orientation. In places, vascularity may be largely reticular B) or laminar D), or a combination of both. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Green boxes in A) and C) indicate enlarged areas shown in B) and D). Scale bars, 1 cm (A and C); 1 mm (B and D).

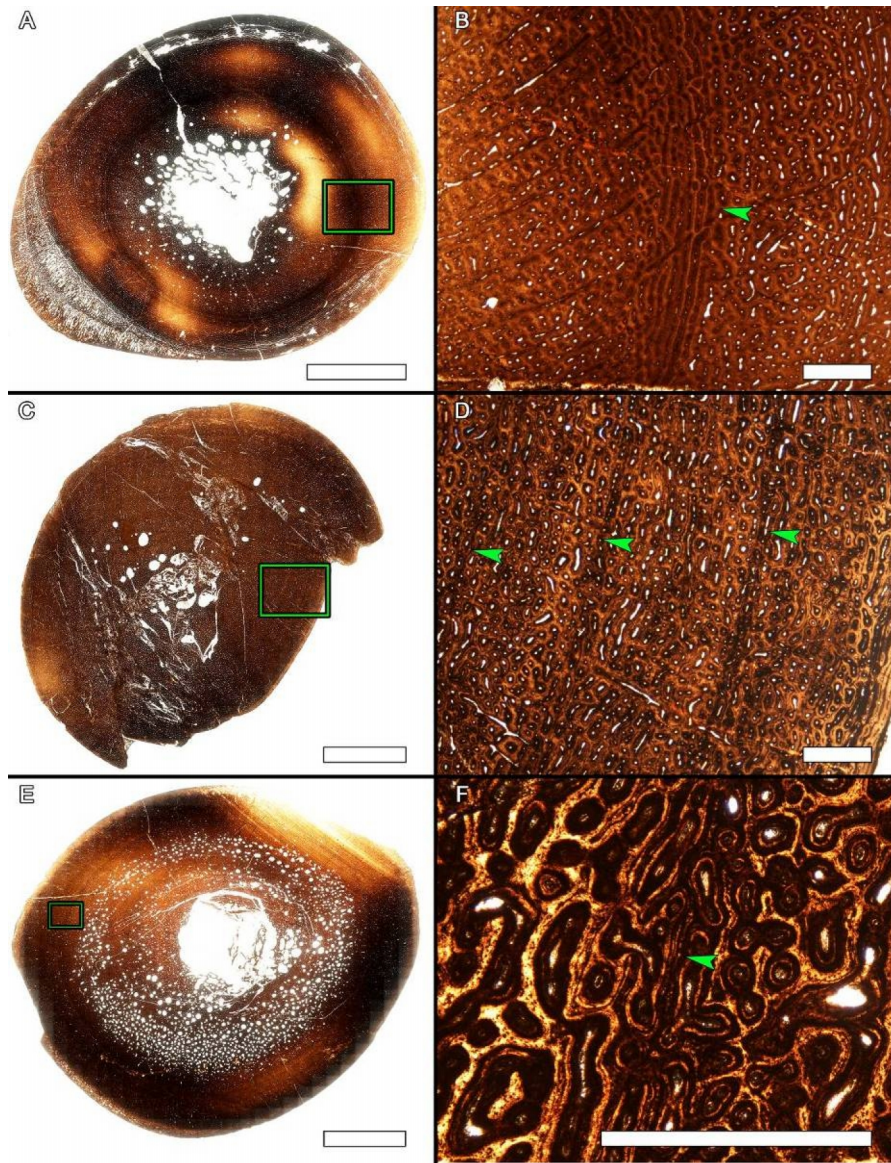


Figure 6.7. An unusual banding pattern is observed in many tibiae less than 50 cm in length. This pattern seems to be caused by variation in vascular canal size and orientation as well as diagenetic staining. Examples of tibiae with this banding include A) T-9, C) T-11, and E) T-16. These faint bands can be seen in the polished transverse sections, but are less evident at high resolutions. One band is indicated by an arrow in B), where decidedly more laminar tissue is bounded on either side by reticular vascularity. Three bands are indicated by arrows in D), where their presence is due in larger part to the reduced size of the vascular canals rather than orientation. A single band at high magnification is indicated by an arrow in F), where again this band appears to be the result of vascular canal diameter reduction. There is no evidence of true LAGs in any of the observed bands. Green boxes in A), C), and E) indicate enlarged areas shown in B), D), and F). Scale bars, 1 cm (A, C, E); 1 mm (B, D, F).

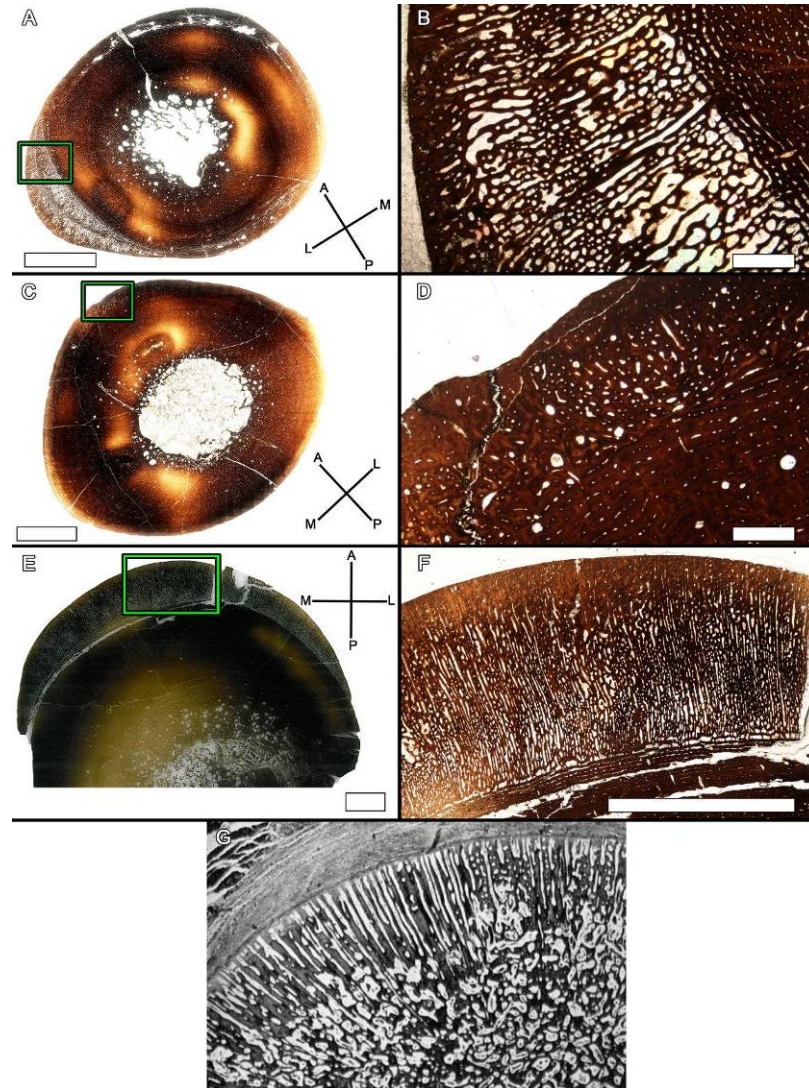


Figure 6.8. Radially oriented vascularity at the periosteal surface suggests possible osteopetrosis in three tibiae of *Maiasaura*. A) At 38.4 cm, T-9 is the smallest tibia within the sample affected. The lateral side of the tibia B) shows an abrupt transition from laminar/reticular vascularity to radial vascularity, interrupted by a narrow band of more longitudinally oriented canals in the middle of the radial tissue. C) The anterior periosteal surface of T-20 has what looks to be the beginnings of this pathology D), but it is not as well developed as in the other tibiae. E) T-42 is the largest tibia (90.5 cm) affected, and radial periosteal growth F) is also the most pronounced in this specimen. G) An example of avian osteopetrosis in a chicken (modified from Campbell, 1966). The periosteal radial vascularity is strikingly similar to what is observed in the *Maiasaura* tibiae. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Green boxes in A), C), and E) indicate enlarged areas shown in B), D), and F). Scale bars, 1 cm (A, C, E); 1 mm (B, D, F).



Figure 6.9. Transverse sections from the twenty-two *Maiasaura* tibiae between 40 cm and 50 cm in length. Parallel streaks are artifacts of imaging the sections on a flatbed scanner. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Right tibia transverse sections were inverted for easier comparison. Scale bar, 5 cm.

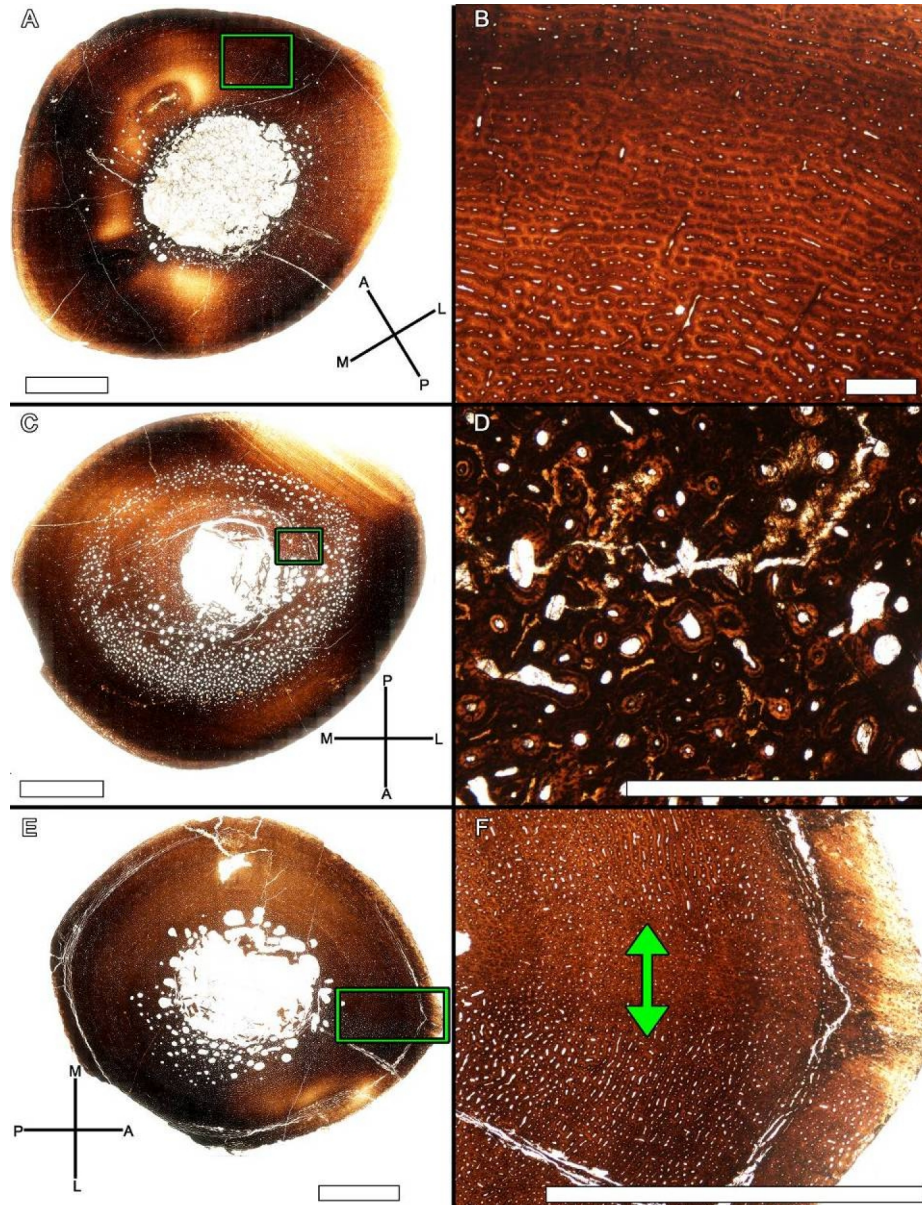


Figure 6.10. Bone tissue features present in tibiae between 40 cm and 50 cm in length. A) As in smaller tibiae, vascularity is quite variable between individuals as well as within transverse sections, but B) is usually some combination of reticular and laminar arrangement. C) The frequency of resorption cavities increases in this size range, as does D) the frequency of secondary osteons within the innermost cortex. E) Also common in this size range, although not present in every specimen, is the appearance of a prominent anterior border. F) A column of more strongly radial tissue spans from the inner cortex to the periosteal surface, associated with the anterior border. The green arrow indicates the width of this column of radial tissue. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Green boxes in A), C), and E) indicate enlarged areas shown in B), D), and F). Scale bars, 1 cm (A, C, E, F); 1 mm (B, D).

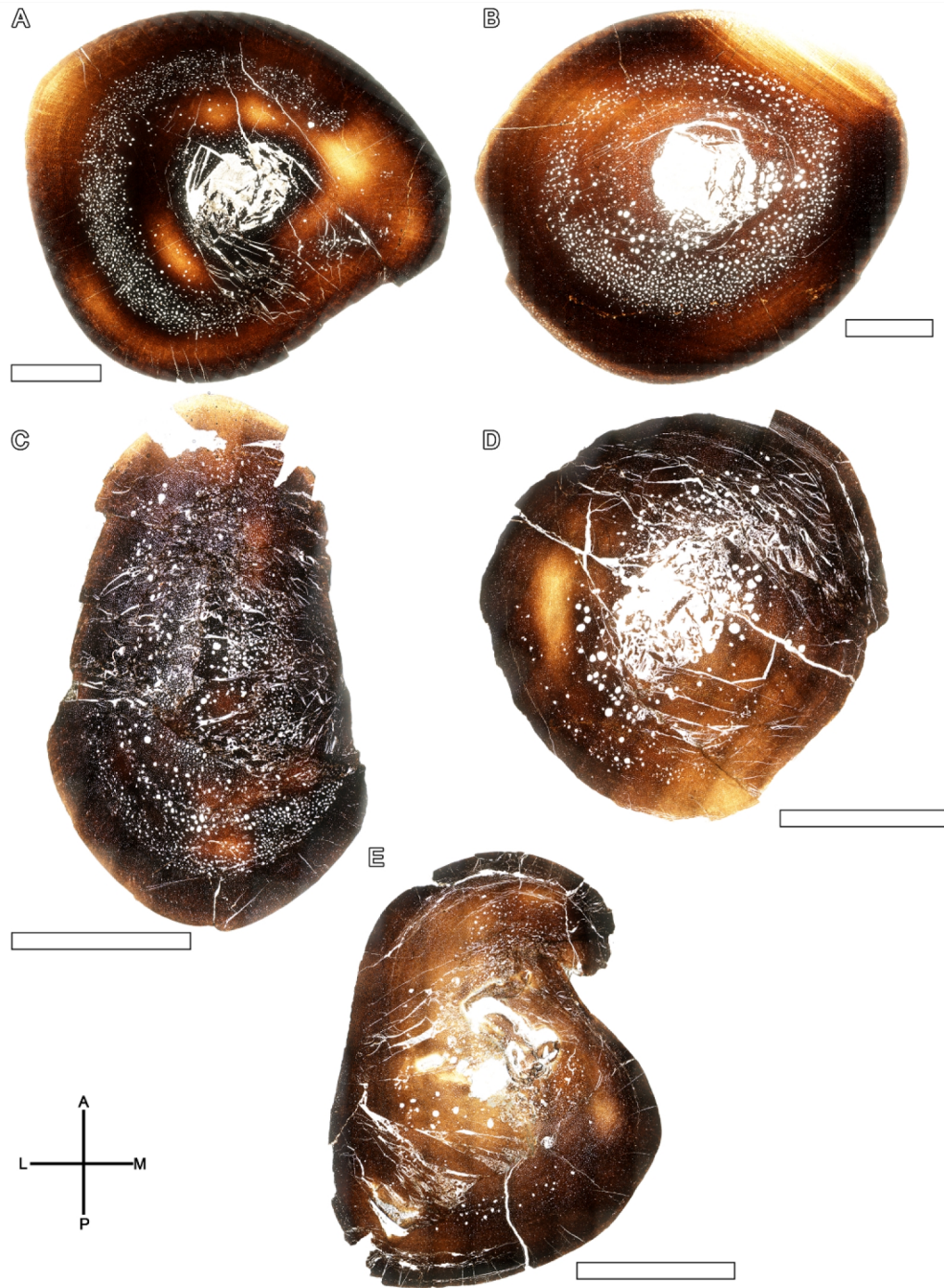


Figure 6.11. Several tibiae within the 40 cm – 50 cm size range possess resorption cavities arranged in one or more circular rings within the mid cortex. These include A) T-6, B) T-16, C) T-19, D), T-25, and E) T-24. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Scale bars, 1 cm.

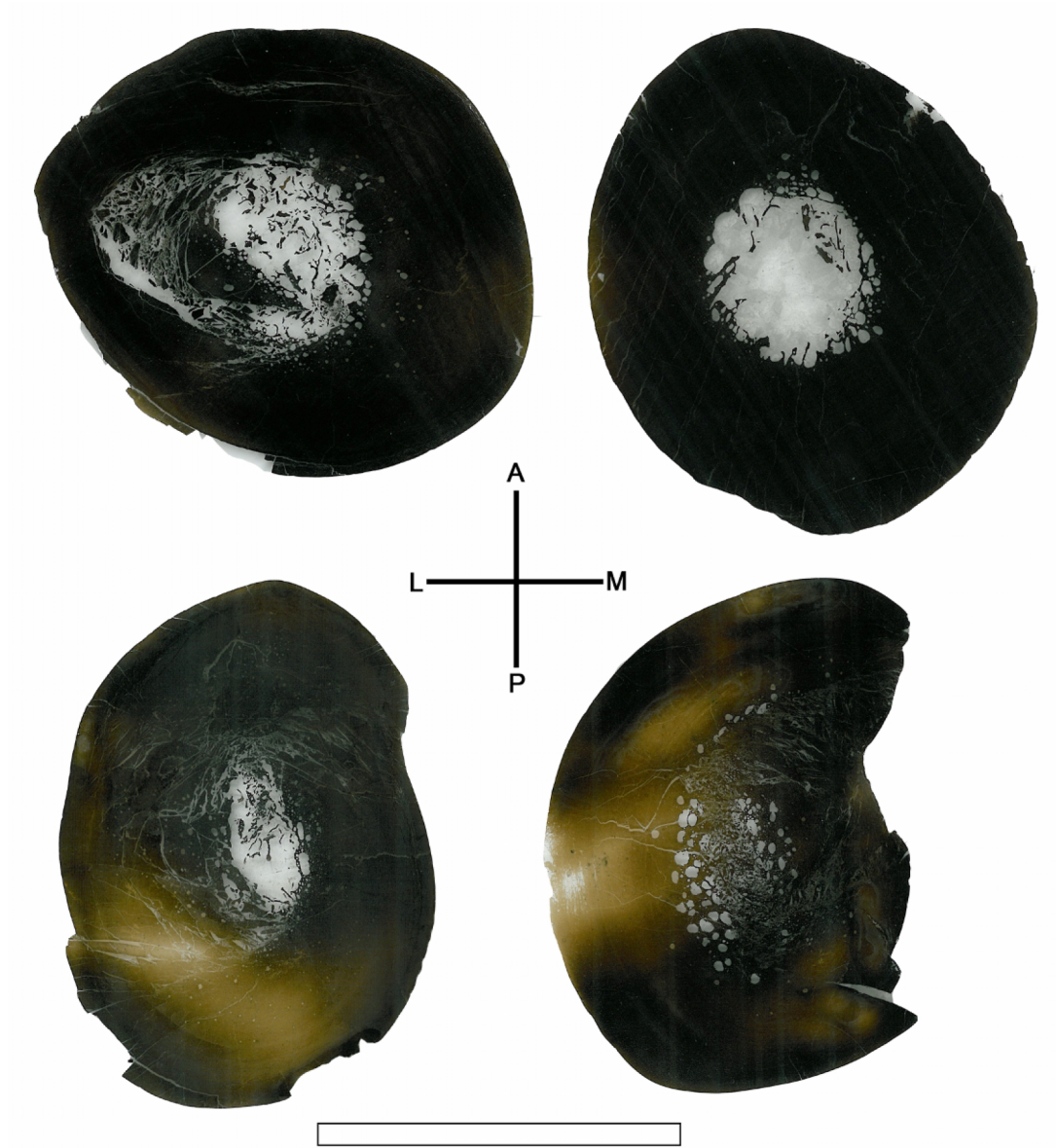


Figure 6.12. Transverse sections from the four *Maiasaura* tibiae between 50 cm and 70 cm in length. Parallel streaks are artifacts of imaging the sections on a flatbed scanner. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Right tibia transverse sections were inverted for easier comparison. Scale bar, 5 cm.

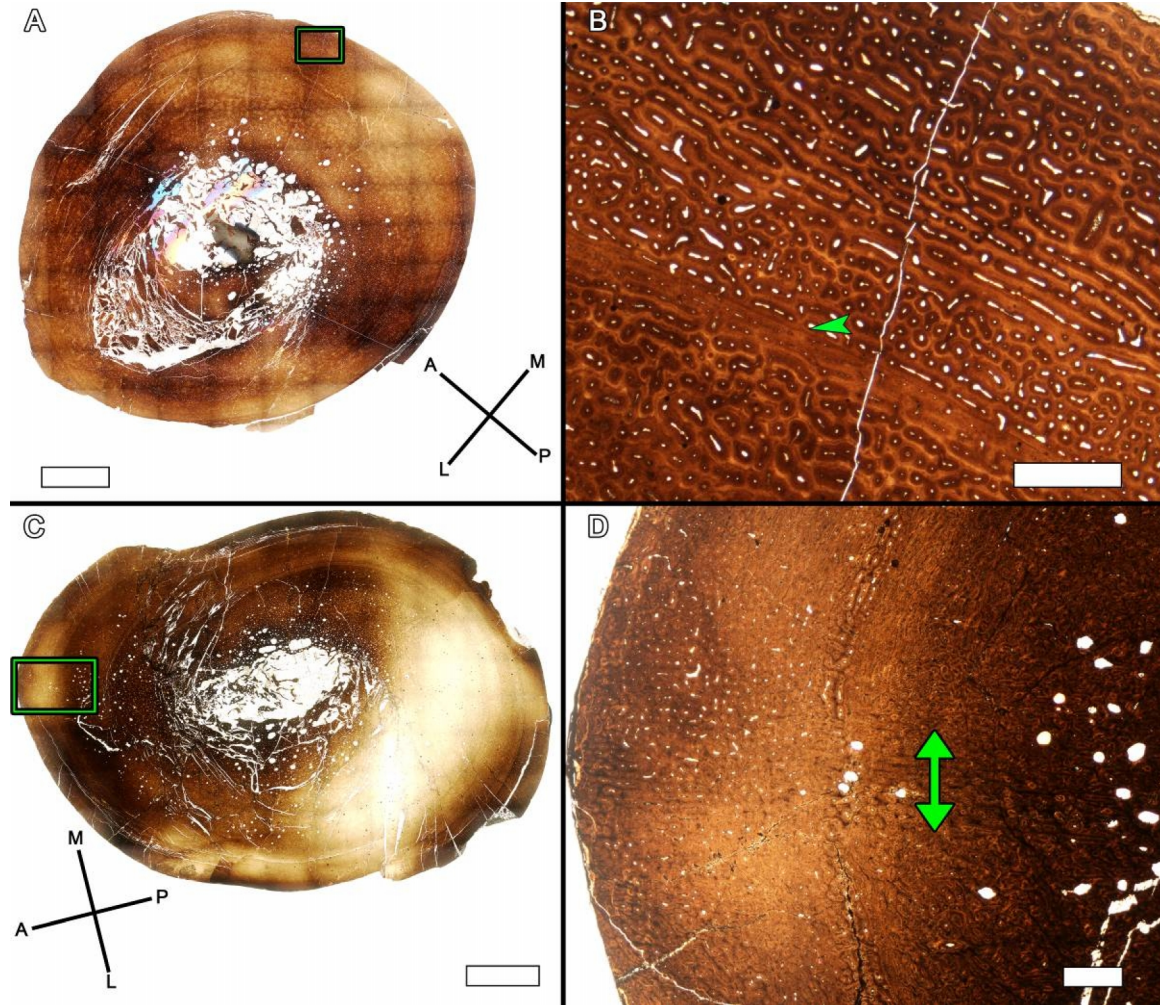


Figure 6.13. Bone tissue features present in tibiae between 50 cm and 70 cm in length. Tibiae in this size range all possess two LAGs. A) T-30 shows some resorption cavities within the inner cortex, and B) vascularity ranges between reticular and laminar. However, immediately after the second LAG (arrow), vascular orientation is longitudinal for several laminae before becoming a mixture of laminar and reticular for the remainder of the zone thickness. C) The cortex of T-32 also possesses scattered resorption cavities. A column of radially vascularized bone is present, corresponding to the anterior border. The green arrow indicates the width of this column. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Green boxes in A) and C) indicate enlarged areas shown in B) and D). Scale bars, 1 cm (A, C); 1 mm (B, D).

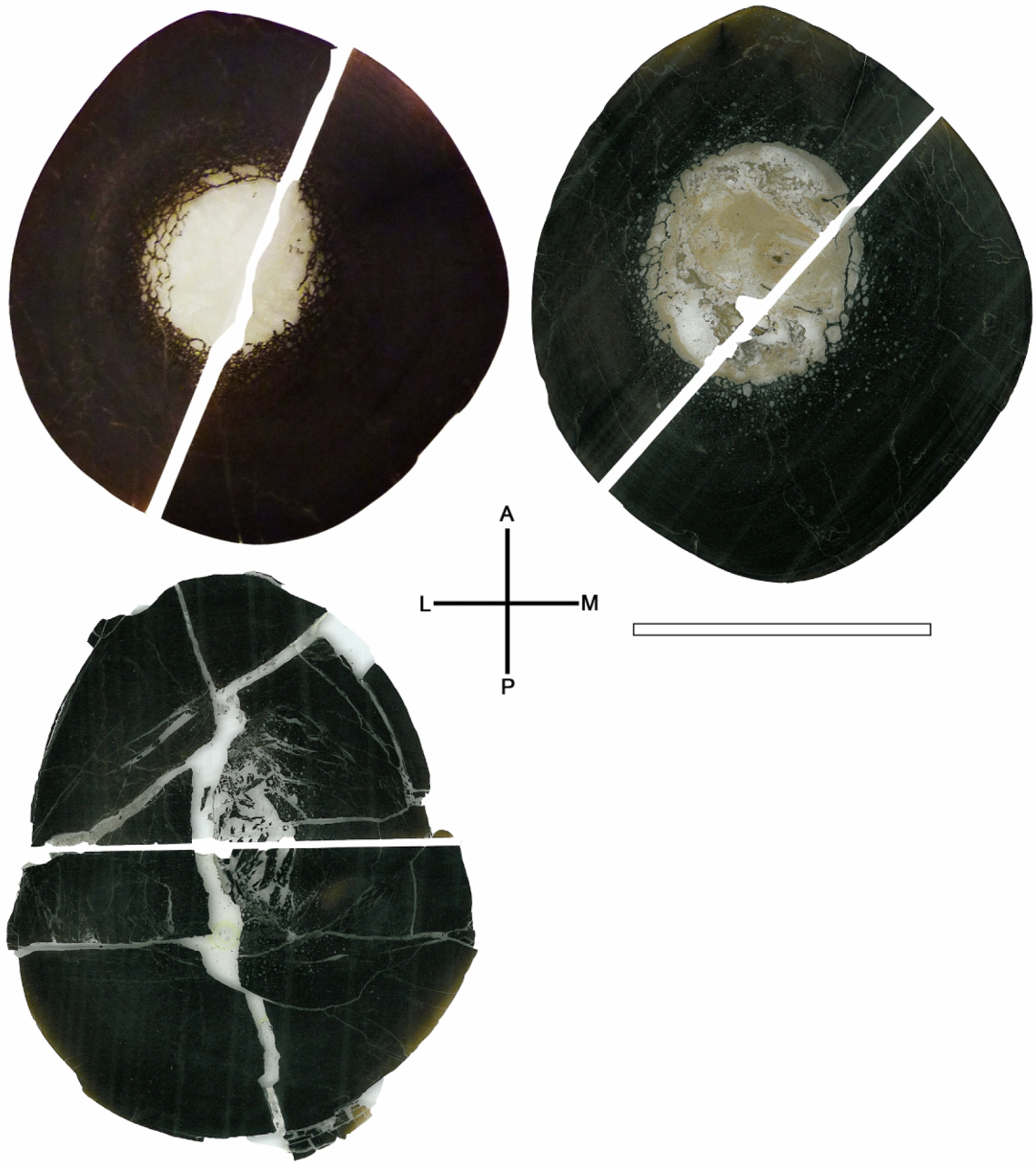


Figure 6.14. Transverse sections from the three *Maiasaura* tibiae between 70 cm and 80 cm in length. Parallel streaks are artifacts of imaging the sections on a flatbed scanner. Transverse sections in this size category were too large for a single slide, so each specimen was bisected and mounted on two slides. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Right tibia transverse sections were inverted for easier comparison. Scale bar, 5 cm.

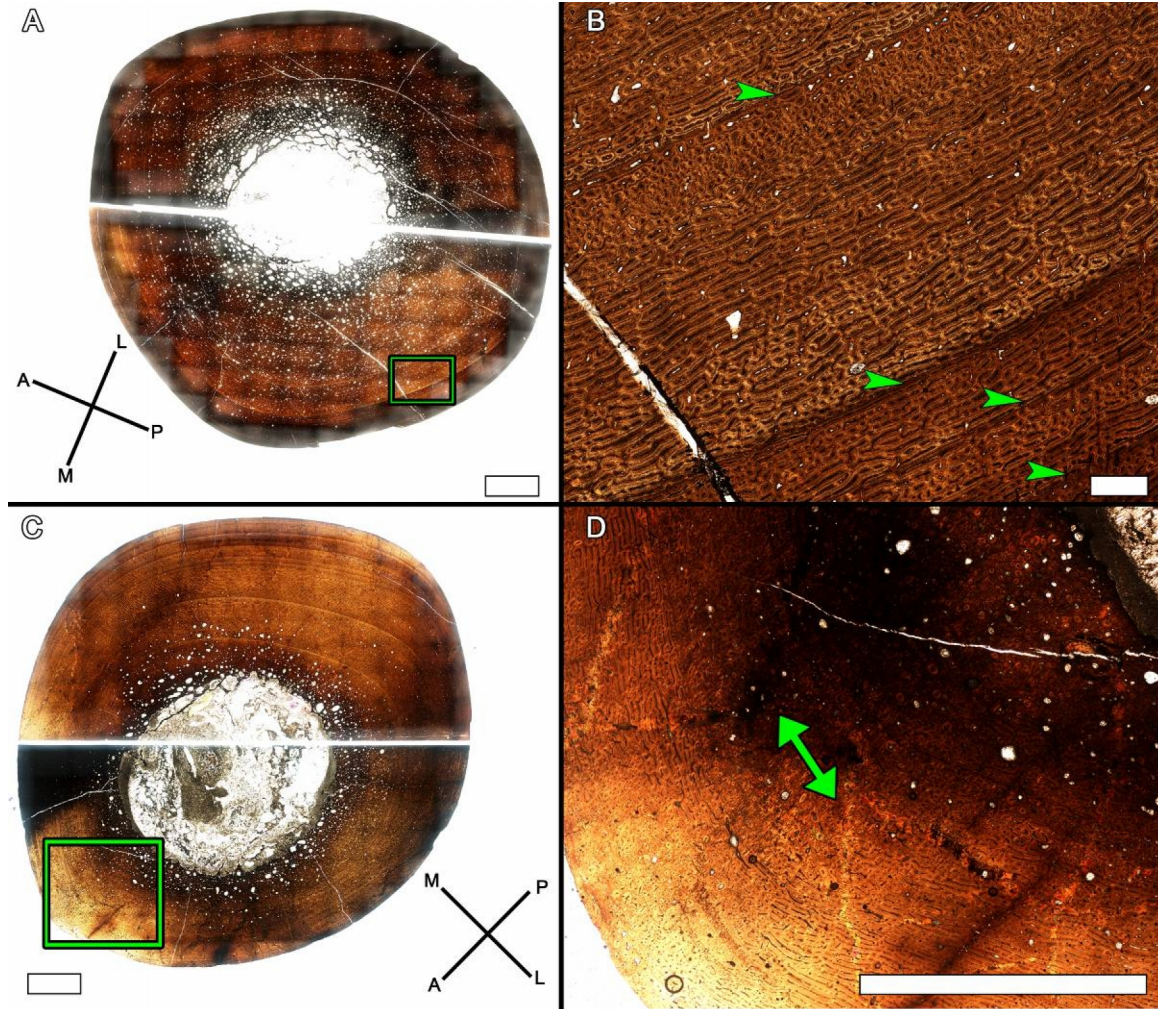


Figure 6.15. Bone tissue features present in tibiae between 70 cm and 80 cm in length. A) The cortex of T-33 is dotted by numerous scattered resorption cavities. The pixilated mosaic appearance of the transverse section is due to the “stitching” process of the software used to compile the image. B) An enlargement of the green box from A) with arrows pointing to the second LAG (top) through the fifth LAG. A repeating pattern of vascularity is observed. Immediately after a LAG, vascular orientation is strongly longitudinal for several laminae before changing to laminar or plexiform vascularity. C) As with T-33, the cortex of T-34 is also invaded by resorption cavities, but they are more concentrated in the inner cortex. D) An enlargement of the green box from C) shows a thick column of reticular primary tissue and secondary osteons stretching from inner to outer cortex, corresponding with the anterior border at the periosteal surface. The green arrow indicates the width of this column. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Scale bars, 1 cm (A, C, D); 1 mm (B).

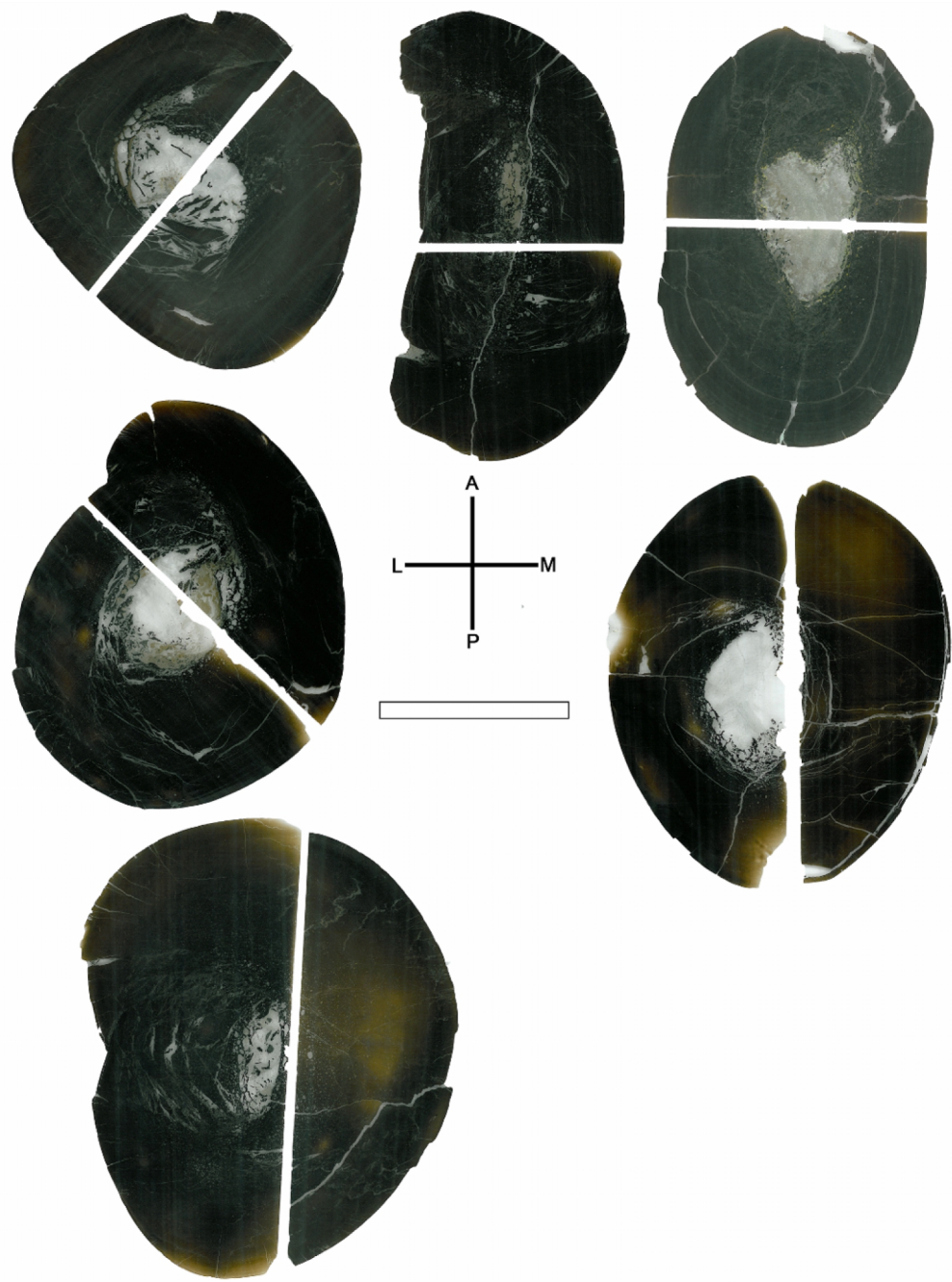


Figure 6.16. Transverse sections from the six *Maiasaura* tibiae between 80 cm and 90 cm in length. Parallel streaks are artifacts of imaging the sections on a flatbed scanner. Transverse sections in this size category were too large for a single slide, so each specimen was bisected and mounted on two slides. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Right tibia transverse sections were inverted for easier comparison. Scale bar, 5 cm.

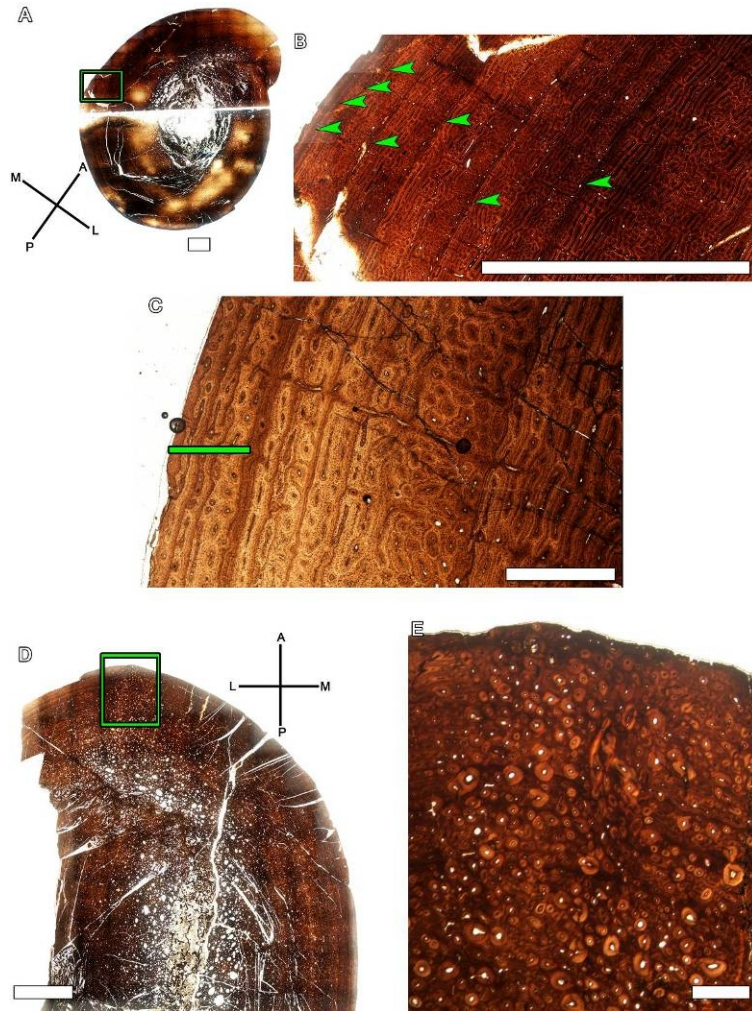


Figure 6.17. Bone tissue features present in tibiae between 80 cm and 90 cm in length. A) Resorption cavities are not as prevalent in T-40 as they are in other tibiae within this size category. B) An enlargement of the area within the green box from A) shows the second LAG (right) through the ninth LAG (left) as indicated by arrows. Even at this low resolution the recurring cyclic vascular pattern observed after the second LAG in most tibiae is evident. C) At higher magnification, the periosteal surface of T-40 is shown to possess an EFS (width denoted by green bar), signaling that growth in the length of the tibia has ceased. D) Scattered resorption cavities are frequent within the cortex of T-37. The pixilated mosaic appearance of the transverse section is due to the “stitching” process of the software used to compile the image. E) An enlargement of the green box in D) shows that instead of a column made of reticular or radial vascular canals at the anterior border, the column in tibiae of this size consists of multiple generations of secondary osteons. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Scale bars, 1 cm (A, B, D); 1 mm (C, E).

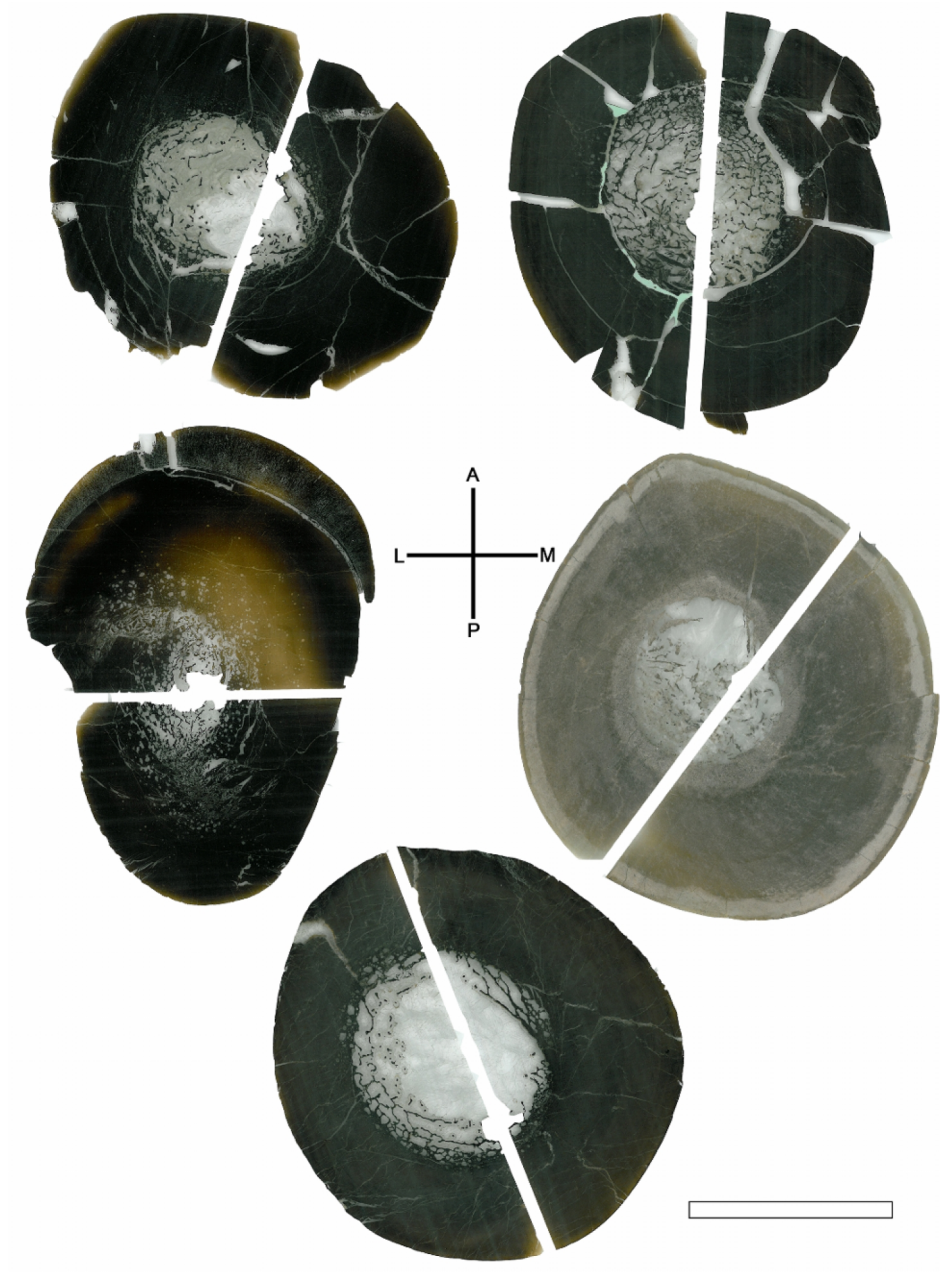


Figure 6.18. Transverse sections from the five *Maiasaura* tibiae between 90 cm and 100 cm in length. Parallel streaks are artifacts of imaging the sections on a flatbed scanner. Transverse sections in this size category were too large for a single slide, so each specimen was bisected and mounted on two slides. The cross marks orientation: A- anterior, P- posterior, L- lateral, M- medial. Right tibia transverse sections were inverted for easier comparison. Scale bar, 5 cm.

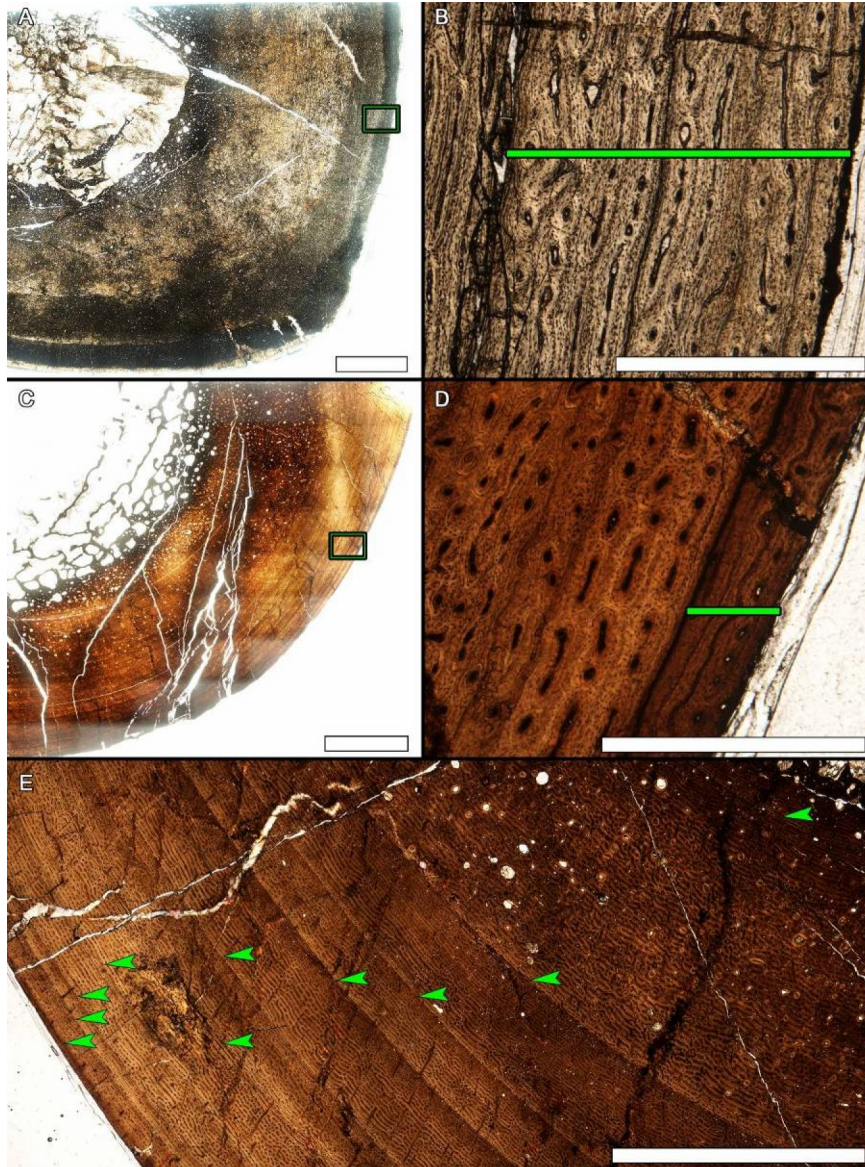


Figure 6.19. Bone tissue features present in tibiae between 80 cm and 90 cm in length. A) The cortex of T-45 is largely destroyed by diagenetic alteration, but when visible the tissue is often a mixture of secondary osteons and interstitially visible primary tissue. B) An enlargement of the green box from A) reveals an EFS (width indicated by green line) is present at the periosteal surface. C) Resorption cavities and secondary osteons are frequent in the inner cortex of T-45, but the majority of the cortex is still primary. D) An enlargement of the area within the green box from C) shows an EFS is present (width indicated by green line), though not as well developed as the EFS from T-46. E) A radial section from inner to outer cortex (right to left). LAGs are marked by arrows. The first LAG (far right) is nearly destroyed by secondary reconstruction and medullar cavity expansion. The spacing of the zones decreases considerably after the second LAG, and then again after the sixth LAG.

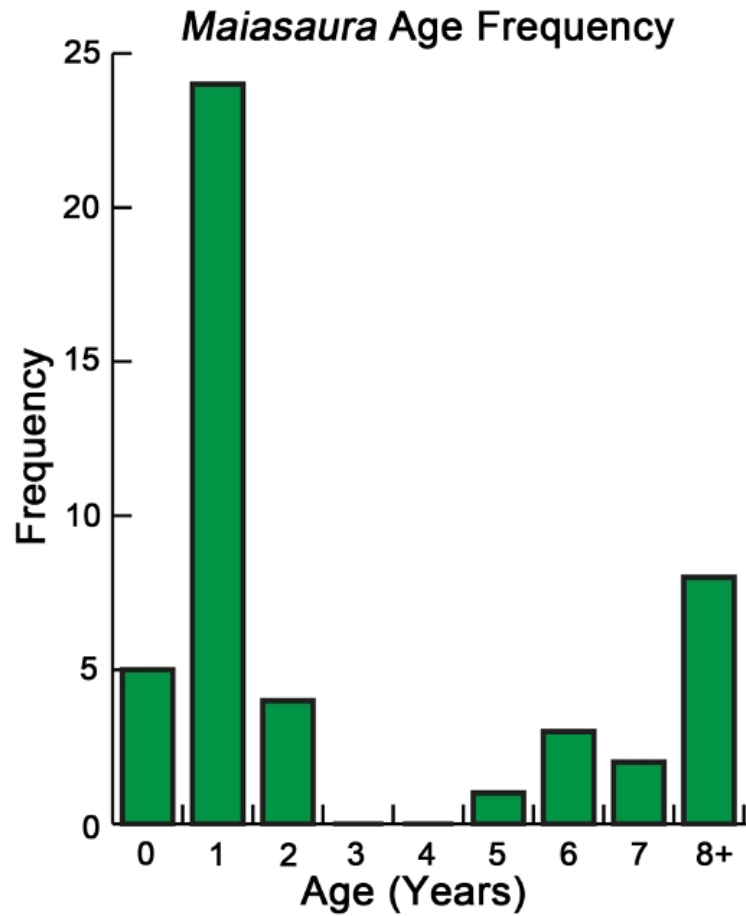


Figure 6.20. An age frequency chart showing the distribution of the *Maiasaura* sample set based on LAG counts. The majority of the sample is composed of yearlings and those less than one year of age, suggesting mortality was high for very immature individuals. Mortality rate is high again for those nearing or having attained skeletal maturity.

Literature Cited

- Anderson, J. F., A. Hall-Martin, and D. A. Russell. 1985. Long bone circumference and weight in mammals, birds, and dinosaurs. *Journal of the Zoological Society of London* 207:53-61.
- Barreto, C. 1997: Dinosaur growth plates and dinosaur bone growth. *Dinofest International: proceedings of a symposium held at Arizona State University, Tempe, Arizona.*
- Bourdon, E., J. Castanet, A. d. Ricqlès, P. Scofield, A. Tennyson, H. Lamrous, and J. Cubo. 2009. Bone growth marks reveal protracted growth in New Zealand kiwi (Aves, Apterygidae). *Biology Letters* 5:639-642.
- Campbell, J. G. 1965. A dinosaur bone lesion resembling avian osteopetrosis and some remarks on the mode of development of the lesions. *Journal of the Royal Microscopical Society* 85:163-174.
- Castanet, J., D. G. Newman, and H. S. Giron. 1988. Skeletochronological data on the growth, age, and population structure of the tuatara, *Sphenodon punctatus*, on Stephens and Lady Alice Islands, New Zealand. *Herpetologica* 44:25-37.
- Castanet, J., A. Grandin, A. Abourachid, and A. d. Ricqlès. 1996. Expression de la dynamique de croissance dans la structure de l'os périostique chez *Anas platyrhynchos*. *Comptes Rendus de l'Académie des sciences - Series III - Sciences de la Vie* 319:301-308.
- Castanet, J., K. C. Rogers, J. Cubo, and J.-J. Boisard. 2000. Periosteal bone growth rates in extant ratites (ostriche and emu). Implications for assessing growth in dinosaurs. *Comptes Rendus de l'Académie des sciences - Series III - Sciences de la Vie* 323:543-550.
- Castanet, J., S. Croci, F. Aujard, M. Perret, J. Cubo, and E. d. Margerie. 2004. Lines of arrested growth in bone and age estimation in a small primate: *Microcebus murinus*. *Journal of Zoology, London* 263:31-39.
- Cormack, D. 1987. *Ham's Histology*. Lippincott Williams & Wilkins, New York, 732 pp.
- Dilkes, D. W. 2001. An ontogenetic perspective on locomotion in the Late Cretaceous dinosaur *Maiasaura peeblesorum* (Ornithischia: Hadrosauridae). *Canadian Journal of Earth Sciences* 38:1205-1227.

- Erickson, G. M., and T. A. Tumanova. 2000. Growth curve of *Psittacosaurus mongoliensis* Osborn (Ceratopsia: Psittacosauridae) inferred from long bone histology. *Zoological Journal of the Linnean Society* 130:551-566.
- Francillon-Vieillot, H., V. d. Buffrenil, J. Castanet, J. Geraudie, F. J. Meunier, J. Y. Sire, L. Zylberberg, and A. d. Ricqlès. 1990. Microstructure and mineralization of vertebrate skeletal tissues; pp. 471-548 in J. G. Carter (ed.), *Skeletal biomineralization patterns, processes and evolutionary trends*. Van Nostrand Reinhold, New York.
- Frylestam, B., and T. von Schantz. 1977. Age determination of European hares based on periosteal growth lines. *Mammal Review* 7:151-154.
- Games, I. 1990. Growth curves for the Nile crocodile as estimated by skeletochronology; pp. 111-121 in *Proceedings of the 10th Working Meeting of the Crocodile Specialist Group Species Survival Commission of the International Union for the Conservation of Nature and Natural Resources*, Gainesville, Florida.
- Hemelaar, A. S. M., and J. J. van Gelder. 1980. Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. *Netherlands Journal of Zoology* 30:129-135.
- Horner, J. R. 2002. Evidence of dinosaur social behavior; pp. 71-78 in J. Scotchmoor (ed.), *Dinosaurs, The Science Behind the Stories*. American Geological Institute.
- Horner, J. R., and R. Makela. 1979. Nest of juveniles provides evidence of family structure among dinosaurs. *Nature* 282:296-298.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 2000. Long bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. *Journal of Vertebrate Paleontology* 20:115-129.
- Horner, J. R., K. Padian, and A. d. Ricqlès. 2001a. Comparative osteohistology of some embryonic and perinatal archosaurs: developmental and behavioral implications for dinosaurs. *Paleobiology* 27:39-58.
- Horner, J. R., J. G. Schmitt, F. Jackson, and R. Hanna. 2001b. Bones and rocks of the Upper Cretaceous Two Medicine-Judith River clastic wedge complex, Montana; pp. 3-13 in C. L. Hill (ed.), *Mesozoic and Cenozoic Paleontology in the Western Plains and Rocky Mountains: Guidebook for the Field Trips*, Society of Vertebrate Paleontology, 61st Annual Meeting. Museum of the Rockies, Bozeman, MT.

- Hübner, T. R. 2012. Bone histology in *Dysalotosaurus lettowvorbecki* (Ornithischia: Iguanodontia) - variation, growth, and implications. Public Library of Science ONE 7:e22958.
- Hutton, J. M. 1986. Age determination of living Nile crocodiles from the cortical stratification of bone. Copeia 2:332-341.
- Joanen, T., and L. McNease. 1987. Alligator farming research in Louisiana, USA; pp. 329-340 in J. W. Grahame, S. Webb, C. Manolis, and P. J. Whitehead (eds.), Wildlife Management: Crocodiles and Alligators. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.
- Klein, N., T. Scheyer, and T. Tütken. 2009. Skeletochronology and isotopic analysis of a captive individual of *Alligator mississippiensis* Daudin, 1802. Fossil Record 12:121-131.
- Lamm, E.-T. 2007. Paleohistology widens the field of view in paleontology; pp. 50-51 in M. Marko, J. H. Scott, E. Vicenzi, S. Dekanich, J. Frafjord, P. Kotula, S. McKernan, and J. Shields (eds.), Proceedings microscopy and microanalysis 2007. Cambridge University Press, New York.
- Lee, A. H., and S. Werning. 2008. Sexual maturity in growing dinosaurs does not fit reptilian growth models. Proceedings of the National Academy of Sciences, USA 105:582-587.
- Lorenz, J. C., and W. Gavin. 1984: Geology of the Two Medicine Formation and the sedimentology of a dinosaur nesting ground. Field Conference Northwestern Montana.
- Margerie, E. d. 2002. Laminar bone as an adaptation to torsional loads in flapping flight. Journal of Anatomy 201:521-526.
- Margerie, E. d., J.-P. Robin, D. Verrier, J. Cubo, R. Groscolas, and J. Castanet. 2004. Assessing a relationship between bone microstructure and growth rate: a fluorescent labelling study in the king penguin chick (*Aptenodytes patagonicus*). The Journal of Experimental Biology 207:869-879.
- Montes, L., J. Castanet, and J. Cubo. 2010. Relationship between bone growth rate and bone tissue organization in amniotes: first test of Amprino's rule in a phylogenetic context. Animal Biology 60:25-41.
- Packard, G. C., T. J. Boardman, and G. F. Brichard. 2009. Allometric equations for predicting body mass of dinosaurs. Journal of Zoology:1-9.

- Peabody, F. E. 1961. Annual growth zones in living and fossil vertebrates. *Journal of Morphology* 108:11-62.
- Ponton, F., A. Elzanowski, J. Castanet, A. Chinsamy, E. d. Margerie, A. d. Ricqlès, and J. Cubo. 2004. Variation of the outer circumferential layer in the limb bones of birds. *Acta Ornithologica* 39:21-24.
- Ricqlès, A. d. 1980. Tissue structures of dinosaur bone: functional significance and possible relation to dinosaur physiology; pp. 103-138 in R. D. K. Thomas and E. C. Olson (eds.), *A Cold Look at the Warm Blooded Dinosaurs*. Westview Press, Boulder.
- Rogers, R. R. 1990. Taphonomy of three dinosaur bone beds in the Upper Cretaceous Two Medicine Formation of northwestern Montana: evidence for drought-related mortality. *Palaios* 5:394-413.
- Starck, J. M., and A. Chinsamy. 2002. Bone microstructure and developmental plasticity in birds and other dinosaurs. *Journal of Morphology* 254:232-246.
- Trexler, D. 2001. Two Medicine Formation, Montana: Geology and fauna; pp. 298-309 in D. H. Tanke and K. Carpenter (eds.), *Mesozoic Vertebrate Life*. Indiana University Press, Indianapolis.
- Trueman, C. L. 1999. Rare earth element geochemistry and taphonomy of terrestrial vertebrate assemblages. *Palaios* 14:555-568.
- Tucker, A. D. 1997. Validation of skeletochronology to determine age of freshwater crocodiles (*Crocodylus johnstoni*). *Australian Journal of Marine and Freshwater Research* 48:343-351.
- Woodward, H. N., and T. M. Lehman. 2009. Bone histology and microanatomy of *Alamosaurus sanjuanensis* (Sauropoda: Titanosauria) from the Maastrichtian of Big Bend National Park, Texas. *Journal of Vertebrate Paleontology* 29:807-821.
- Woodward, H. N., J. R. Horner, and J. O. Farlow. 2011. Osteohistological evidence for determinate growth in the American alligator. *Journal of Herpetology* 45:339-342.
- Woodward, H. N., J. R. Horner, and J. O. Farlow. *In prep.* Intra-skeletal histovariability in *Alligator mississippiensis* and its applications for non-avian dinosaur osteohistology.

CHAPTER SEVEN

CONCLUSIONS

The validity of dinosaur paleohistological interpretations often suffers due to a lack of adequate sample sizes as well as from a lack of extant studies on which to base those interpretations. To help remedy the problem, this dissertation quantified individual bone microstructural variation present within both extant and extinct populations of archosaurs, and demonstrated the value of increasing quantitative histological analyses on extant animals to provide a stronger framework from which to investigate the growth of extinct taxa.

Regarding alligators, results demonstrated that: no statistical difference in bone microstructure exists between left and right elements; there is strong evidence that each skeletal element deposits a growth mark each year; the femur, humerus, and tibia grow the fastest in each alligator; and the presence of an EFS shows that contrary to popular belief, alligators do attain skeletal maturity. Extrapolated to dinosaur studies, these results suggest that: homologous elements from one individual should display similar growth rates and tissue structures; an inconsistent number of growth marks across elements is due to medullary expansion; if attempting to obtain maximum growth rates, the femur, humerus, or tibia are likely the best options for analysis; and because both birds and crocodylians achieve skeletal maturity, it is likely that non-avian dinosaurs did as well.

The observations and conclusions of the alligator studies were then applied to the analysis of forty eight tibiae of the hadrosaur *Maiasaura*. In general, the results supported

earlier observations of Horner et al. (2000) but also provided stronger quantitative analyses of growth: *Maiasaura* grew rapidly for most of its life, attaining skeletal maturity and a body length of 7 m in as little as nine years. But there was considerable variation in individual growth rates, and over half of the specimens examined from the bone bed were a year or less in age. Thus if only a random handful of tibiae was selected for analysis even from this comparatively large sample set, gross misinterpretations regarding the growth and development of this taxon might result.

Therefore, this dissertation emphasizes the dangers associated with drawing conclusions about the growth of either an extant or extinct taxon based on small sample sizes. For example, reexamination of previous histologic studies on polar dinosaurs and “*Raptorex*” revealed problems with interpreting behavior based on extremely small samples for the former and the dangers of misinterpreting bone microstructural organization for the latter. Additionally, the alligators used for intraskeletal analyses demonstrated so much variability in growth rates that it became obvious more than three individuals would be required to make any significant conclusions regarding the range of growth for alligators. Lastly, although the *Maiasaura* study represents the largest single-element paleohistological study to date, forty eight tibiae were still insufficient to make robust growth curves. This is because over half of the sample turned out to be a year or less in age and of the nine *Maiasaura* used to make growth curves, only a single skeletally mature individual was included.

Despite the obvious need for more extensive histological studies on both extinct and extant taxa, paleohistology has provided scientists with a wealth of information about

extinct life. However, paleohistology is often negatively viewed as a destructive sampling technique, restricting the access to suitable sample sizes. The results of this dissertation should demonstrate what a valuable tool bone microstructure can be, as this field provides details about the growth of a taxon that cannot be obtained from morphology alone. In this sense, paleohistology should not be perceived as a destructive method, but rather a method for *data recovery*. Viewed in this light, the importance of large sample sets to enable statistical significance becomes clear.

Literature Cited

- Horner, J. R., A. d. Ricqlès, and K. Padian. 2000. Long bone histology of the hadrosaurid dinosaur *Maiaasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. *Journal of Vertebrate Paleontology* 20:115-129.

REFERENCES CITED

- Achenbach, J. Unveiled: The Surprisingly Small Precursor of T. Rex. Washington Post 18th September 2009. <http://www.washingtonpost.com/wp-dyn/content/article/2009/09/17/AR2009091702573.html?hpid=topnews&sid=ST2009091800725>. Retrieved 2011 March 8.
- Agnolin, F. L., M. D. Ezcurra, D. F. Pais, and S. W. Salisbury. 2010. A reappraisal of the Cretaceous non-avian dinosaur faunas from Australia and New Zealand: evidence for their Gondwanan affinities. *Journal of Systematic Palaeontology* 8:257-300.
- Anderson, J. F., A. Hall-Martin, and D. A. Russell. 1985. Long bone circumference and weight in mammals, birds, and dinosaurs. *Journal of the Zoological Society of London* 207:53-61.
- Andrews, R. M. 1982. Patterns of growth in reptiles; pp. 273-320 in C. Gans and F. H. Pough (eds.), *Biology of the Reptilia*. Academic Press, London, England.
- Barreto, C. 1997: Dinosaur growth plates and dinosaur bone growth. *Dinofest International: proceedings of a symposium held at Arizona State University, Tempe, Arizona*.
- Benson, R. B. J. 2008. New information on *Stokesosaurus*, a tyrannosauroid (Dinosauria: Theropoda) from North America and the United Kingdom. *Journal of Vertebrate Paleontology* 28:732-750.
- Benson, R. B. J., P. M. Barrett, T. H. Rich, and P. Vickers-Rich. 2010. A Southern Tyrant Reptile. *Science* 327:1613.
- Bonnan, M. F., J. O. Farlow, and S. L. Masters. 2008. Using linear and geometric morphometrics to detect intraspecific variability and sexual dimorphism in femoral shape in *Alligator mississippiensis* and its implications for sexing fossil archosaurs. *Journal of Vertebrate Paleontology* 28:422-431.
- Bourdon, E., J. Castanet, A. d. Ricqlès, P. Scofield, A. Tennyson, H. Lamrous, and J. Cubo. 2009. Bone growth marks reveal protracted growth in New Zealand kiwi (Aves, Apterygidae). *Biology Letters* 5:639-642.
- Boyle, A. Tiny T. rex? Big surprise! MSNBC News. <http://cosmiclog.msnbc.msn.com/archive/2009/09/17/2072603.aspx>. Retrieved 2011 March 8.

- Brinkman, D. B., and A. G. Neuman. 2002. Teleost centra from uppermost Judith River Group (Dinosaur Park Formation, Campanian) of Alberta. *Canadian Journal of Paleontology* 76:138-155.
- Brochu, C. A. 1996. Closure of neurocentral sutures during crocodilian ontogeny: implications for maturity assessment in fossil archosaurs. *Journal of Vertebrate Paleontology* 16:49-62.
- Brochu, C. A. 2003. Osteology of *Tyrannosaurus rex*: Insights from a nearly complete skeleton and high-resolution computed tomographic analysis of the skull. *Journal of Vertebrate Paleontology Memoir* 7:1-138.
- Bronikowski, A. M., and S. J. Arnold. 1999. The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. *Ecology* 80:2314-2325.
- Brown, B., and E. Schlaikjer. 1940. The structure and relationships of *Protoceratops*. *Annals of the New York Academy of Sciences* 40:133-266.
- Brown, C. M., A. P. Russell, and M. J. Ryan. 2009. Pattern and transition of surficial bone texture of the Centrosaurine frill and their ontogenetic and taxonomic implications. *Journal of Vertebrate Paleontology* 29:132-141.
- Brusatte, S. L., T. D. Carr, G. M. Erickson, G. S. Bever, and M. A. Norell. 2010. A long-snouted, multihorned tyrannosaurid from the Late Cretaceous of Mongolia. *Proceedings of the National Academy of Science* 106:17261-17266.
- Buffrénil, V. d. 1980. Mise en évidence de l'incidence des conditions de milieu sur la croissance de *Crocodylus siamensis* (Schneider, 1801), et valeur des marques de croissance squelettiques pour l'évaluation de l'âge individuel. *Archives de Zoologie Experimentale et Generale* 121:63-76.
- Buffrénil, V. d., and J. Castanet. 2000. Age estimation by skeletochronology in the Nile monitor (*Varanus niloticus*), a highly exploited species. *Journal of Herpetology* 34:414-424.
- Bybee, P. J., A. H. Lee, and E. Lamm. 2006. Sizing the Jurassic theropod dinosaur *Allosaurus*: assessing growth strategy and evolution of ontogenetic scaling of limbs. *Journal of Morphology* 267:347-359.
- Campbell, J. G. 1965. A dinosaur bone lesion resembling avian osteopetrosis and some remarks on the mode of development of the lesions. *Journal of the Royal Microscopical Society* 85:163-174.

- Carpenter, K., C. Miles, and K. Cloward. 2005. New small theropod from the Upper Jurassic Morrison Formation of Wyoming; pp. 23-48 in K. Carpenter (ed.), *The Carnivorous Dinosaurs*. Indiana University Press, Bloomington.
- Carr, T. D. 1999. Craniofacial ontogeny in Tyrannosauridae (Dinosauria, Coelurosauria). *Journal of Vertebrate Paleontology* 19:497-520.
- Carr, T. D., and T. E. Williamson. 2004. Diversity of Late Maastrichtian Tyrannosauridae (Dinosauria: Theropoda) from western North America. *Zoological Journal of the Linnean Society-London* 142:419-523.
- Carr, T. D., T. E. Williamson, and D. E. Schwimmer. 2005. A new genus and species of tyrannosaurid from the Late Cretaceous (Middle Campanian) Demopolis Formation of Alabama. *Journal of Vertebrate Paleontology* 25:119-143.
- Carrano, M. T., J. R. Hutchinson, and S. D. Sampson. 2005. New information on *Segisaurus halli*, a small theropod dinosaur from the Early Jurassic of Arizona. *Journal of Vertebrate Paleontology* 25:835-849.
- Case, T. J. 1978. Speculation on the growth rate and reproduction of some dinosaurs. *Paleobiology* 4:320-328.
- Castanet, J., D. G. Newman, and H. S. Girons. 1988. Skeletochronological data on the growth, age, and population structure of the tuatara, *Sphenodon punctatus*, on Stephens and Lady Alice Islands, New Zealand. *Herpetologica* 44:25-37.
- Castanet, J., H. Francillon-Vieillot, P. J. Meunier, and A. d. Ricqlès. 1993. Bone and individual aging; pp. 245-283 in B. K. Hall (ed.), *Bone*. CRC Press, London.
- Castanet, J., A. Grandin, A. Abourachid, and A. d. Ricqlès. 1996. Expression de la dynamique de croissance dans la structure de l'os périostique chez *Anas platyrhynchos*. *Comptes Rendus de l'Académie des sciences - Series III - Sciences de la Vie* 319:301-308.
- Castanet, J., K. C. Rogers, J. Cubo, and J.-J. Boisard. 2000. Periosteal bone growth rates in extant ratites (ostriche and emu). Implications for assessing growth in dinosaurs. *Comptes Rendus de l'Académie des sciences - Series III - Sciences de la Vie* 323:543-550.
- Castanet, J., S. Croci, F. Aujard, M. Perret, J. Cubo, and E. d. Margerie. 2004. Lines of arrested growth in bone and age estimation in a small primate: *Microcebus murinus*. *Journal of Zoology, London* 263:31-39.

- Chabreck, R. H., and T. Joanen. 1979. Growth rates of American alligators in Louisiana. *Herpetologica* 35:51-57.
- Charnov, E. L., T. F. Turner, and K. O. Winemiller. 2001. Reproductive constraints and the evolution of life histories with indeterminate growth. *Proceedings of the National Academy of Sciences, USA* 98:9460-9464.
- Chinsamy-Turan, A. 2005a. *The Microstructure of Dinosaur Bone: Deciphering Biology with Fine-Scale Techniques*. The Johns Hopkins University Press, Baltimore.
- Chinsamy-Turan, A. 2005b. *The microstructure of dinosaur bone: deciphering biology with fine-scale techniques*. Johns Hopkins University Press, Baltimore, 216 pp.
- Chinsamy, A. 1990. Physiological implications of the bone histology of *Syntarsus rhodesiensis* (Saurischia: Theropoda). *Palaeontologia Africana* 27:77-82.
- Chinsamy, A. 1993a. Bone histology and growth trajectory of the prosauropod dinosaur *Massospondylus carinatus* owen. *Modern Geology* 18:319-329.
- Chinsamy, A. 1993b. Image analysis and the physiological implications of the vascularisation of femora in archosaurs. *Modern Geology* 19:101-108.
- Chinsamy, A., T. Rich, and P. Vickers-Rich. 1998. Polar dinosaur bone histology. *Journal of Vertebrate Paleontology* 18:385-390.
- Clark, J. 2009. Becoming *T. rex*. *Science* 326:373-374.
- Congdon, J. D., and R. C. van Loben Sels. 1993. Relationships of reproductive traits and body size with attainment of sexual maturity and age in Blanding's turtles (*Emydoidea blandingii*). *Journal of Evolutionary Biology* 6:547-557.
- Congdon, J. D., R. D. Nagle, O. M. Kinney, and R. C. van Loben Sels. 2001. Hypotheses of aging in a long-lived vertebrate, Blanding's turtle (*Emydoidea blandingii*). *Experimental Gerontology* 36:813-827.
- Cormack, D. 1987. *Ham's Histology*. Lippincott Williams & Wilkins, New York, 732 pp.
- Coulson, T. D., R. A. Coulson, and T. Hernandez. 1973. Some observations on the growth of captive alligators. *Zoologica*:47-52.
- Csiki, Z., M. Vremir, S. L. Brusatte, and M. A. Norell. 2010. An aberrant island-dwelling theropod dinosaur from the Late Cretaceous of Romania. *Proceedings of the National Academy of Science* 107:15357-15361.

- Currie, P. J. 2003a. Allometric growth in tyrannosaurids (Dinosauria: Theropoda) from the Upper Cretaceous of North America and Asia. *Canadian Journal of Earth Sciences* 40:651-665.
- Currie, P. J. 2003b. Cranial anatomy of tyrannosaurid dinosaurs from the Late Cretaceous of Alberta, Canada. *Acta Palaeontologica Polonica* 48:191-226.
- Currie, P. J., and D. A. Eberth. 1993. Palaeontology, sedimentology, and palaeoecology of the Iren Dabasu Formation (Upper Cretaceous), Inner Mongolia, People's Republic of China. *Cretaceous Research* 14:127-144.
- Currie, P. J., and Z. Dong. 2001. New information on *Shanshanosaurus huoyanshanensis*, a juvenile tyrannosaurid (Theropoda, Dinosauria) from the Late Cretaceous of China. *Canadian Journal of Earth Sciences* 38:1729-1737.
- Currie, P. J., and D. A. Russell. 2005. The geographic distribution of articulated and associated dinosaur remains; pp. 537-569 in P. J. Currie and E. B. Koppelhaus (eds.), *Dinosaur Provincial Park*. Indiana University Press, Bloomington.
- Currie, P. J., W. Langston, and D. H. Tanke. 2008. A new species of *Pachyrhinosaurus* (Dinosauria, Ceratopsidae) from the Upper Cretaceous of Alberta, Canada; pp. 1-108 in P. J. Currie, W. Langston, and D. H. Tanke (eds.), *A New Horned Dinosaur from an Upper Cretaceous Bone Bed in Alberta*. NRC Research Press, Ottawa.
- Dilkes, D. W. 2001. An ontogenetic perspective on locomotion in the Late Cretaceous dinosaur *Maiasaura peeblesorum* (Ornithischia: Hadrosauridae). *Canadian Journal of Earth Sciences* 38:1205-1227.
- Dodson, P. 1975. Taxonomic implications of relative growth in Lambeosaurine hadrosaurs. *Systematic Zoology* 24:37-54.
- Dodson, P. 1976. Quantitative aspects of relative growth and sexual dimorphism in *Protoceratops*.
- Dodson, P., and P. J. Currie. 1988. The smallest ceratopsid skull - Judith River Formation of Alberta. *Canadian Journal of Earth Sciences* 25:926-930.
- Dong, Z. 1977. On the dinosaurian remains from Turpan, Xinjiang. *Vertebrata Palasiatica* 15:59-66.
- Doube, M., M. M. Kłosowski, I. Arganda-Carreras, F. Cordelières, R. P. Dougherty, J. Jackson, B. Schmid, J. R. Hutchinson, and S. J. Shefelbine. 2010. BoneJ: free and extensible bone image analysis in ImageJ. *Bone* 47:1076-1079.

- Enlow, D. H. 1969. The bone of reptiles; pp. 45-80 in A. d. A. Bellairs and T. S. Parsons (eds.), *Biology of the Reptilia*. Academic Press, New York.
- Enlow, D. H., and S. O. Brown. 1956. A comparative histological study of fossil and recent bone tissues, Part I. *The Texas Journal of Science* 8:405-443.
- Enlow, D. H., and S. O. Brown. 1957. A comparative histological study of fossil and recent bone tissues, Part II. *The Texas Journal of Science* 8:405-443.
- Erickson, G. M. 2005. Assessing dinosaur growth patterns: a microscopic revolution. *TRENDS in Ecology and Evolution* 20:677-684.
- Erickson, G. M., and T. A. Tumanova. 2000. Growth curve of *Psittacosaurus mongoliensis* Osborn (Ceratopsia: Psittacosauridae) inferred from long bone histology. *Zoological Journal of the Linnean Society* 130:551-566.
- Erickson, G. M., K. Curry Rogers, D. J. Varricchio, M. A. Norell, and X. Xu. 2007. Growth patterns in brooding dinosaurs reveals the timing of sexual maturity in non-avian dinosaurs and genesis of the avian condition. *Biology Letters: Paleontology*.
- Erickson, G. M., P. J. Makovicky, P. J. Currie, M. A. Norell, S. A. Yerby, and C. A. Brochu. 2004. Gigantism and comparative life-history parameters of tyrannosaurid dinosaurs. *Nature* 430:772-775.
- Evans, D. C., C. A. Forster, and R. R. Reisz. 2005. The type specimen of *Tetragonosaurus erectofrons* (Ornithischia: Hadrosauridae) and the identification of juvenile Lambeosaurines; pp. 349-366 in P. J. Currie and E. B. Koppelhaus (eds.), *Dinosaur Provincial Park*. Indiana University Press, Bloomington.
- Evans, D. C., R. R. Reisz, and K. Dupuis. 2007. A juvenile *Parasaurolophus* (Ornithischia: Hadrosauridae) braincase from Dinosaur Provincial Park, Alberta, with comments on crest ontogeny in the genus. *Journal of Vertebrate Paleontology* 27:642-650.
- Everhart, M. J. 2011. Rediscovery of the *Hesperornis regalis* Marsh 1871 holotype locality indicates an earlier stratigraphic occurrence. *Transactions of the Kansas Academy of Science* 114:59-68.
- Farlow, J. O., and A. Britton. 2000. Size and body proportions in *Alligator mississippiensis*: implications for archosaurian ichnology. *Paleontological Society of Korea Special Publication* 4:189-206.

- Farlow, J. O., S. Hayashi, and G. J. Tattersall. 2010. Internal vascularity of the dermal plates of *Stegosaurus* (Ornithischia, Thyreophora). *Swiss Journal of Geoscience* 103:173-185.
- Farlow, J. O., G. R. Hurlburt, R. M. Elsey, A. R. C. Britton, and W. Langston Jr. 2005. Femoral dimensions and body size of *Alligator mississippiensis*: estimating the size of extinct mesoeucrocodylians. *Journal of Vertebrate Paleontology* 25:354-369.
- Fowler, D. W., H. N. Woodward, E. A. Freedman, P. L. Larson, and J. R. Horner. 2011. Reanalysis of "*Raptorex kriegsteini*": a juvenile tyrannosaurid dinosaur from Mongolia. *Public Library of Science ONE* 6:e21376.
- Francillon-Vieillot, H., V. d. Buffrenil, J. Castanet, J. Geraudie, F. J. Meunier, J. Y. Sire, L. Zylberberg, and A. d. Ricqlès. 1990. Microstructure and mineralization of vertebrate skeletal tissues; pp. 471-548 in J. G. Carter (ed.), *Skeletal biomineralization patterns, processes and evolutionary trends*. Van Nostrand Reinhold, New York.
- Frey, T. v. E. 1988. Das Tragsystem der Krododile- eine biomechanische und phylogenetische Analyse. *Stuttgarter Beiträge zur Naturkunde Serie A (Biologie)* 426:1-60.
- Frylestam, B., and T. von Schantz. 1977. Age determination of European hares based on periosteal growth lines. *Mammal Review* 7:151-154.
- Games, I. 1990. Growth curves for the Nile crocodile as estimated by skeletochronology; pp. 111-121 in *Proceedings of the 10th Working Meeting of the Crocodile Specialist Group/Species Survival Commission of the International Union for the Conservation of Nature and Natural Resources*, Gainesville, Florida.
- Gibson, C. W. D., and J. Hamilton. 1984. Population processes in a large herbivorous reptile: the giant tortoise of Aldabra atoll. *Oecologia* 61:230-240.
- Gregory, R. T., C. B. Douthitt, I. R. Duddy, P. Vickers-Rich, and T. H. Rich. 1989. Oxygen isotopic composition of carbonate concretions from the lower Cretaceous of Victoria, Australia: implications for the evolution of meteoric waters on the Australian continent in a paleopolar environment. *Earth and Planetary Science Letters* 92:27-42.
- Gross, W. 1934. Die Typen des mikroskopischen Knochenbaues bei fossilen Stegocephalen und Reptilien. *Zeitschrift für Anatomie und Entwicklungsgeschichte*:731-764.

- Hall, B. K. 2005. *Bones and Cartilage: Developmental and Evolutionary Skeletal Biology*. Elsevier Academic Press, San Diego, 760 pp.
- Hallam, A. 1985. A review of Mesozoic climates. *Journal of the Geological Society (London)* 142:433-445.
- Heino, M., and V. Kaitala. 1999. Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. *Journal of Evolutionary Biology* 12:423-429.
- Hemelaar, A. S. M., and J. J. van Gelder. 1980. Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. *Netherlands Journal of Zoology* 30:129-135.
- Herne, M. C., J. P. Nair, and S. W. Salisbury. 2010. Comment on "A Southern Tyrant Reptile". *Science* 329:1013.
- Holmes, R. B., C. A. Forster, M. Ryan, and K. M. Shepherd. 2001. A new species of *Chasmosaurus* from the Dinosaur Park Formation of Southern Alberta. *Canadian Journal of Earth Sciences* 38:1423-1438.
- Holtz Jr., T. R. 2001. The phylogeny and taxonomy of the Tyrannosauridae; pp. 64-83 in D. H. Tanke and K. Carpenter (eds.), *Mesozoic Vertebrate Life*. Indiana University Press, Bloomington.
- Holtz Jr., T. R. 2004. Tyrannosauroidae; pp. 111-136 in D. Weishampel, P. Dodson, and H. Osmolska (eds.), *The Dinosauria*. University of California Press, Berkeley.
- Hone, D. W., K. Wang, C. Sullivan, X. Zhao, S. Chen, D. Li, S. Ji, Q. Ji, and X. Xu. 2011. A new tyrannosaurine theropod, *Zhuchengtyrannus magnus* is named based on a maxilla and dentary. *Cretaceous Research* doi: 10.1016/j.cretres.2011.03.005.
- Horner, J. R. 1983. Cranial osteology and morphology of the type specimen of *Maiasaura peeblesorum* (Ornithischia: Hadrosauridae), with discussion of its phylogenetic position. *Journal of Vertebrate Paleontology* 3:29-38.
- Horner, J. R. 2002. Evidence of dinosaur social behavior; pp. 71-78 in J. Scotchmoor (ed.), *Dinosaurs, The Science Behind the Stories*. American Geological Institute.
- Horner, J. R., and R. Makela. 1979. Nest of juveniles provides evidence of family structure among dinosaurs. *Nature* 282:296-298.

- Horner, J. R., and P. J. Currie. 1994. Embryonic and neonatal morphology and ontogeny of a new species of *Hypacrosaurus* (Ornithischia, Lambeosauridae) from Montana and Alberta; pp. in K. Carpenter, K. F. Hirsch, and J. R. Horner (eds.), *Dinosaur Eggs and Babies*. Cambridge University Press, New York.
- Horner, J. R., and K. Padian. 2004. Age and growth dynamics of *Tyrannosaurus rex*. *Proceedings of the Royal Society of London Series B* 271:1875-1880.
- Horner, J. R., and M. B. Goodwin. 2006. Major cranial changes during *Triceratops* ontogeny. *Proceedings of the Royal Society of London Series B* 273:2757-2761.
- Horner, J. R., and M. B. Goodwin. 2008. Ontogeny of cranial epi-ossifications in *Triceratops*. *Journal of Vertebrate Paleontology* 28:134-144.
- Horner, J. R., and M. B. Goodwin. 2009. Extreme cranial ontogeny in the Upper Cretaceous dinosaur *Pachycephalosaurus*. *Public Library of Science ONE* 4:e7626.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 1999. Variation in dinosaur skeletochronology indicators: implications for age assessment and physiology. *Paleobiology* 25:295-304.
- Horner, J. R., A. d. Ricqlès, and K. Padian. 2000. Long bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. *Journal of Vertebrate Paleontology* 20:115-129.
- Horner, J. R., K. Padian, and A. d. Ricqlès. 2001a. Comparative osteohistology of some embryonic and perinatal archosaurs: developmental and behavioral implications for dinosaurs. *Paleobiology* 27:39-58.
- Horner, J. R., J. G. Schmitt, F. Jackson, and R. Hanna. 2001b. Bones and rocks of the Upper Cretaceous Two Medicine-Judith River clastic wedge complex, Montana; pp. 3-13 in C. L. Hill (ed.), *Mesozoic and Cenozoic Paleontology in the Western Plains and Rocky Mountains: Guidebook for the Field Trips*, Society of Vertebrate Paleontology, 61st Annual Meeting. Museum of the Rockies, Bozeman, MT.
- Horner, J. R., A. d. Ricqlès, K. Padian, and R. D. Scheetz. 2009. Comparative long bone histology and growth of the "hypsilophodontid" dinosaurs *Orodromeus makelai*, *Dryosaurus altus*, and *Tenontosaurus tilletii* (Ornithischia: Euornithodpoda). *Journal of Vertebrate Paleontology* 29:734-747.

- Hübner, T. R. 2012. Bone histology in *Dysalotosaurus lettowvorbecki* (Ornithischia: Iguanodontia) - variation, growth, and implications. Public Library of Science ONE 7:e22958.
- Hurum, J. H., and K. Sabath. 2003. Giant theropod dinosaurs from Asia and North America: skulls of *Tarbosaurus bataar* and *Tyrannosaurus rex* compared. Acta Palaeontologica Polonica 48:161-190.
- Hutt, S., D. W. Naish, D. M. Martill, M. J. Barker, and P. Newberry. 2001. A preliminary account of a new tyrannosauroid theropod from the Wessex Formation (Early Cretaceous) of southern England. Cretaceous Research 22:227-242.
- Hutton, J. M. 1986. Age determination of living Nile crocodiles from the cortical stratification of bone. Copeia 2:332-341.
- Joanen, T., and L. McNease. 1987. Alligator farming research in Louisiana, USA; pp. 329-340 in J. W. Grahame, S. Webb, C. Manolis, and P. J. Whitehead (eds.), Wildlife Management: Crocodiles and Alligators. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.
- Klein, N., and P. M. Sander. 2008. Ontogenetic stages in the long bone histology of sauropod dinosaurs. Paleobiology 34:248-264.
- Klein, N., T. Scheyer, and T. Tütken. 2009. Skeletochronology and isotopic analysis of a captive individual of *Alligator mississippiensis* Daudin, 1802. Fossil Record 12:121-131.
- Knoll, F., K. Padian, and A. d. Ricqlès. 2010. Ontogenetic change and adult body size of the early ornithischian dinosaur *Lesothosaurus diagnosticus*: implications for basal ornithischian taxonomy. Gondwana Research 17:171-179.
- Köhler, M. 2011. On the significance of fibrolamellar zonal bone in bovids; pp. 54 in First International Symposium on Paleohistology, Vol. 1, I. C. d. P. M. Crusafont ed Gràfica Masana, Sabadell, Spain.
- Kozłowski, J. 1996. Optimal allocation of resources explains interspecific life-history patterns in animals with indeterminate growth. Proceedings of the Royal Society of London Series B 263:559-566.
- Lailvaux, S. P., A. Herrel, B. VanHooydonck, J. J. Meyers, and D. J. Irschick. 2004. Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). Proceedings of the Royal Society of London Series B 271:2501-2508.

- Lamm, E.-T. 2007. Paleohistology widens the field of view in paleontology; pp. 50-51 in M. Marko, J. H. Scott, E. Vicenzi, S. Dekanich, J. Frafjord, P. Kotula, S. McKernan, and J. Shields (eds.), *Proceedings microscopy and microanalysis 2007*. Cambridge University Press, New York.
- Lee, A. H. 2004. Histological organization and its relationship to function in the femur of *Alligator mississippiensis*. *Journal of Anatomy* 204:197-207.
- Lee, A. H., and S. Werning. 2008. Sexual maturity in growing dinosaurs does not fit reptilian growth models. *Proceedings of the National Academy of Sciences, USA* 105:582-587.
- Li, P.-X., D.-Y. Su, Y.-G. Li, and J.-X. Yu. 1994. Age assignment of the *Lycopera*-bearing bed. *Acta Geologica Sinica-English Edition* 7:329-349.
- Lorenz, J. C., and W. Gavin. 1984. Geology of the Two Medicine Formation and the sedimentology of a dinosaur nesting ground. *Field Conference Northwestern Montana*.
- Lovich, J. E., C. H. Ernst, and J. F. McBreen. 1990. Growth, maturity, and sexual dimorphism in the wood turtle, *Clemmys insculpta*. *Canadian Journal of Zoology* 68:572-677.
- Ma, F. 1987. Review of *Lycopera davidi*. *Vertebrata Palasiatic* 25:8-19.
- Madsen, J. H. 1974. A new theropod dinosaur from the Upper Jurassic of Utah. *Journal of Paleontology* 48:27-31.
- Maleev, E. A. 1955. Giant carnivorous dinosaurs of Mongolia. *Doklady Akademii Nauka SSSR* 104:634-637.
- Mantell, G. A. 1850. On the Pelorosaurus; an undescribed gigantic terrestrial reptile whose remains are associated with those of the iguanodon and other saurians in the strata of Tilgate Forest, in Sussex. *Philosophical Transactions of the Royal Society of London* 140:379-390.
- Margerie, E. d. 2002. Laminar bone as an adaptation to torsional loads in flapping flight. *Journal of Anatomy* 201:521-526.
- Margerie, E. d., J.-P. Robin, D. Verrier, J. Cubo, R. Groscolas, and J. Castanet. 2004. Assessing a relationship between bone microstructure and growth rate: a fluorescent labelling study in the king penguin chick (*Aptenodytes patagonicus*). *The Journal of Experimental Biology* 207:869-879.

- Margottini, L. 2011. Is it time to declutter the dinosaur roster? *Science* 332:782.
- Martin, A. J. 2009. Dinosaur burrows in the Otway Group (Albian) of Victoria, Australia, and their relation to Cretaceous polar environments. *Cretaceous Research* 30:1223-1237.
- Maryanska, T., and H. Osmolska. 1979. Aspects of hadrosaurian cranial anatomy. *Lethaia* 12:265-273.
- Montes, L., J. Castanet, and J. Cubo. 2010. Relationship between bone growth rate and bone tissue organization in amniotes: first test of Amprino's rule in a phylogenetic context. *Animal Biology* 60:25-41.
- Moore, I. T., M. P. Lemaster, and R. T. Mason. 2000. Behavioural and hormonal responses to capture stress in the male red-sided garter snake, *Thamnophis sirtalis parietalis*. *Animal Behaviour* 59:529-534.
- Mullen, W. Fossil identified as mini-T.rex. *Chicago Tribune*, 18th September 2009. http://articles.chicagotribune.com/2009-09-18/news/0909170802_1_fossil-bones-tyrannosaurus-rex-chicago-paleontologist-paul-sereno. Retrieved 2011 March 8.
- Newbrey, M. G., A. M. Murray, D. B. Brinkman, M. V. H. Wilson, and A. G. Neuman. 2010. A new articulated freshwater fish (Clupeomorpha, Ellimmichthyiformes) from the Horseshoe Canyon Formation, Maastrichtian, of Alberta, Canada. *Canadian Journal of Earth Sciences* 47:1183-1196.
- Nopcsa, F. B., and E. Heidsieck. 1933. On the histology of the ribs in immature and half-grown trachodont dinosaurs. *Proceedings of the Zoological Society of London*:221-226.
- Olsson, M., and R. Shine. 1996. Does reproductive success increase with age or with size in species with indeterminate growth? A case study using sand lizards (*Lacerta agilis*). *Oecologia* 105:175-178.
- Osborn, H. F. 1905. *Tyrannosaurus* and other Cretaceous carnivorous dinosaurs. *Bulletin of the American Museum of Natural History* 21:259-265.
- Packard, G. C., T. J. Boardman, and G. F. Brichard. 2009. Allometric equations for predicting body mass of dinosaurs. *Journal of Zoology*:1-9.
- Padian, K., and J. R. Horner. 2002. Typology versus transformation in the origin of birds. *TRENDS in Ecology & Evolution* 17:120-124.

- Padian, K., and J. R. Horner. 2004. Dinosaur Physiology; pp. in D. B. Weishampel, P. Dodson, and H. Osmólska (eds.), *The Dinosauria*. University of California Press, Berkeley.
- Padian, K., A. d. Ricqlès, and J. R. Horner. 2001. Dinosaurian growth rates and bird origins. *Nature* 412:405-408.
- Padian, K., J. R. Horner, and A. d. Ricqlès. 2004. Growth in small dinosaurs and pterosaurs: the evolution of archosaurian growth strategies. *Journal of Vertebrate Paleontology* 24:555-571.
- Peabody, F. E. 1961. Annual growth zones in living and fossil vertebrates. *Journal of Morphology* 108:11-62.
- Ponton, F., A. Elzanowski, J. Castanet, A. Chinsamy, E. d. Margerie, A. d. Ricqlès, and J. Cubo. 2004. Variation of the outer circumferential layer in the limb bones of birds. *Acta Ornithologica* 39:21-24.
- Rauhut, O. W. M. 2003. A tyrannosauroid dinosaur from the Upper Jurassic of Portugal. *Palaeontology* 46:903-910.
- Reid, R. H. 1981. Lamellar-zonal bone with zones and annuli in the pelvis of a sauropod dinosaur. *Nature* 292:49-51.
- Reid, R. H. 1984. Primary bone and dinosaurian physiology. *Geological Magazine* 121:589-598.
- Reid, R. H. 1990. Zonal "growth rings" in dinosaurs. *Modern Geology* 15:19-48.
- Reisz, R. R., D. Scott, H.-D. Sues, D. C. Evans, and M. A. Raath. 2005. Embryos of an early Jurassic prosauropod dinosaur and their evolutionary significance. *Science* 309:761-764.
- Rich, T. H., and P. V. Rich. 1989. Polar dinosaurs and biotas of the Early Cretaceous of southeastern Australia. *National Geographic Research* 5:15-53.
- Rich, T. H., and P. Vickers-Rich. 1994. Neoceratopsians and ornithomimosaurs: dinosaurs of Gondwana origin? *National Geographic Research and Exploration* 10:129-131.
- Rich, T. H., and P. Vickers-Rich. 1999. The Hypsilophodontidae from Southeastern Australia; pp. 167-180 in Y. Tomida, T. H. Rich, and P. Vickers-Rich (eds.), *Proceedings of the Second Gondwanan Dinosaur Symposium*. National Science Museum Monographs, Tokyo.

- Rich, T. H., P. V. Rich, B. Wagstaff, J. McEwen-Mason, C. B. Douthitt, and R. T. Gregory. 1989. Early Cretaceous biota from the northern side of the Australo-Antarctic rift valley. *Geological Society Special Publications* 47:121-130.
- Ricqlès, A. d. 1968. Recherches paléohistologiques sur les os longs des tétrapodes I. - origine du tissu osseux plexiforme des dinosauriens sauropodes. *Annales de Paléontologie (Vertébrés)* 54:133-145 with one plate.
- Ricqlès, A. d. 1969. Recherches paléohistologiques sur les os longs des tétrapodes II. - quelques observations sur la structure des os longs des Thériodontes. *Annales de Paléontologie (Vertébrés)* 55:3-52 with eight plates.
- Ricqlès, A. d. 1972. Recherches paléohistologiques sur les os longs des tétrapodes III. - Titanosuchiens, Dinocéphales et Dicynodontes. *Annales de Paléontologie (Vertébrés)* 58:17-60 with eight plates.
- Ricqlès, A. d. 1974a. Recherches paléohistologiques sur les os longs des tétrapodes IV. - Éothériodontes et Pélycosaures. *Annales de Paléontologie (Vertébrés)* 60:3-39 with eight plates.
- Ricqlès, A. d. 1974b. Recherches paléohistologiques sur les os longs des tétrapodes V. - Cotylosaures et Mésosaures. *Annales de Paléontologie (Vertébrés)* 60:171-216 with seven plates.
- Ricqlès, A. d. 1974c. Evolution of endothermy: histological evidence. *Evolutionary Theory* 1:51-80.
- Ricqlès, A. d. 1975. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (première partie.). *Annales de Paléontologie (Vertébrés)* 61:51-129 with nine plates.
- Ricqlès, A. d. 1976. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux de tétrapodes (deuxième partie.). *Annales de Paléontologie (Vertébrés)* 62:71-119 with four plates.
- Ricqlès, A. d. 1977a. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (deuxième partie, suite.). *Annales de Paléontologie (Vertébrés)* 63:33-55.
- Ricqlès, A. d. 1977b. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux

- des tétrapodes (deuxième partie, fin.). *Annales de Paléontologie (Vertébrés)* 63:133-159.
- Ricqlès, A. d. 1978a. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (troisième partie.). *Annales de Paléontologie (Vertébrés)* 64:85-111.
- Ricqlès, A. d. 1978b. Recherches paléohistologiques sur les os longs des tétrapodes VII. - sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes (troisième partie, fin.). *Annales de Paléontologie (Vertébrés)* 64:153-184 with six plates.
- Ricqlès, A. d. 1980. Tissue structures of dinosaur bone: functional significance and possible relation to dinosaur physiology; pp. 103-138 in R. D. K. Thomas and E. C. Olson (eds.), *A Cold Look at the Warm Blooded Dinosaurs*. Westview Press, Boulder.
- Ricqlès, A. d. 1983. Cyclical growth in the long limb bones of a sauropod dinosaur. *Acta Palaeontologica Polonica* 28:225-232.
- Ricqlès, A. d., K. Padian, and J. R. Horner. 2001: The bone histology of basal birds in phylogenetic and ontogenetic perspectives. *New Perspectives on the Origin and Early Evolution of Birds: Proceedings of the International Symposium in Honor of John H. Ostrom*.
- Ricqlès, A. d., K. Padian, and J. R. Horner. 2003. On the bone histology of some Triassic pseudosuchian archosaurs and related taxa. *Annales de Paléontologie* 89:67-101.
- Ricqlès, A. d., F. J. Meunier, J. Castanet, and H. Francillon-Vieillot. 1991. Comparative microstructure of bone; pp. 1-78 in B. K. Hall (ed.), *Bone: Bone Matrix and Bone Specific Products*. CRC Press, Boca Raton.
- Ricqlès, A. d., K. Padian, F. Knoll, and J. R. Horner. 2008. On the origin of high growth rates in archosaurs and their ancient relatives: complementary histological studies on Triassic archosauriforms and the problem of a "phylogenetic signal" in bone histology. *Annales de Paléontologie* 94:57-76.
- Rogers, R. R. 1990. Taphonomy of three dinosaur bone beds in the Upper Cretaceous Two Medicine Formation of northwestern Montana: evidence for drought-related mortality. *Palaios* 5:394-413.
- Rozhdestvensky, A. K. 1965. Growth changes in Asian dinosaurs and some problems of their taxonomy. *Paleontologicheskii Zhurnal* 3:95-109.

- Russell, D. A. 1970. Tyrannosaurs from the Late Cretaceous of western Canada. National Museums of Canada Publications in Paleontology 1:1-34.
- Ryan, M. J., and D. C. Evans. 2005. Ornithischian dinosaurs; pp. 312-348 in P. J. Currie and E. B. Koppelhaus (eds.), Dinosaur Provincial Park. Indiana University Press, Bloomington.
- Ryan, M. J., and A. P. Russell. 2005. A new centrosaurine ceratopsid from the Oldman Formation of Alberta and its implications for centrosaurine taxonomy and systematics. Canadian Journal of Earth Sciences 42:1369-1387.
- Ryan, M. J., A. P. Russell, D. A. Eberth, and P. J. Currie. 2001. The taphonomy of a Centrosaurus (Ornithischia: Ceratopsidae) bone bed from the Dinosaur Park Formation (Upper Campanian), Alberta, Canada, with comments on cranial ontogeny. Palaios 16:482-506.
- Sampson, S. D. 1995. Two new horned dinosaurs from the Upper Cretaceous Two Medicine Formation of Montana; with a phylogenetic analysis of Centrosaurinae (Ornithischia: Ceratopsidae). Journal of Vertebrate Paleontology 15:743-760.
- Sampson, S. D., M. J. Ryan, and D. Tanke. 1997. Craniofacial ontogeny in centrosaurine dinosaurs (Ornithischia: Ceratopsidae): taxonomic and behavioral implications. Zoological Journal of the Linnean Society 121:293-337.
- Sander, P. M., and N. Klein. 2005. Developmental plasticity in the life history of a prosauropod dinosaur. Science 310:1800-1802.
- Sander, P. M., and P. Andr  ssy. 2006. Lines of arrested growth and long bone histology in Pleistocene large mammals from Germany: what do they tell us about dinosaur physiology? Palaeontographica A 277:143-159.
- Scannella, J. B., and D. W. Fowler. 2009. Anagenesis in *Triceratops*: evidence from a newly resolved stratigraphic framework for the Hell Creek Formation. Cincinnati Museum Center Science Contributions.
- Scannella, J. B., and J. R. Horner. 2010. *Torosaurus* Marsh, 1891, is *Triceratops* Marsh, 1889 (Ceratopsidae: Chasmosaurinae): synonymy through ontogeny. Journal of Vertebrate Paleontology 30:1157-1168.
- Schweitzer, M. H., R. M. Elsey, C. G. Dacke, J. R. Horner, and E.-T. Lamm. 2007. Do egg-laying crocodilian (*Alligator mississippiensis*) archosaurs form medullary bone? Bone 40:1152-1158.

- Seitz, A. L. 1907. Vergleichende Studien über den mikroskopischen Knochenbau fossiler und rezenter Reptilien, Nova Acta, Volume 87. Abhandlungen der Kaiserlichen Leopold-Carolignischen deutschen Akademie der Naturforscher, 230-370 pp.
- Sereno, P. C., L. Tan, S. L. Brusatte, H. J. Kriegstein, X. Zhao, and K. Cloward. 2009. Tyrannosaurid skeletal design first evolved at small body size. *Science* 326:418-422.
- Snoover, M. L., and A. A. Hohn. 2004. Validation and interpretation of annual skeletal marks in loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles. *Fishery Bulletin* 102:682-692.
- Sparkman, A. M., S. J. Arnold, and A. M. Bronikowski. 2007. An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the garter snake *Thamnophis elegans*. *Proceedings of the Royal Society of London Series B* 274:943-950.
- Spicer, R. A., and R. M. Corfield. 1992. A review of terrestrial and marine climates in the Cretaceous with implications for modelling the 'Greenhouse Earth'. *Geological Magazine* 129:169-180.
- Stamps, J. A. 2007. Growth-mortality tradeoffs and 'personality traits' in animals. *Ecology Letters* 10:355-363.
- Starck, J. M., and A. Chinsamy. 2002. Bone microstructure and developmental plasticity in birds and other dinosaurs. *Journal of Morphology* 254:232-246.
- Tanke, D. H. 2005. Identifying lost quarries; pp. 34-53 in P. J. Currie and E. B. Koppelhaus (eds.), *Dinosaur Provincial Park*. Indiana University Press, Bloomington.
- Trexler, D. 2001. Two Medicine Formation, Montana: Geology and fauna; pp. 298-309 in D. H. Tanke and K. Carpenter (eds.), *Mesozoic Vertebrate Life*. Indiana University Press, Indianapolis.
- Trueman, C. L. 1999. Rare earth element geochemistry and taphonomy of terrestrial vertebrate assemblages. *Palaios* 14:555-568.
- Trueman, C. N., and M. J. Benton. 1997. A geochemical method to trace the taphonomic history of reworked bones in sedimentary settings. *Geology* 25:263-266.
- Trueman, C. N., A. K. Behrensmeyer, R. Potts, and N. Tuross. 2006. High-resolution records of location and stratigraphic provenance from the rare earth element composition of fossil bones. *Geochimica et Cosmochimica Acta* 70:4343-4355.

- Trusler, P., P. Vickers-Rich, and T. H. Rich. 2010. The artist and the scientists: bringing prehistory to life. Cambridge University Press, Melbourne, 308 pp.
- Tsuihiji, T., M. Watabe, K. Tsogtbaatar, R. Barsbold, S. Suzuki, A. H. Lee, R. C. Ridgely, Y. Kawahara, and L. M. Witmer. 2011. Cranial osteology of a juvenile specimen of *Tarbosaurus bataar* from the Nemegt Formation (Upper Cretaceous) of Bugin Tsav, Mongolia. *Journal of Vertebrate Paleontology* 31:497-517.
- Tucker, A. D. 1997. Validation of skeletochronology to determine age of freshwater crocodiles (*Crocodylus johnstoni*). *Australian Journal of Marine and Freshwater Research* 48:343-351.
- Tumarkin-Deratzian, A. R. 2007. Fibrolamellar bone in wild adult *Alligator mississippiensis*. *Journal of Herpetology* 41:341-345.
- Turvey, S. T., O. R. Green, and R. Holdaway. 2005. Cortical growth marks reveal extended juvenile development in New Zealand moa. *Nature* 435:940-943.
- Tutken, T., H.-U. Pfretzschner, T. W. Vennemann, G. Sun, and Y. D. Wang. 2004. Paleobiology and skeletochronology of Jurassic dinosaurs: implications from the histology and oxygen isotope compositions of bone. *Palaeogeography, Palaeoclimatology, Palaeoecology* 206:217-238.
- Varricchio, D. J. 1993. Bone microstructure of the Upper Cretaceous theropod dinosaur *Troodon formosus*. *Journal of Vertebrate Paleontology* 13:99-104.
- Veevers, J. J., C. M. Powell, and S. B. Roots. 1991. Review of seafloor spreading around Australia, I. Synthesis of the patterns of spreading. *Australian Journal of Earth Science* 38:373-389.
- Wagstaff, W. E., and J. McEwen-Mason. 1989. Palynological dating of Lower Cretaceous coastal vertebrate localities, Victoria, Australia. *National Geographic Research* 5:54-63.
- Wedel, M. J. 2003. The evolution of vertebral pneumaticity in sauropod dinosaurs. *Journal of Vertebrate Paleontology* 23:344-357.
- Weishampel, D. B., D. E. Fastovsky, M. Watabe, D. Varricchio, F. Jackson, K. Tsogtbaatar, and R. Barsbold. 2008. New oviraptorid embryos from Bugin-Tsav, Nemegt Formation (Upper Cretaceous), Mongolia, with insights into their habitat and growth. *Journal of Vertebrate Paleontology* 28:1110-1119.

- Whitlock, J. A., J. A. Wilson, and M. C. Lamanna. 2010. Description of a nearly complete juvenile skull of *Diplodocus* (Sauropoda: Diplodocoidea) from the Late Jurassic of North America. *Journal of Vertebrate Paleontology* 30:442-457.
- Wilkinson, P. M. 2008: American Alligator growth: determinate or indeterminate? Proceedings of the 19th Working Meeting of the IUCN-SSC Crocodile Specialist Group, Santa Cruz, Bolivia.
- Wilkinson, P. M., and W. E. Rhodes. 1997. Growth rates of American alligators in coastal South Carolina. *The Journal of Wildlife Management* 61:397-402.
- Witmer, L. M. 1995. The extant phylogenetic bracket and the importance of reconstructing soft tissues in fossils; pp. 296 in J. Thomason (ed.), *Functional morphology in vertebrate paleontology*. Cambridge University Press.
- Woodward, H. N., and T. M. Lehman. 2009. Bone histology and microanatomy of *Alamosaurus sanjuanensis* (Sauropoda: Titanosauria) from the Maastrichtian of Big Bend National Park, Texas. *Journal of Vertebrate Paleontology* 29:807-821.
- Woodward, H. N., J. R. Horner, and J. O. Farlow. 2011. Osteohistological evidence for determinate growth in the American alligator. *Journal of Herpetology* 45:339-342.
- Woodward, H. N., J. R. Horner, and J. O. Farlow. *In prep.* Intra-skeletal histovariability in *Alligator mississippiensis* and its applications for non-avian dinosaur osteohistology.
- Xu, X., M. A. Norell, X. Kuang, X. Wang, Q. Zhao, and C. Jia. 2004. Basal tyrannosauroids from China and evidence for protofeathers in tyrannosauroids. *Nature* 431:680-684.
- Xu, X., C. A. Forster, M. A. Norell, G. M. Erickson, D. A. Eberth, C. Jia, and Q. Zhao. 2006. A basal tyrannosauroid dinosaur from the Late Jurassic of China. *Nature* 439:715-718.
- Zaragüeta, B. 2004. Basal clupeomorphs and ellimmichthyiform phylogeny; pp. 391-404 in G. Arratia and A. Tintori (eds.), *Mesozoic Fishes 3 - Systematics, Paleoenvironments, and Biodiversity*. Verlag Dr. Friedrich Pfeil, Munich.
- Zhang, J.-Y. 2002. A new species of *Lycoptera* from Liaoning, China. *Vertebrata Palasiatic* 40:257-266.