

OSTEOHISTOLOGY OF SUTURAL FUSION IN THE SKULLS OF ARCHOSAURS:
IMPLICATIONS FOR MATURITY ASSESSMENT IN NON-AVIAN DINOSAURS
AND FOR THE EVOLUTION OF SKELETAL TISSUES

by

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ABSTRACT

Sutures are fibrous tissues that unite the skull bones of vertebrates. The degree of sutural closure is often used in paleontology to assess maturity in mammals and dinosaurs. Surprisingly, little is known about the biology of sutures in the closest evolutionary groups to non-avian dinosaurs: birds and crocodilians (extant archosaurs). The purpose of this dissertation is to assess, by means of morphological observations, if the degree of sutural closure is indeed an accurate method for maturity assessment in non-avian dinosaurs, and to gain a better understanding of archosaurian sutures at the microscopic scale.

The order in which sutures fuse in the skulls of emus ($n=24$) and American alligators ($n=50$) reveals that sutural closure is a useful proxy for maturity in the former species but not in the latter. As growth progresses in alligators, sutures become relatively wider and more open in larger, older individuals compared to smaller, younger specimens. This pattern is previously unreported in alligators and it likely reflects skull mechanics related to feeding and not exclusively ontogeny. This indicates sutural closure is not a robust proxy for maturity in non-avian dinosaurs.

Next, the histology of craniofacial sutures in these same extant species and in some non-avian dinosaurs is studied. Comparisons with mammalian sutural histology from the literature are also made. Emus and mammals possess a sutural periosteum, but it disappears rapidly during ontogeny in American alligators. The histology of the sutural mineralized tissues of non-avian dinosaurs suggest that they also lack a sutural periosteum and that their primary mode of ossification involves the direct mineralization of the fibrous soft-tissues of their sutures. The microstructural differences between sutures in archosaurs and mammals are undeniable, indicating that extant mammals are a poor analogue for investigating the growth of non-avian dinosaurs.

Finally, the sutural borders of embryonic and nestling hadrosaurs are investigated. They are composed of chondroid bone, a tissue that allows rapid sutural growth in some extant species, but these sutural mineralized fronts lack secondary cartilage. Instead, secondary cartilage was only observed at jaw articulations. This tissue is found exclusively in birds within extant sauropsids, reflecting the dinosaurian origin of birds.

CHAPTER ONE

INTRODUCTION

“We have studied so little of what constitutes skeletogenesis in the animal kingdom, expecting all vertebrates to conform to the mammalian pattern discussed in most textbooks” Brian K. Hall, 2005.

Maturity assessment is a very important matter in vertebrate paleontology (Brochu, 1996; Fowler et al., 2011; Irmis, 2007; Maisano, 2002; Scannella and Horner, 2010). It is crucial to know whether variation between specimens is ontogenetic or phylogenetic in order to have the most accurate estimation of species diversity through time, and to use specimens of appropriate ontogenetic stages in phylogenetic analyses (i.e., skeletally mature individuals, Irmis, 2007). The sub-field of paleohistology has been contributing greatly to dinosaur paleontology for the last 30 years. Indeed, paleohistology provides an accurate method for the maturity assessment of non-avian dinosaur specimens. This method involves histological sectioning and analysis of limb bone elements (e.g., Horner et al., 2000; Padian et al., 2001; Woodward-Ballard, 2012). Limb bones are the best bones in the tetrapod body to assess maturity because they undergo less modeling and cortical drift than other skeletal elements (such as skull or jaw bones). Although it is subject to some debates, as of today, paleohistological analyses provide the most robust and accurate estimations of maturity in non-avian dinosaurs. Other maturity assessment methods (that do not involve paleohistological examinations) exist and are

extensively used nowadays. One of these methods uses the degree of sutural closure (also known as sutural fusion).

Sutures are the fibrous tissues found between the skull bones of vertebrates (Herring, 2000; Kokich, 1986; Opperman, 2000). Sutures are not joints because they lack an articular cavity. The joints found in the skull-base are known as synchondroses (which are cartilaginous instead of fibrous, Marieb, 2014). However, in paleontology, the term ‘suture’ is often very general and also (incorrectly) designates the cartilaginous articulations of the skull base.

Generally, the sutures of extant mammal embryos, babies and juveniles are open, and they progressively close as the animals mature into adults (Cole et al., 2003; Herring, 2000). This progressive closure has been used for decades to assess the maturity of fossil mammals (e.g., Black et al., 2010; Marsh, 1887; Sanchez-Villagra, 2010) and non-avian dinosaurs (e.g., Bakker and Williams, 1988; Rauhut, 2004; Sereno et al., 2009; Yates, 2003). However, **mammals are not the correct extant taxon for comparison with non-avian dinosaurs**, and this method has never been tested in the extant phylogenetic bracket (EPB) of the Dinosauria (Witmer, 1995). The EPB of non-avian dinosaurs are birds and crocodilians, also referred to as extant archosaurs. Since progressive sutural closure has been essentially observed in mammals and not in the closest extant relatives of dinosaurs, it appears erroneous to assume that this condition was present in the Dinosauria. Mammals and dinosaurs belong to clades that are very distant phylogenetically and the cranial mechanics of these two groups are undeniably different.

Therefore, the purpose of this dissertation is to assess, by means of morphological and histological observations, if the degree of sutural closure (i.e., progressive sutural closure through ontogeny) is indeed an accurate method for maturity assessment in non-avian dinosaurs. This was done by 1) examining the sequences of sutural closure (i.e., the order in which sutures close during ontogeny) in the skulls of some extant archosaurs, and 2) by examining the histology of sutures in both extant and fossil archosaurs, including non-avian dinosaurs.

In Chapter Two, the sequences of sutural closure in the emu, *Dromaius novaehollandiae*, and the American alligator, *Alligator mississippiensis* are determined. Dozens of dry skulls (forming two growth series) were analyzed morphologically for each species. The timings of sutural closure were also plotted on each sequence (i.e., the onset of sexual and skeletal maturity). The main objective of this chapter was to test whether young specimens indeed showed open sutures and more mature specimens showed closed sutures. It is the only chapter that does not present microstructural analyses.

Chapter Three examines the sutural histology of these same extant species (the emu and the American alligator). The ontogeny of some cranial and facial sutures, and some skull-base synchondroses were retraced and after understanding how these structures formed and grew in these extant species some non-avian dinosaur sutures and synchondroses were analyzed. Modes of formation and growth were inferred in these fossils based on the results provided by the extant archosaurs. Finally, archosaurian sutural histology was compared to mammalian sutural histology (i.e., the only point of

comparison available in the literature). The major aims of this chapter were to 1) gain an understanding of archosaurian suture biology from a histological perspective, and 2) test whether the microstructure of archosaurian and mammalian sutures are comparable (and whether it is right to assume that the sutures of these two groups form and grow similarly). If these two chapters show that sutural closure is not a good indicator for maturity in the Dinosauria, all previous maturity assessments of non-avian dinosaur specimens based on the degree of closure of their sutures should be reconsidered.

The last three chapters deal with histological analyses (Chapters Four and Five) and microradiographic examinations (Chapter Six) of sutures (but also of tooth insertion sites and other areas of the skull and jaws) of non-avian dinosaur specimens of early ontogenetic stages: hatchlings and embryos. The goals of these chapters were to investigate the presence (or absence) of some sutural mineralized tissues, secondary cartilage and chondroid bone, that have been found in the skull of their closest extant relatives (in embryos and juveniles only). Secondary cartilage is a tissue that originates secondarily from the periosteum after the formation of skull bones. Within the Sauropsida, this tissue is exclusively found in birds. Therefore, finding this tissue in non-avian dinosaurs would further cement the dinosaurian origins of birds (see Chapters Four and Five). Chondroid bone is an intermediate tissue between bone and cartilage that grows extremely rapidly at the sutural borders of many vertebrates. The discovery of chondroid bone in a non-avian dinosaur would indicate that it is more abundant in the Vertebrata than previously thought (see Chapter Six). Even though these three chapters do not directly assess whether sutural closure is an adequate proxy for maturity in the

Dinosauria, they are important to understanding the early stages of dinosaurian sutural histology, to understanding the mechanisms of suture closure in non-avian dinosaurs and to provide insights on the evolution of skeletal tissues within the Archosauria.

In summary, this dissertation aims to 1) assess whether the degree of sutural closure is a good proxy for maturity in extant archosaurs, and by analogy in non-avian dinosaurs, 2) gain an understanding of the general biology and histology of archosaurian sutures (and how they compare to those of mammals), 3) retrace the evolution of sutural skeletal tissues within the Archosauria. Before revealing the results of this dissertation, a brief account on the history of studies reporting sequences of sutural closure and sutural histology follows.

Background and Literature Review

General Biology of Sutures

According to Persson (1973), the main biological functions of sutures are to unite bones, to act as areas of growth and to absorb mechanical stress in order to protect osteogenic tissues. The fibrous tissues of sutures are known as the sutural ligament (Kokich, 1986). It is often compared to the periodontal ligament that anchors teeth in alveolar bone, or the periosteal membrane that covers intramembranous bones (Enlow, 1990). While sutures are patent (i.e., open and unossified), they function as compensatory growth sites during craniofacial development, i.e., they allow rapid bone formation at the edges of the bone fronts in response to their soft-tissue environment (mostly the expanding brain in mammals, Kokich, 1986). In other words, bone deposition and

resorption at the sutural margins is not a primary growth force of the skull, but it is secondary (or adaptive). Obliteration may occur by fusion of bone fronts across the suture (Opperman, 2000).

Ontogenetic Sequences of Sutural Closure

Sequences in Mammals: Ontogenetic sequences (or ontogenetic patterns) of sutural closure have been reported exclusively in mammals since the end of the 1800s. Sequences are assessed systematically with a high number of dry skulls of unknown age and sex in almost all cases (see Wang et al., 2006 for discussion). The relative ontogeny of these dry specimens is assessed by size and/or the degree of tooth wear. The first ontogenetic sequence published was that of humans (Dwight, 1890; Todd and Lyon, 1925). It was shortly followed by comparisons with primates (Krogman, 1930) and monkeys (Chopra, 1957). Kokich summarized the studies concerning humans and made a ‘consensus’ sequence (Kokich, 1986, table 4-1 p. 94). He stated that cranial sutures close at relatively young ages, while facial sutures remain open until late in life (for e.g., the zygomaticomaxillary, frontomaxillary, and frontonasal sutures may not fuse until the 7th decade of life). Sequences have also been investigated in hyenas (Schweikher, 1930), pigs (Herring, 1972; Herring, 1974), fur seals and sea lions (Brunner et al., 2004), pteropodid bats (Giannini et al., 2006), the florida manatee (Hoson et al., 2009), hystricognath rodents (Wilson and Sánchez-Villagra, 2009), a fossil marsupial (Black et al., 2010), cetarciodactyls (Bärmann and Sánchez-Villagra, 2012; Sánchez-Villagra, 2010), other primates (Cray et al., 2008; Flores and Barone, 2012; Wang et al., 2006) and

carnivores (Goswami et al., 2013). The order in which groups of sutures close is generally well conserved across these mammals, and the sequence is simplified as: vault, base, circum-meatal, palatal, facial and craniofacial (established by Schweikher, 1930 and Krogman, 1930). This pattern is however not invariable (e.g., Herring, 1972) and sometimes intra and interspecific variations are high (e.g., Wilson and Sanchez-Villagra, 2009). The main aim of these studies is to decipher phylogenetic patterns within and between clades. Single outliers or different patterns between clades are usually explained by ecological adaptations and/or heterochronies (e.g., Bärmann and Sanchez-Villagra, 2012; Goswami et al., 2013). Other authors investigate the sequence of sutural closure to assess maturity in mammals of unknown age caught in the wild. In general, multiple methods are tested in these studies (for e.g., the count of cementum annuli, the degree of tooth wear) in addition to the degree of sutural closure (see the review by Schroeder et al., 2005). The results are mixed: in some species, a few sutures (usually one or two) may help placing animals in age categories. It is the case for the cottontail (Hoffmeister and Zimmerman, 1967) and the raccoon (Junge and Hoffmeister, 1980). However, in other cases, even though it is subject to some debates, sutural closure is so variable within one same species that it is not reliable for age estimation, e.g., in gray wolves, foxes and humans (Landon et al., 1998; Sahni and Jit, 2005; Schroeder et al., 2005).

Sequences in Archosaurs: Outside of this mammalian monopoly, very little is known about skull sutures in sauropsids (but see Maisano 2002), and specifically in archosaurs. In birds, no complete sequence has ever been investigated (but see the emu sequence in Chapter Two). Only Jollie (1957) and Marshall (2000) have made some

observations in chickens and ostriches respectively. In chickens, fusion begins in the occipital region at 75 days of age and becomes complete at about 100 days (when skeletal maturity is attained). In ostriches, 4 sutures in the skull roof fuse by the time of sexual maturity (at about 3 years of age). Concerning the neurocentral synchondroses (between the neural arch and the vertebral centrum), Starck (1993) found that they were all closed at hatching for precocial birds, but open at this same time in altricial birds (with a cranio-caudal post-hatching pattern). In crocodilians, no sequence has been reported in the skull either (but see the American alligator sequence in Chapter Two). However the closure of neurocentral synchondroses was studied through ontogeny in different species of crocodilians by Brochu (1996). He found that closure follows a distinct caudal-to-cranial sequence (opposite to that of altricial birds). This study is a pioneer for the assessment of maturity in fossils using sutural closure as a proxy. However, like Irmis mentioned (Irmis, 2007), this sequence was not calibrated using absolute ages of individuals, sexual or skeletal maturity. This was partly done in a more recent study by Ikejiri (2012) who found that before sexual maturity, completely closed neurocentral synchondroses were restricted to the caudal vertebrae. In non-avian dinosaurs, relatively complete sequences of sutural closure have only been reported in the skulls of three species: *Tyrannosaurus* (Carr and Williamson, 2004), *Triceratops* (Longrich and Field, 2012) and *Centrosaurus* (Frederickson and Tumarkin-Deratzian, 2014).

Sutural Histology

Histological studies describing sutures and their ontogeny are surprisingly rare (Herring, 2000). Moreover, there is no literature on the comparative histology of sutures

within vertebrates, only mammalian sutures have been described (Herring, 2000) (but for the histology of the gekkotan fronto-parietal suture, see Payne et al., 2011). The microscopic structure of craniofacial sutures through ontogeny is known in humans (Kokich, 1976; Koskinen et al., 1976; Latham, 1971; Miroue and Rosenberg, 1975; Persson and Thilander, 1977; Sitsen, 1933) and some laboratory mammals, such as rats and rabbits (Moss, 1958; Persson, 1973; Persson and Roy, 1979; Persson et al., 1978; Pritchard et al., 1956). Only a few of these studies follow precisely the postnatal development of a suture until its fusion (see discussion in Cohen, 1993). Within the last 30 years, there has been a lack of studies describing age-related histological changes in sutures and sutural bone. Only two studies mention some changes through ontogeny, but although they use histological procedures, documenting age-related histological changes is not their main focus (Knaup et al., 2004; Sun et al., 2004).

Sutures are composed of (1) the periosteum of two separated bones, (each having a cambial layer containing collagenous fibers, osteoprogenitor cells and osteoblasts, and a rarely observed capsular layer composed mostly of collagenous fibers, small blood vessels, and fibroblasts) and (2) a middle layer that is non-osteogenic and composed mostly of collagenous fibers, mesenchymal cells and fibroblasts (Pritchard et al. 1956, Persson 1973). They are encapsulated in dense fibrous connective tissues called uniting layers (Pritchard et al. 1956). The borders of the bone in contact with a suture have been described by various terms such as “juxta-sutural bone” (Enlow 1990), “sutural bony margins” (Kokich 1986) or “sutural bone fronts” (Opperman 2000). Extensive details

about sutural (and synchondroseal) histology through ontogeny are exposed in Chapter Three.

Special Calcified Tissues in Sutural Areas:

Secondary Cartilage and Chondroid Bone: Secondary cartilage and chondroid bone (or chondroid tissue) are two calcified tissues found throughout the skulls of mammals and birds. The former arises secondarily (i.e., after bone formation) on pre-existing membrane bones, and is therefore called secondary cartilage (Hall, 2005), and the latter is an intermediate tissue between bone and cartilage. Secondary cartilage and chondroid bone are often found together in various areas of the skull, including sutures. Lengelé et al., (1996) showed that they were both full autonomous tissue entities, able to arise independently from the periosteal mesenchymal stem cells, from different biomechanical inductions (Lengelé, 1997). Chondroid bone is induced by tension and therefore is present mostly in the sutural areas (e.g., Manzanares et al., 1988). Secondary cartilage is induced by compression and shear movements and therefore is mostly present at joints and articulations (Hall, 1967). Ontogenetically, these two tissues disappear rapidly by resorption and/or replacement by bone, but they may arise again after hatching/birth if mechanical forces are still acting. The last two sections of this introduction deal with these two sutural mineralized tissues.

Secondary Cartilage: Secondary (or adventitious) cartilage has been reported in the skull articulations and muscle insertions of extant birds (e.g., Bock, 1960; Hall, 1967; Hall, 1968; Lengelé et al., 1996a; Murray and Smiles, 1965; Murray, 1963), mammals (e.g., Goret-Nicaise et al., 1984; Moss, 1958; Vinkka-Puhakka, 1991) and teleosts

(e.g., Benjamin 1989, Witten and Hall 2002, Gillis et al 2006). It arises in order to accommodate mechanical stress. Sutural secondary cartilage (i.e., found directly within cranio-facial sutures) has essentially been reported in mammals (i.e., humans, rats, mice, rabbits, cats and hamsters). In humans, the first report is that of Sitsen (1933) in the lambdoid suture (parieto-occipital) of infants under 6 months old. Later, it was reported in the sagittal (interparietal) and mid-palatal sutures of fetuses (Pritchard et al., 1956), in the premaxillary-vomerine suture in 9 month-old infant with a cleft palate (Friede, 1973), in other various facial sutures (Persson, 1973), in the lambdoid suture (Hinton et al., 1984, echoing the first study by Sitsen, 1933), and in the metopic suture (interfrontal; Manzanares et al., 1988). In experimental animals, secondary cartilage was found in rats in the interfrontal suture (Moss, 1958; Vinkka, 1982), in the intermaxillary suture (Mohammed, 1957; Persson, 1973), in the mid-palatal suture (Pritchard et al., 1956), and in the fronto-parietal suture (Markens, 1975). It was also found in the mid-palatal suture of post-natal mice and cats (Bloore et al., 1969; Griffiths et al., 1967; Linder-Aronson and Larsson, 1965). More recently, it was found in the intermaxillary suture of hamsters before 25 days post-natal (Vinkka-Puhakka, 1991). Regardless of where it forms (sutures, articulations or muscle insertions) this mineralized tissue is important because it carries a phylogenetic signal. As of today, the most parcimonious interpretation states that the secondary cartilages found in birds, mammals and teleosts are not homologous and arose three times independently (Hall, 2000). Secondary cartilage is not found in extant lissamphibians nor in non-avian sauropsids (Hall, 2000; Hall and Hanken, 1985; Irwin and Ferguson, 1986; Vickaryous and Hall, 2008). Therefore, within the clade including

Lissamphibia and Sauropsida, this tissue appears to be restricted to Aves. In other words, it is an avian tissue, hence the term ‘avian secondary cartilage’ (Hall, 2000; see Chapters Four and Five for further elaboration).

Chondroid Bone: Chondroid tissue is a term proposed by Goret-Nicaise and Dhem (1982), but it was previously known and referred to as “chondroid bone” (Beresford, 1981; Schaffer, 1888; Vickaryous and Hall, 2008). Chondroid bone has cartilage-like rounded cells that are closely packed together in clusters in a bone like matrix (Lengelé et al., 1996b). It is the privileged vector for growth in the sutures, where mechanical stresses result from the rapid expansion of the underlying brain (Lengelé, 1997; Lengelé et al., 1990; Manzanares et al., 1988). The first study to report the presence of chondroid tissue in a suture was that of Dhem et al., (1983). They found some in the ‘normal’ metopic suture of a 9 month-old infant, and in the fronto-parietal suture of an 8 month-old infant that had suffered from brachycephaly. Goret-Nicaise et al., (1988) reported that it was present in all cranial sutures, from 20 weeks of gestation embryos until at least 9 months post-natal. They concluded that it was the driving force for longitudinal sutural growth (i.e., bone lengthening at the sutural borders). The same year, Manzanares et al., (1988) investigated the role of chondroid bone in sutural closure and they found that fusion involves the formation of chondroid bone bridges across the metopic sutures of 6-month-old infants. In a more recent study, it was found in all the cranio-facial sutural edges in chick embryos (quail-chick chimeras) at the 9th, 12th, and 14th day of incubation (Lengelé et al., 1996b). More recently, Rafferty and Herring (1999) found this tissue in the nasofrontal suture of 4 to 6 month-old miniature pigs. They

hypothesized that the presence of this tissue may explain the ability of this suture to grow rapidly, despite high compressive loadings. “Chondroid tissue” was recently described during the formation of the gekkotan notochord (Jonasson et al., 2012). However, its mode of formation (differentiating from the chordoid tissue) seems to be very different from that of ‘chondroid tissue’ *sensu* Goret-Nicaise and Dhem (1982).

From a phylogenetic perspective, chondroid bone seems to be widely distributed in extant vertebrates (Beresford, 1981). Besides from mammals and birds, it had been found in agnathans (Ørvig, 1951) teleosteans (Gillis et al., 2006; Huysseune and Verraes, 1986; Taylor et al., 1994), and more recently in the skull of *Alligator mississippiensis* (Vickaryous and Hall, 2008). Since it is present in the EPB of the Dinosauria, it is likely that it was present in the sutural borders of non-avian dinosaurs (see Chapter Six).

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CHAPTER TWO

ONTOGENY OF SUTURAL CLOSURE IN THE SKULLS OF EXTANT ARCHOSAURS: RECONSIDERING MATURITY ASSESSMENT IN NON-AVIAN DINOSAURS

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

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Contributions: Conceived the study, analyzed data, interpreted results and edited early versions of the manuscript.

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Contributions: Provided funding and access to specimens. Assisted with study design and discussed implications of results.

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Abstract

Sutures are the fibrous tissues uniting the bones in the skulls of vertebrates. It is generally observed that vertebrate sutures are open early in ontogeny (i.e., the entire life span) and progressively close as maturity is attained. This trend has been used for decades in paleontology to assess maturity in non-avian dinosaurs. However, since it is not known if skull sutures are indicators of maturity in extant archosaurs, which are the closest extant relatives of non-avian dinosaurs, it cannot be assumed that suture closure can be employed as proxies of maturity in these fossils. Therefore, in the present study, we investigate the sequence and degree of sutural closure in the skulls of two extant archosaurs: the emu, *Dromaius novaehollandiae* (n=24) and the American alligator, *Alligator mississippiensis* (n=50). By means of a modified cladistic analysis (with characters describing sutural closure and interdigitation) we report that sutural closure is a good proxy for maturity in *D. novaehollandiae* (42 characters), but not in *A. mississippiensis* (80 characters). Almost all the sutures in the skull of *D. novaehollandiae* progressively obliterate through ontogeny (15 out of 20 sutures) and the obliteration of some sutures can be used as benchmarks for sexual and skeletal maturity. In *A. mississippiensis*, only two sutures out of 36 obliterate completely (the interfrontal and interparietal sutures), and they do so during embryonic development. Therefore, they cannot be used as benchmarks for sexual or skeletal maturity in this species. Moreover, as maturity progresses in alligators, sutures become wider, and appear more open in large, old individuals than in smaller, younger individuals. We hypothesize that the pattern observed in American alligators does not reflect ontogeny, but instead, accommodates the

increasing amounts of stress received by the skull during feeding. This study suggests that the use of sutural closure as a proxy for maturity in non-avian dinosaurs should be carefully reconsidered, and even avoided in some instances. Limb bone histology remains the most accurate method to assess maturity in the Dinosauria.

Keywords: Sutures, Skull, Braincase, Maturity assessment, Epigenetic signals, Archosauria, *Alligator mississippiensis*, *Dromaius novaehollandiae*, Dinosauria.

Background

Sutures are the fibrous tissues uniting bones in the skulls of vertebrates (Herring, 2000; Kokich, 1986; Opperman, 2000). They play two important roles in the skull: they allow its growth during development (also known as ontogeny) and they determine its mechanics (Herring, 2000). Sutures are shock absorbers and reduce the magnitude of stress and strain in the skull bones, preventing them from fracturing (Rafferty et al., 2003). In medicine, sutures have been extensively studied in order to understand premature fusion of the skull bones of human newborns and infants, a condition known as craniosynostosis (Cohen, 1993; Kokich, 1986). It is generally observed that vertebrate sutures are open (or patent) early in development and progressively close as maturity is attained (Cole et al., 2003). During this process, the sutural borders of two opposite bones advance and the unossified suture becomes progressively smaller. Finally, when the bone fronts come into close contact, the suture may close (or fuse), and when it is no longer visible on the ectocranial side of the skull, the suture is known to have obliterated (Opperman, 2000). Some sutures that are straight early in development often become

more and more interdigitated during ontogeny (e.g., see Herring, 1972; Herring, 2000; Jaslow, 1989).

These general ‘trends’ seen in living species (essentially mammals) have been used for decades by paleontologists, i.e., they have assumed that the degree of sutural closure can be used as a proxy for maturity in fossil specimens (e.g., Marsh, 1887). If a fossil exhibits open sutures, it is often concluded that it is an *immature* specimen, while the presence of closed sutures leads to the conclusion that it is *mature*. Such conclusions can be found in many paleontological descriptions, including (but not exclusively) those of two charismatic groups of animals: mammals (e.g., Black et al., 2010; Marsh, 1887; Sánchez-Villagra, 2010) and non-avian dinosaurs (e.g., Bakker and Williams, 1988; Chinsamy-Turan, 2001; Longrich and Field, 2012; Rauhut, 2004; Sampson et al., 1997; Sereno et al., 2009; Yates, 2003). While the sequences of sutural closure in the skulls of extant mammals are fairly well studied and understood (see Bärmann and Sánchez-Villagra, 2012; Cray et al., 2008; Goswami et al., 2013; Wilson and Sánchez-Villagra, 2009) it is not the case for extant archosaurs i.e., birds and crocodilians, the closest living relatives of non-avian dinosaurs. In fact, sequences of sutural closure have been reported in the vertebral column of some members of the extant phylogenetic bracket (EPB, Witmer, 1995) of the Dinosauria (in some crocodilian species Brochu, 1996; Ikejiri, 2012) and some birds (Starck, 1993) but no sequence has ever been reported in their skull. It is still unknown if the closure of any suture or any particular anatomical group of sutures, coincides with the onset of sexual or skeletal maturity.

Since there is a lack of understanding concerning the sutures of living archosaurs, and since it is not known if they are indicators of juvenescence or adulthood in these species, it cannot be assumed that suture closure can be employed as proxies of maturity in their extinct relatives, non-avian dinosaurs. Therefore, in the present study, we investigated the sequences and the degrees of sutural closure in the skulls of two members of the EPB of the Dinosauria: the emu, *Dromaius novaehollandiae* and the American alligator, *Alligator mississippiensis* (Tables 1-3). We employed a modified version of cladistic methodology originally developed by Brochu (1992, 1996), using characters describing the degree of sutural closure and interdigitation (Fig. 1 and see Methods section). We included characters describing sutural interdigitation as well because this parameter often increases during ontogeny in some extant species (Herring, 2000) and it has previously been used to estimate maturity in some fossil dinosaurs (e.g., Evans and Catherine, 2005).

Below is a rapid overview of some aspects of the biology of *D. novaehollandiae* and *A. mississippiensis*, two species with drastically different growth trajectories.

Dromaius novaehollandiae

Emus are ratites belonging to the Family Dromaiidae that originated in Australia, and are now farmed in various countries such as the United States and Canada (e.g., Goonewardene et al., 2003). In most living birds, skeletal maturity is attained before sexual maturity (i.e., the skeleton reaches its final size and then the ability to reproduce follows; (Lee and Werning, 2008). Half of the full height of emus is attained within 3 to 5 months (Davies, 2002), and full height (up to 190 cm) is attained in about 12 months

(Davies, 2002; Minnaar and Minnaar, 1992). Sexual characteristics develop later, from 12 to 20 months in most emus (Minnaar and Minnaar, 1992). Different ages at which they can breed have been reported: 16 to 18 months (Minnaar and Minnaar, 1992), 20 months (Davies, 2002), 2 to 3 years (Grzimek et al., 2003) or 3 to 5 years (D. Collins, personal communication). Egg laying and production of viable eggs become more and more regular as emus get older (D. Collins, personal communication), hence, the age at sexual maturity can be subjective. However, it is safe to assume that emus older than 12 months are skeletally mature, and those older than 18 months old are sexually mature and able to produce viable eggs (with more or less consistency). Individuals have lived for as many as 30 years in captivity (Minnaar and Minnaar, 1992) but typically only live six or seven years in the wild (Grzimek et al., 2003). Emus eat seeds, fruits, flowers, grass and a few insects such as grasshoppers and beetles in the wild (Davies, 2002). To our knowledge, no significant changes in diet have been reported during the ontogeny of the emu. All the specimens of this study were domestic and were fed soft food pellets while alive (D. Collins, personal communication).

Alligator mississippiensis

The American alligator belongs to the Alligatoridae and is present in the southeastern United States. It can be found in the wild and in alligator farms (Elsey et al., 1992). The most studied populations are those in Louisiana (e.g., Chabreck and Joanen, 1979), Florida (e.g., Woodward et al., 1992; Woodward et al., 1995) and South Carolina (e.g., Wilkinson and Rhodes, 1997). All show slight differences in their growth dynamics. Since our sample is mostly composed of alligators from Louisiana and Florida

(only three small alligators are from South Carolina), we will focus on these two populations here. Alligators hatch at total length of about 25 cm. They primarily grow fast and then undergo a reduction in growth at 122 cm due to a diet shift (Woodward et al., 1992). Sub-adults have been designated by Woodward et al., (1992) as individuals between 122 and 183 cm. The latter is the approximate length at which sexual maturity is attained, which is similar in populations from Louisiana (Joanen and McNease, 1987) and Florida (Woodward et al., 1992). Note that alligators (as well as non-avian dinosaurs) reach sexual maturity before skeletal maturity, unlike birds where the opposite occurs due to the increase of their growth rate that occurred during evolution (Padian et al., 2001).

Alligators from the Florida Everglades grow more slowly and mature at smaller size and greater age than Louisiana alligators (Dalrymple, 1996): it requires 18 years for females to reach sexual maturity in Florida (Abercrombie, 1989), but only about 10 years in Louisiana (Joanen and McNease, 1987). At a length of 270 cm, female alligators in Louisiana reach a significant plateau of growth (Chabreck and Joanen, 1979), which corresponds to their skeletal maturity. Female alligators in Florida may keep growing to larger sizes (to about 300 cm, e.g., the longest female ever reported was 309.9 cm; (Woodward et al., 1995). Males in Louisiana are skeletally mature at around 400 cm (Chabreck and Joanen, 1979), but the longest alligator male ever reported was 426.9 cm in Florida (Woodward et al., 1995). Chabreck and Joanen (1979) estimated that this length at skeletal maturity is reached in about 45 years for females and only 40 years for males in Louisiana. Note that domestic alligators can grow and mature faster than wild alligators (Coulson et al., 1973; Elsey et al., 2000). It has commonly been assumed that

American alligators have an indeterminate growth, but by means of histological analysis, Woodward et al., (2011) showed that they eventually stop growing. Finally, they are known to undergo shifts in diet, eating progressively larger and tougher preys during their ontogeny (Delany, 1990; Delany and Abercrombie, 1986).

Results

Ontograms

Dromaius novaehollandiae: The cladistic analysis of 24 specimens for 42 multistate characters gave a strict consensus that collapsed into the hypothetical embryo and one large polytomy for all the other specimens. Therefore, in order to provide some structured data we show the 50% majority-rule consensus tree (Fig. 2A). The tree is linear and pectinate, with three groups collapsing into polytomies. It has a length of 117 steps, a consistency index of 0.58, a homoplasy index of 0.42 and a retention index of 0.74. The color code (Fig. 2B) shows the ontogenetic categories that were estimated prior to phylogenetic analysis in each specimen (based on age or skull length). The direction of ontogeny shown in figure 2B could be considered the ‘correct’ pattern of ontogeny. The most important parameter to note is that the ontogram (Fig. 2A) shows a pattern consistent with ontogeny: the least mature individuals are near the base of the tree (in blue and green) and the progressively more mature individuals are further away from the root (in yellow and orange, also see the known ages of some specimens). Only three groups of specimens seem to not follow this ‘correct’ pattern: MOR OST 1298 or ROM R7644, ROM R7945 or MOR OST 1809, and ROM R7654. Regardless of these

inconsistencies (that could be due to intraspecific variation and consequent misidentification from our part of the ontogenetic category of some specimens when their age was unknown), the resulting ontogram shows that the degree of sutural closure indeed produces a pattern consistent with ontogeny, and in turn this suggest that this parameter is a good indicator of maturity in this species. The ontogram also produces a ‘clade’ that includes all the skeletally mature individuals (see the onset of skeletal maturity mapped on the ontogram, Fig. 2A). This ‘clade’ includes a smaller clade formed of all the sexually mature individuals (see the onset of sexual maturity mapped on the ontogram, Fig. 2A). During ontogeny, more and more sutures close (i.e., with their suture line still visible on the outside of the skull) and then completely obliterate (i.e., with complete disappearance of the suture line). Figure 2A shows selected synontomorphies concerning sutural obliteration, i.e., the sutures that obliterate consistently in all of the specimens of the analysis (coded as a three as in Fig. 1C). Synontomorphies involving closed sutures (coded as a two, Fig. 1B) are not shown because it appears that obliteration is a better indicator of sutural closure. Indeed, while obliteration seems to be final, closure does not. During this research, we have observed sutures that close early in ontogeny (coded as a two) but then ‘re-open’ and become wider (e.g., coded as a one, or even a zero, see next section concerning *A. mississippiensis* and the Discussion section).

The sequence of obliteration of the sutures and synchondroses in *D. novaehollandiae* is described as follows (Fig. 2A): (1) the parieto-squamosal suture, (2) the basioccipital-basiparasphenoid synchondrosis and the parietal-laterosphenoid and the squamosal-laterosphenoid sutures, (3) the fronto-parietal, interparietal, parietal-

supraoccipital and the squamosal-exoccipital sutures, and the exoccipital-supraoccipital synchondrosis, (4) the naso-frontal suture, (5) the naso-prefrontal suture, (6) the interfrontal suture, and (7) the nasal-mesethmoid and frontal-mesethmoid sutures. These last two obliterations occur in the specimen MOR OST 1803, which is the furthest away from the root and the oldest of our sample (a 20 year-old male). The onset of skeletal maturity coincides with the obliteration of the sutures of group (3) listed above, while the onset of sexual maturity corresponds to the obliteration of the Nasal-Prefrontal suture (but could also correspond to that of the naso-frontal suture depending on where sexual maturity is mapped on the ontogram). To roughly summarize this sequence, the braincase synchondroses and the cranial sutures are the first parts of the skull to obliterate completely, while facial sutures obliterate last. No palatal suture obliterated in a consistent pattern within all the specimens examined (no synontomorphies for the obliteration of palatal sutures exist).

Alligator mississippiensis. The cladistic analysis of 49 specimens for 80 multistate characters gave a strict consensus that collapsed into the hypothetical embryo and multiple polytomies. Therefore, just as we did for the emu ontogram we document the 50% majority rule consensus tree (Fig. 3A, but see the strict consensus tree in Appendix A). The tree is linear and pectinate, with four groups collapsing into polytomies. It has a length of 563 steps, a consistency index of 0.25, a homoplasy index of 0.75 and a retention index of 0.62. The most important parameter to note is that this ontogram shows the opposite trend to that presented previously in the emus (i.e. opposite to the ‘correct’ direction of ontogeny; see the color code in Fig. 3B with the estimated ontogenetic

categories of each specimen based on total length). Here the most mature individuals are near the base of the tree (in yellow and orange) and the less mature individuals are further away from the root (in blue and green). For example, the specimen that falls right next to the root is the enormous *Alligator* skull (66 cm long) from the Pleistocene, while the animals furthest away from the root are an embryo (MOR OST 1645) and a one day-old hatchling (ROM R 7964). Since the degree of sutural closure reconstructed a pattern opposite to that of expected through ontogeny, this suggests that sutural closure is not an indicator of maturity in this species. In fact, sutures are more open in the most mature individuals (which explain why they are placed at the base of the tree, next to the all-zero hypothetical embryo), and sutures are more closed in the least mature individuals (which explains their location further away from the root). Moreover, only two sutures obliterate completely during the ontogeny of *A. mississippiensis*, the interfrontal and the interparietal sutures (Fig. 3A). Obliteration of these two sutures occurs during embryonic development (see Fig. 3C that shows the fusion of the interfrontal bones in an unhatched embryo, MOR OST 1646, not included in this cladistic analysis). The full-term unhatched embryo (MOR OST 1645) had already obliterated these two sutures.

Attention was not drawn to the synontomorphies concerning the degree of interdigitation in both the emu and the American alligator because this parameter is not used as frequently in paleontology for assessing maturity. However, the averaged degrees of interdigitation will be discussed later in the following section.

Averaged Degrees of Sutural Closure and Interdigitation

As mentioned in the Background and the Methods sections, we ‘transformed’ the character states of each suture into a set score (Fig. 1) in order to calculate the global degree of sutural closure for each individual specimen and also for each ontogenetic category (Figs 4 and 5). An average of zero in one specimen would mean that all its sutures are open, while an average of three would mean that all its sutures are obliterated. Averages were also calculated for each anatomical group of sutures (Fig. 6). The global averaged degree of interdigitation was also calculated for each ontogenetic category (Fig. 7), but not for each individual. Assigning scores to sutures provides a separate type of visualization of the data compared to that provided by the two ontograms (Figs. 2 and 3). Before showing the results of this section, we would like to emphasize that our quantification of sutural closure might not be the most robust method to do so. However, since we already had used a way to describe sutures for the phylogenetic analysis, time was lacking to create a new and more accurate way to quantify each sutural state (i.e., such as that found by Wilson and Sanchez-Villagra (2009)). Nevertheless, this coding still helped visualize trends in the degree of closure or interdigitation of sutures during ontogeny in these two species.

Sutural Closure: The relationship between the averaged degree of sutural closure per individual and ontogeny is shown using skull length as a proxy in 24 emus (Fig. 4) and 46 American alligators (Fig. 5). Three alligators were excluded from this data set because of their incompleteness (only their braincases remained and had been scored).

Although age would be a better proxy for maturity than skull length, we did not use age because only 9 out of the 24 emus and only 3 out of the 46 alligators had accompanying age data. Total length is a better proxy for maturity in the American alligators than skull length, but only 38 out of 46 specimens had a known total length. Therefore skull length was chosen in figure 5 in order to present as many data points as possible (but see this same graph using total length in the X axis of Appendix B).

Note that the categories show moderate overlap in Figure 4. This discrepancy is probably due to intraspecific variation. The graph shows a clear linear relationship between sutural closure and maturity, with a Pearson's correlation coefficient of 0.86. Just as it was presented by the ontogram, the degree of sutural closure increases during ontogeny, with the lowest average (0.32) present in the juvenile ROM R7360 (estimated to be a few weeks old) and the highest average (2.73) present in what we estimated to be a skeletally mature adult (of unknown age, MOR OST 186). The second highest average (2.67) was that of MOR OST 1803, the twenty year-old male. (See Appendix C for the averages of all the specimens). The averaged degrees of sutural closure were also calculated for each ontogenetic category: the juveniles altogether show a value of 0.91, the sub-adults show 1.45, the skeletally mature individuals show 2.10, and the sexually mature individuals show 2.52 (see Appendix C). In Figure 5, the ontogenetic categories are labeled under the trend line, and the estimated diet shifts of the specimens (based on data previously published by Delany, 1990; Delany and Abercrombie, 1986) are exposed above the trendline. The graph shows a clear inverse relationship between the degree of sutural closure and skull length, with a Pearson's correlation coefficient of -0.79 for a

linear relationship. However, note that this relationship could potentially be logarithmic instead, since it shows a slightly better Pearson's coefficient of -0.82 (data not shown). This data supports what was documented by the ontogram (Fig. 3A). The highest averaged degree of sutural closure (1.8) is present in ROM R6252, a hatchling, while the lowest (0.26) is presented by a skeletally mature domestic male ROM R4411 (see Appendix D for the averages of all the specimens). The averaged degrees of sutural closure were also calculated for each ontogenetic category: the juveniles altogether have the highest value of 1.45, the sub-adults have 1.02, the sexually mature individuals have 0.68, and the skeletally mature individuals have the lowest value of 0.53 (see Appendix C). Note that these averages are smaller than what was observed in the ontogenetic categories of the emu listed previously.

It is difficult to see if there are significant differences between the averages of sutural closure of males, females, domestic and wild animals without more rigorous statistical analyses. However, it appears that in general the values of domestic alligators are smaller than those of the wild alligators (Fig. 5, see the empty circles generally below the regression line). It is interesting to note because there are known osteological differences between wild and domestic alligators: domestic alligators have broader skulls than wild alligators of the same skull length. Perhaps these characteristics are responsible for the apparent differences in the average degrees of sutural closure between wild and farmed specimens (see discussion for further elaboration).

Sutural Closure per Anatomical Group: The averaged degrees of sutural closure were also calculated for each anatomical group presented in Table 3. Figures 6A and 6B

show these averages during the ontogeny of the emu and the American alligator, respectively. In the emu (Fig. 6A) it appears that facial and palatal sutures are more open than the cranial sutures and braincase synchondroses in juveniles and sub-adults. At some point between sub-adulthood and skeletal maturity, this pattern switches and the cranial sutures and braincase synchondroses have a higher averaged degree of sutural closure. This pattern stays the same for the remainder of ontogeny. All the braincase sutures are the first to be completely obliterated (at skeletal maturity, with an average of three), while cranial sutures reach their highest degree of sutural closure later, around sexual maturity (with an average of 2.93). The least open sutures are the palatal sutures (with an average of 1.75 at sexual maturity). The degree of closure of facial sutures (with an average of 2.33 at sexual maturity) is intermediate between these two extremes. This sequential pattern is in accordance with the synontomorphies presented in Fig. 2A.

In the alligators (Fig. 6B), the pattern is more simple: all anatomical groups have averages that decrease during ontogeny. Cranial sutures are always more closed than any other anatomical sutural group (with an average of 0.95 at skeletal maturity), followed by the braincase synchondroses (0.71 at skeletal maturity), and finally the facial and palatal sutures (0.14 at skeletal maturity). These averages are much lower than those of the emus. It also appears that braincase synchondroses reach their final degree of closure during sexual maturity (0.72) since this number does not increase nor decrease consistently until after skeletal maturity (0.71) is reached.

Sutural Interdigitation: The averaged degree of sutural interdigitation is always higher in *A. mississippiensis* than in *D. novaehollandiae* during ontogeny (Fig. 7, also see

Appendices E and F for all the interdigitation scores and averages). In general, sutures start straight then become slightly interdigitated and finally very interdigitated in the alligators. However, this is not the case for all sutures, as some straight sutures never increase their degree of interdigitation through ontogeny (e.g., the internasal (Fig. 1D) and naso-maxillary sutures, as well as the exoccipital-quadrates articulation). These sutures were straight in wild alligators, but slightly or very interdigitated in almost all the domestic alligators (data not shown). In the emu, the degree of interdigitation increases through ontogeny from juveniles to sub-adults and then decreases in skeletally and sexually mature individuals. This decrease is most likely an artifact of the coding used in the phylogenetic analysis. Indeed, when a suture was obliterated (coded as a three), the degree of interdigitation was not visible and interdigitation was coded as ‘non applicable’ with a question mark (?). All of these question marks associated with obliterated sutures in the mature individuals decreased the averaged degree of sutural interdigitation in the last two ontogenetic categories. The ‘correct’ trend would show that the maximum degree of sutural interdigitation is reached in sub-adults and that this degree remains constant for the remainder of ontogeny.

Discussion

Sutural Closure through Ontogeny in *D. novaehollandiae* and *A. mississippiensis*

We report two opposing trends in the emu and the American alligator: while sutural closure is a good proxy for maturity in *D. novaehollandiae* (Figs. 2, 4, 5A), it is not the case for *A. mississippiensis* (Figs. 3, 5, 5B). The modified cladistics methodology

using characters related to sutural closure did produce a pattern consistent with ontogeny in the emu (Fig. 2), but not in the American alligator (Fig. 3). While more and more sutures fuse and obliterate during ontogeny in the emu (i.e., 70% of the sutures; Figs. 2, and also Fig. 4), only 2 sutures obliterate in the American alligator (i.e., 5% of the sutures; Fig. 3). In fact, in the alligators, sutures are more open in old, mature individuals than in young, immature individuals (Fig. 5). In other words, the skulls of American alligators curiously ‘loosen up’ during ontogeny. We will discuss this matter in the following section.

For the emus, not only did sutural closure reproduce a pattern consistent with ontogeny, but the obliteration of a group of sutures also corresponds to the onset of skeletal maturity: the fronto-parietal, the interparietal, the parietal-supraoccipital and the squamosal-exoccipital sutures and the exoccipital-supraoccipital synchondrosis (Fig. 2). The obliteration of some facial sutures (the naso-frontal, nasal-prefrontal and interfrontal sutures) coincides with the onset (or the imminent onset) of sexual maturity (Fig. 2). Therefore, the obliteration of these sutures could potentially be used as a benchmark for skeletal and sexual maturity in this species. It appears that some obliterations may also indicate maturity in other ratites, such as the ostrich, since it has been reported that four sutures in their skull roof are fused by the time of sexual maturity at about 3 years of age (Marshall, 2000). In the alligators, the two sutures that obliterate do so during embryonic development, or shortly after hatching and show an antero-posterior gradient of fusion (the frontals fuse first, followed by the parietals; Fig. 3C). This antero-posterior gradient has also been reported in a species of lizard, *Cyrtodactylus pubisulcus* (Rieppel, 1992),

but note that in the emu, the parietals fuse first and do so much later than in the alligators during their relative ontogeny (Fig. 2). The obliteration of the interfrontal and interparietal sutures in the American alligator cannot be used as a benchmark for maturity. These results show that the sequences as well as the timings of sutural closure are drastically different between *D. novaehollandiae* and *A. mississippiensis*. This is not surprising since it is known that even between closely related clades (or even within clades), the sequences and the amount of sutures that obliterate can be highly variable (Cole et al., 2003; Goswami et al., 2013; Herring, 2000; Maisano, 2002). For example, all the sutures in the skulls of grizzly bears (*Ursus arctos*) close, while the closely related polar bear (*Ursus maritimus*) only closes half of its sutures (Goswami et al., 2013).

In the emu, both the ontogram (Fig. 2) and the degree of sutural closure for different anatomical groups of sutures (Fig. 6A) reveal an overall anatomical pattern of sutural closure: (1) in general, all the braincase synchondroses are obliterated first, (2) followed by cranial sutures and (3) finally facial sutures. It appears that palatal sutures do not obliterate. Jollie also found that fusion begins in the occipital region in chickens and becomes complete when skeletal maturity is attained (Jollie, 1957). These patterns are however different from the one generally observed in mammals (established in Krogman, 1930 and Schweikher, 1930). A modified version of this mammalian sequence could be described as follows: (1) cranial sutures, (2) braincase synchondroses, (3) palatal sutures and finally (4) facial sutures (see Fig. 2 in Wilson and Sánchez-Villagra, 2009). These differences are not surprising due to the large phylogenetic distance between birds and mammals. In *A. mississippiensis*, at any given time during ontogeny, cranial sutures

present the highest degree of closure, followed by the braincase synchondroses, the facial sutures, and finally the palatal sutures are the most opened of all (Fig. 6B). This hierarchy is rather similar to that presented in the emu (Fig. 6A), and perhaps this suggests some pattern conservation between these two species. However there is not enough data to make any conclusions concerning their respective clades, or whether or not this could be designated as the ‘archosaurian pattern’ of sutural closure. Perhaps investigating sequences in more species of extant birds and crocodilians will reveal if this ‘pattern’ is conserved (like it is in general in most mammals, (Wilson and Sánchez-Villagra, 2009)). Finally, we found that interdigitation slightly increases during the ontogeny of the emu, but increases drastically during that of *A. mississippiensis* (Fig. 7). It could potentially be used as a proxy for maturity in the latter species, but alas, a repeatable and practical way to quantify sutural interdigitation through ontogeny is yet to be determined. Moreover, interdigitations play a mechanical role in the skull and might reflect epigenetic signals rather than ontogenetic signals in some cases (Herring, 2000 and see next section in the discussion).

Note that all of our emu specimens were domestic, while the alligator specimens included both wild and captive animals. It would be interesting to test if all the patterns shown by the domestic emus are the same in wild emus, since slight differences have been observed between these two specimen categories in the American alligators (Fig. 5). Moreover, captive animals have different diets than those of wild animals, which could be reflected in their sutures. It is now important to discuss the inverse relationship between the degree of sutural closure and ontogeny that is observed in *A. mississippiensis*

(Figs. 3 and 5). Why do the sutures of American alligators ‘re-open’ during development? The following section will discuss some epigenetic signals that could ‘overpower’ the ontogenetic signals in this species.

Possible Epigenetic Signals in the Skull and Sutures of *Alligator mississippiensis*

Sutures are not only sites of bone deposition during growth (i.e., with an ontogenetic signal), but they also determine the mechanics of the skull in terms of movement and force transmission (i.e., with epigenetic signals; Herring, 2000). In other words, and this is a very important statement, sutures might stay open in some species not because their skull is still growing, but because the sutural ligament reduces the magnitude of stress in the juxta-sutural bone and prevents fractures (Rafferty et al., 2003). This has been observed in species with head-butting behavior, (*Ovis orientalis* and *Capra hircus*, Jaslow and Biewener, 1995; Jaslow, 1989; Jaslow, 1990) and during mastication and rooting in miniature pigs (*Sus scrofa*, Rafferty et al., 2003). Moreover, the ‘re-opening’ of some mammalian sutures has been observed, and it is made possible by bone resorption during the initial events of sutural fusion (Persson et al., 1978). Theoretically, if the bone fronts never meet during ontogeny (which is probably what happens in American alligators), then no resorption is necessary to increase the degree of closure of a suture. We hypothesize that almost all the sutures of *A. mississippiensis* remain open and increase their degree of patency during ontogeny to accommodate the increasing amounts of stress that their skulls receive during feeding. Indeed, it is known that American alligators undergo shifts in their diet to progressively larger and more

robust, bony prey. Insects are the predominant food in small individuals from 25 through 60 cm long (Delany, 1990) while the proportions of crustaceans, fish and reptiles increase as they reach a total length of 122 cm (Delany, 1990). Up until about 300 cm long, fish are predominant in their diet (Delany and Abercrombie, 1986) and finally the largest alligators (longer than 300 cm) eat mostly large mammals (such as deer and hogs), turtles, and other alligators (Delany and Abercrombie, 1986). These diet shifts are illustrated in Figure 5. Moreover, it has been shown that the bite force of American alligators increases through ontogeny (Erickson et al., 2003) and that they are able to get bigger and more robust preys due to cranial modifications (Dodson, 1975). Obviously, one could say that the sutures of these animals remain open for a long time because they are slow-growing ectotherms (they can take up to 40 years to reach skeletal maturity, Chabreck and Joanen, 1979). However, this explanation would be incomplete since even the skeletally mature individuals have open sutures, and neither can this account for the inverse relationship seen in Fig. 5. Two observations support our hypothesis: the degree of sutural interdigitation increases drastically during the ontogeny of *A. mississippiensis* (Fig. 7), and it has been previously shown that interdigitations absorb strain energy and dampen compressive impact forces more effectively than straight sutures (Herring, 1972; Herring, 2000) and normal cranial bone (Jaslow, 1990). For example, the sutures at the base of the antlers in deer, or in the areas that host the incisors of rodents are more interdigitated than the sutures of the rest of their skulls (Herring, 2000). Note that in the emu, a species that only eats softer types of food, the degree of interdigitation did not increase drastically, and its values were always much lower than those of the alligators

(Fig. 6). Second, we have mentioned that it appears that the sutures of domestic alligators present a lower average of sutural closure (i.e., were more open) than those of the wild alligators of the same skull size (Fig. 5). Although we have not tested if the values of the domestic alligators were statistically smaller than those of the wild alligators, in general, the former specimens (indicated by the open circles) were below the trend line (Fig. 5). This would correlate well with the findings of Erickson et al., (2004), who found that domestic alligators bite more forcefully than their wild counterparts with respect to jaw length. One of their explanations was that the relatively broader heads of domestic alligators compared to wild alligators afford more space for the adductor muscles of their jaws. Perhaps including more domestic alligators in this sample could help confirm that the apparent differences in sutural closure observed here are indeed due to a phenotypic plasticity taking place during captivity.

A. mississippiensis may represent an example of a species where epigenetic signals are stronger than ontogenetic signals in skull sutures. These epigenetic signals could explain why their degree of sutural closure does not increase during ontogeny, but instead decreases. These results are opposite to the general assumption that states that vertebrate sutures are open early in development and fuse progressively with growth. In fact, there are many more examples reported in the literature that contradict this general assumption. Some sutures may remain open even well after growth has stopped, while others may obliterate very early as soon as some minimal growth has been reached (Cohen, 1993; Herring, 2000). For example, all the sutures in rats and mice never fuse during their ontogeny (except for the posterior interfrontal suture, Mehrara et al., 1999).

Many sutures never close completely in carnivorous mammals as well (the average of closure of 32 sutures across 25 species studied was 28%, which is about 9 sutures out of 32), with the most extreme example being the Northern elephant seal that only shows 14% of closure (approximately 5 sutures out of 32, Goswami et al., 2013). The overall suture closure of ruminants is also low, with the majority of species closing less than 25% of their sutures (in that case approximately 7 sutures out of 29; Bärmann and Sánchez-Villagra, 2012). The most extreme examples would be *Capreolus* (the European roe deer) and *Rubicapra* (the chamois) that only close 5 sutures out of the 29 studied (Bärmann and Sánchez-Villagra, 2012; which corresponds to 17% of closure). Recall that the American alligator showed an average of 5% of closure, and this is much smaller than any number presented in these mammals. Perhaps snakes would show an even smaller number due to their extreme jaw adaptations (see Herring, 2000).

In the opposite case, some individual sutures or groups of sutures may close very rapidly. For example, the interfrontal suture in humans obliterates between the second and fifth postnatal year (Kokich, 1986; and recall that the interfrontal suture of the American alligator fuses earlier, during embryonic development, Fig. 3C). The posterior interfrontal suture of rats and mice also fuses very early, shortly after weaning (Mehrara et al., 1999). Among squamates, closure of synchondroses in the skull base may occur before the animal reaches 30% of its maximum size (Maisano, 2002). This is particularly the case in *Bipes biporus* (the Mexican mole lizard), where the braincase is already closed but almost triples in length until skeletal maturity (Maisano, 2002). Another example not pertaining to the skull but that should still be mentioned here is the case of

the caudal vertebrae of *A. mississippiensis* that are already fully closed in hatchlings (Brochu, 1996).

It is a fact that growth is still possible after the closure of synchondroses (e.g., Brochu, 1996; Maisano, 2002) or even after sutural closure (Cohen, 1993), but these “provocative” facts (named so in Cohen, 1993) are rarely mentioned in paleontological studies. The results showed by *A. mississippiensis* and the few examples presented above concerning some extant vertebrates suggest that it is incorrect to assume that closed sutures indicate maturity and open sutures indicate juvenescence. Consequently, we conclude that the future use of sutural closure as a proxy for maturity in extinct archosaurs, including non-avian dinosaurs, should be carefully reconsidered (and so should every previous maturity assessment that was made in these species).

Implications for Maturity Assessment in Non-Avian Dinosaurs

Maturity assessment of fossil specimens is extremely important for systematics and paleobiology (Brochu, 1996; Irmis, 2007; Maisano, 2002; Scannella and Horner, 2010). It is crucial to know whether the variation between specimens is ontogenetic or phylogenetic in order to have the most accurate estimation of species diversity through time, and use the appropriate ontogenetic stages (i.e., skeletally mature individuals) in phylogenetic analyses (Irmis, 2007). Non-avian dinosaurs were the dominant terrestrial animals during the Mesozoic, and it is essential to employ accurate methods of maturity assessment in these charismatic animals. Maturity assessment using sutures as a proxy in the vertebral column of fossil archosaurs has already been introduced by two recent

pioneering studies (Brochu, 1996; Irmis, 2007). Here, by examining the skull sutures of *A. mississippiensis* and *D. novaehollandiae*, and by reviewing the literature concerning this same matter on some extant species, we show that sequences of sutural closure are species specific and that their relationship with ontogeny is not always simple. Various epigenetic signals may encroach the primary ontogenetic signal carried by sutures, such as feeding habits. In fact, just like in *A. mississippiensis*, there are already known examples of non-avian dinosaurs where diet ‘masks’ the ontogenetic signal of their sutures: some theropod dinosaurs, *Tyrannosaurus rex* and *Allosaurus fragilis* (Rayfield, 2005; Rayfield, 2004; Rayfield et al., 2001). Almost all of their craniofacial sutures remain open late in ontogeny. Using finite element analysis, it was found that they retain open sutures to resist really high biting and tearing forces, more precisely a “puncture-pull” feeding habit in *T. rex* (Rayfield, 2004). Perhaps other theropods that employed these “puncture-pull” techniques and had tremendous bite forces would also show such a pattern of sutural patency.

It is also possible that this is the case for some herbivorous dinosaurs as well, such as hadrosaurs and ceratopsians. Indeed, like *A. mississippiensis*, many hadrosaurs went through drastic changes in length and height from hatching to skeletal maturity (Codron et al., 2013), and they took more than a year to reach skeletal maturity (e.g., 7-8 years in *Maiasaura*, 12 years in *Hypacrosaurus*, 10-15 years in *Edmontosaurus*; Cooper et al., 2008; Horner et al., 2000; Vandervan et al., 2014). Moreover, the dental batteries of hadrosaurs acquired new tooth rows and became more and more efficient at grinding during ontogeny, which in turn allowed them to grind tougher material (Horner et al.,

2004). It is not implausible to think that these drastic size changes and jaw modifications were accompanied by some sort of diet shifts that modified (or had an impact on) the degree of sutural closure of their sutures. Concerning ceratopsians, these horned dinosaurs are known to present a diverse array of cranial ornamentations and adaptations, called “bizarre” structures (Padian and Horner, 2011). These “bizarre” structures have been hypothesized to be linked to species recognition (Padian and Horner, 2011) and social selection (Hieronymus et al., 2009) (but see also Knell and Sampson, 2011 for sexual selection hypotheses). Perhaps, in the case of ceratopsian dinosaurs, these epigenetic factors (such as species recognition) were encroaching on the ontogenetic signal of their sutures. In fact, it has already been observed that this degree is too variable intraspecifically to be a safe criterion for ontogenetic determination in the skulls of *Protoceratops* (Brown and Schlaikjer, 1940) and *Agujaceratops* (Lehman, 1989). Just like *A. mississippiensis*, some of the smallest specimens of these species had closed sutures, while some of largest individuals retained open sutures. More recently, it has also been found that the largest known specimens of *Triceratops* (Scannella and Horner, 2011) and *Centrosaurus* (Frederickson and Tumarkin-Deratzian, 2014) showed open sutures. The latter studies concluded that sutural closure should not be relied upon as the sole criterion when determining relative maturity in ceratopsian dinosaurs (Frederickson and Tumarkin-Deratzian, 2014; Scannella and Horner, 2011). While we agree with these conclusions, we would like to emphasize that in some instances, the use of sutural closure as a maturity indicator should be completely avoided. It would be likely to find varied sources of epigenetic signals encroaching on the primary ontogenetic signal of skull

sutures in dinosaurian species that 1) undergo extreme size changes through ontogeny and reach skeletal maturity over the course of several years (e.g., hadrosaurs), 2) possess extensive cranial ornamentation (e.g., some ceratopsians), and 3) have extreme skull or jaw adaptations (e.g., some theropods). Perhaps the use of sutural closure for maturity assessment should be avoided primarily in such species. However, on the other hand we have seen that the degree of sutural patency is a good proxy for maturity in the emu, *D. novaehollandiae*. This species does not go through such drastic changes in size during its ontogeny and grows very rapidly, reaching skeletal maturity in one year. Moreover, emus do not undergo diet shifts nor present any specialized beak adaptations. It would be likely that the sutural ontogenetic signal stays pristine in dinosaurian species that present similar life characteristics (i.e., with a relatively small size, who reach skeletal maturity relatively rapidly and who do not present any complex diet shifts or cranial ornamentations). An example could be that of small theropods such as *Troodon* who has been estimated to reach full height in 3 to 5 years (Varricchio, 1993).

It is beyond the scope of this paper to test these hypotheses, but we suggest that the use of sutural closure to assess maturity in non-avian dinosaurs should be carefully reconsidered since sequences of sutural closure in the skull of extant archosaurs are species specific and do not solely reflect growth and ontogeny. We have shown that it is erroneous to assume that open sutures indicate juvenescence and closed sutures indicate adulthood, and perhaps this statement also relates to other fossils and not uniquely to non-avian dinosaurs.

Conclusions

Sutures are influenced by genetic, epigenetic, cellular and molecular factors, but the way in which they interact together is still unresolved (Opperman, 2000). There is a considerable lack of data concerning the sutures of non-mammalian animals, including archosaurs. The sequences of sutural closure in the skulls of a high number of avian and crocodilian species from different clades have to be investigated in order to understand what factors govern these sequences and whether or not there might be a conserved ‘archosaurian pattern’, potentially useful for maturity assessment in their extinct relatives. Those sequences should always be calibrated with sexual and skeletal maturity in order to be of any use for the maturity assessment of extinct species (as it was suggested by Irmis, 2007). Perhaps the relationship between sutural closure in the skull and the vertebral column should also be investigated. Different quantitative methods could be used for these studies, including cladistic methodology. However, a unified and standardized way to code sutures should be established, as well as a way to quantify sutural closure (perhaps similar to the methods presented in Wilson and Sánchez-Villagra, 2009). Concerning non-avian dinosaurs, before a further understanding of sutural closure is acquired, it is important to avoid the use of this method for assessing maturity in any new isolated specimen. First, sutural closure has to pass the test as a good maturity indicator by producing an ontogram consistent with ontogeny, and this can only be done on species that have relatively complete and robust ontogenetic series. Finally, even though sutural closure still has the potential to be used for maturity assessment in fossils, limb-bone histology remains the best method to reveal maturity in non-avian dinosaurs (e.g., Horner

et al., 2000; Padian et al., 2001). A histological study is underway to document the types of tissues found in archosaurian sutures (both extant and extinct), to compare sutural morphology and sutural histology, and to investigate growth mechanisms at the microscopic scale (Chapter Three).

Methods

Specimen Collection and Preparation

Almost all of the dry emu skulls (Table 1) belong to the osteology collections of the Museum of the Rockies (MOR) and were collected from emu farms in 2013 and 2014 (MOR OST 1799 through 1813). Fleshy heads were sent frozen to the MOR (from specimens that had died of various natural causes) and defleshed by dermestid beetles (Skull Taxidermy, Deer Lodge, MT). Four other emu skulls were collected earlier and were already present at the MOR. A few emu specimens also came from the osteology collections of the Royal Ontario Museum (ROM). Nine out of the twenty-four skulls were aged by an emu rancher (D. Collins, MER) and only one of these specimens had a known sex (Table 1).

The ROM provided almost all the American alligator skulls presented in this study, with sex, total length, weight and geographic data. Most of them were wild, but a few alligators were domestic. Only three hatchlings had a known age (Table 2). One fossil *Alligator* from the Pleistocene of Canada, with a skull length of 66 cm, was also examined (ROM R51011, Table 2). Some alligator specimens of the osteology collections of the MOR were also included in this study.

All the emu specimens were prepared by beetles, but not all the alligator specimens were skeletonized with this method (see Table 2). This information is important because the method of skull preparation has an impact on how the sutures appear on dry skulls. Dermestid beetles are thought to be the best agents to conserve articulation. Maceration or boiling often leads to disarticulation (personal communications of various taxidermists) and may result in a sutural morphology (or ‘gap’ size) that is accurate. We eliminated this bias by using mostly specimens skeletonized by beetles (or other invertebrates) and limited the use of maceration (Table 2).

Ontogenetic Categories

Dromaius novaehollandiae: Specimens of *D. novaehollandiae* were divided into four different ontogenetic categories (Table 1) using age, or skull length (SL) and already-published growth patterns on this species (see Background section) when ages were unknown. The categories are as follows: 1) juveniles (from the time at hatching to a few weeks old, which corresponds in our sample to a SL between about 55 mm and 65 mm); 2) sub-adults (from a few months-old to 12 months-old, corresponding to a SL of 97 cm to about 150 cm); 3) skeletally mature individuals (from 13 to 18 months old, corresponding in our sample to skulls between 143 and 158 cm long) and 4) sexually mature individuals (older than 18 months old, which correspond in our sample to skulls between 152 cm to 166 cm). Note that these categories overlap due to intraspecific variation.

Alligator mississippiensis: We also distributed the specimens of *A.*

mississippiensis into four different ontogenetic categories (Table 2). Since the ages of the specimens were unknown (except for three hatchlings), these categories were based on total length (TL) and on the growth patterns and dynamics already published for this species (see Background section). When the TL was unknown, it was estimated using SL and the equations of Chabreck and Joanen (1979) and Woodward et al., (1995). The four categories are as follows: 1) juveniles (from the length at hatching through 121 cm); 2) sub-adults (from 122 to 200 cm), 3) sexually mature (from 201 to 270 cm) and 4) skeletally mature individuals (larger than 271 cm). Three matters are important to mention: 1) a full term embryo (MOR OST 1645) was treated as a juvenile; 2) since Woodward et al., (1992) found that only 64% of females larger than 183 cm long are sexually mature, we assumed a TL of 200 cm for sexual maturity (J. Farlow, personal communication); and 3) it is safe to assume that all the females larger than 270 cm are skeletally mature since they all come from Louisiana (see Background section), but it is not guaranteed that all males larger than 270 cm are skeletally mature.

Modified Cladistics Methodology

This modified cladistic methodology was developed by Brochu (Brochu, 1992; Brochu, 1996) and it has seen an increase in use within the last few years, especially among paleobiologists interested in maturity assessment (e.g., Carr, 1999; Carr and Williamson, 2004; Frederickson and Tumarkin-Deratzian, 2014; Longrich and Field, 2012). In this method, immature character states are analogous to the plesiomorphic conditions of phylogenetic analyses, while mature character states are analogous to the

apomorphic conditions. Shared traits represent synontomorphies, following the terminology of Frederickson and Tumarkin-Deratzian (2014). It is a powerful method because unlike classic cladistics which generate a multi-taxa cladogram, cladistics ontogeny can be used to produce a single-species ontogram (e.g., see Brochu, 1992; Brochu, 1996; Carr and Williamson, 2004; Longrich and Field, 2012). With this ontogenetic pattern, it is possible to identify ontogenetic hierarchies, such as a potential hierarchy of sutural closure in the present study. By using specimens of extant species with known age and/or relative maturity, this method can help test if the degree of sutural closure is indeed a proxy of maturity. If the phylogenetic analysis produces an ontogram that is consistent with ontogeny (i.e., if the least mature individuals are near the base of the tree and the progressively more mature individuals get further away from the root), this would indicate that the degree of sutural closure is a reliable indicator of maturity in this species. Secondly, the timing of sexual and skeletal maturity can be mapped onto this hierarchy.

Two analyses were employed on 24 dry emus skulls (Table 1) and 49 dry alligator skulls (Table 2) in order to explore the hierarchy of obliteration for their skull sutures and skull-base synchondroses (see Table 3). Immature character states were coded as zeroes and mature character states were coded as ones or higher numbers (Fig. 1). A hypothetical embryo where all character states were immature (all-zero), was designated as the outgroup to polarize the characters. A total of 20 and 37 sutures were examined in the emu and American alligator respectively, and each suture was coded according to its degree of sutural closure (Fig. 1A-C) and its degree of interdigitation (Fig. 1D). Sutural

closure (or sutural patency) refers to the width of the gap seen externally between adjacent bones. Scoring for these characters is a modified version of Ryan et al., (2001) and Frederickson and Tumarkin-Deratzian (2014) and is as follows: sutures were considered open (coded as a zero) if bones were clearly separate along their margin of contact; partially closed (coded as a one) if bones were still separate along their margin of contact, but were significantly closer to each other than in the previous state; closed (coded as a two) if the bones were conjoined into a single unit, with a surface nearly level with the surrounding bone, but whose sutural line was still visible externally and could still be traced; and finally, completely obliterated (coded as a three) when there was absolutely no trace of the suture line on the surface of the bones (Fig. 1A-C). For the degree of interdigitation, sutures were coded based on their morphology and sinuosity: straight (coded as a zero), slightly interdigitated (coded as a one), and very interdigitated (coded as a two; Fig. 1D). A few times, one same suture was coded in different views (e.g., palatal, ectocranial or endocranial views when pieces of skull roof had been previously extracted for other studies). This gave a total of 42 characters for the emus and 80 characters for the alligators. All specimens and characters (Appendices G and H) were compiled into two data matrices using Mesquite (Maddison and Maddison, 2011; see Appendices I and J). Polymorphic characters were coded by scoring all states in the same cell of the character matrix (e.g., '0 & 1 & 2' if all three states were present in one same suture). The cladistic analyses were implemented with Phylogenetic Analysis Using Parsimony* v. 4.0b10 (PAUP*; Swofford, 2003). The sizes of the data set were too large to efficiently run a branch and bound algorithm, therefore a heuristic search was used

with ACCTRAN character optimization methods. This heuristic search implemented the random addition sequence with tree-bisection-reconnection (TBR) branch swapping and 1000 replicates. All characters were equally weighted and left unordered. Maxtrees was set up to 5000 for the alligator analysis. The emu analysis exceeded 5000 trees and thus 10000 Maxtrees were set in this case. Support for ontogenetic grouping was determined using non-parametric bootstrap resampling in PAUP* (Felsenstein, 1985). Five thousand bootstrap replicates were analyzed with one tree per replicate retained. Trees were then visualized with FigTree v. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Averaged Degrees of Sutural Closure and Interdigitation

After the cladistic analyses were performed, we re-used the phylogenetic matrices for another purpose: we treated the character state(s) found in each cell as a ‘score’, in order to calculate averages of sutural closure (between zero and three) and interdigitation (between zero and two) for each specimen, and observe how these averages change through ontogeny. This scoring (Fig. 1) is a modified version from that found in Wilson and Sánchez-Villagra (2009) concerning the sutures of hystricognath rodents (see their Figure 4). In the present study, since the scores came from matrices previously used for cladistics, if a suture presented multiple states at once (e.g., open (0) in one area, and partially closed (1) in another) the score of the suture became an average of each character state (e.g., score (0.5) for this example). In the case of Wilson and Sanchez Villagra (2009), such sutures had specific set scores (see their figure 4B,C for sutures that are $\frac{1}{4}$ or $\frac{1}{2}$ closed; also see Irmis, 2007). We calculated these averaged degrees of sutural

closure and interdigitation in each specimen and in each of the four ontogenetic categories for each species.

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Table 2.1. List of the emu specimens used in this study. The age is provided for 9 out the 24 specimens. The rest of the ages were estimated (those followed by a question mark) using their skull length. Abbreviations: (e), estimated.

Ontogenetic category	Specimen number	Skull length (mm)	Age	Sex	Geographic origin
Juveniles	ROM R7945	54.00 (e)	A few weeks (?)	?	?
	MOR OST 1799	54.99	A few weeks (?)	?	Montana
	ROM R7644	55.00 (e)	A few weeks (?)	?	?
	MOR OST 1805	55.82	A few weeks	?	Montana
	ROM R7630	61.00 (e)	A few weeks (?)	?	?
	MOR OST 1806	62.27	A few weeks	?	Montana
	MOR OST 1807	63.08	A few weeks	?	Montana
Sub-adults	MOR OST 1800	97.07	A few months (?)	?	Montana
	MOR-OST-1298	100.00 (e)	A few months (?)	?	Montana
	MOR OST 1808	116.67	8 to 10 months	?	Montana
	MOR OST 1802	128.36	A few months (?)	?	Montana
	MOR OST 1809	149.11	8 to 10 months	?	Montana
Skeletally mature adults	MOR OST 1810	143.94	18 months	?	California
	MOR OST 1814	151.90	18 months	?	California
	MOR OST 1815	153.13	18 months	?	California
	MOR OST 1811	154.39	18 months	?	California
	MOR OST 1813	154.54	18 months	?	California
	MOR OST 1297	155.00 (e)	> 10 months (?)	?	Montana
	MOR OST 186	155.00 (e)	> 10 months (?)	?	Montana
	MOR OST 1812	157.90	18 months	?	California
Sexually mature adults	MOR OST1803	152.23	20 years	M	Montana
	MOR OST 232	158.17	> 18 months (?)	?	Montana
	ROM R6843	163.00	> 18 months (?)	?	Australia
	ROM R7654	166.00	> 18 months (?)	?	?

Table 2.2. List of the American alligator specimens used in this study. When total length was unknown (or if a specimen was incomplete), total length was estimated using two equations (Chabreck and Joanen, 1979; Woodward et al., 1995). The results from Female (F) and Male (M) equations are shown. Abbreviations: D, domestic; e, estimated; Invert. Biota, invertebrate biota; W, wild.

Ontogenetic category	Specimen number	Skull length (mm)	Total length (cm)	SEX	W or D	Geographic origin	Preparation method
Juveniles	MOR OST 1646 (embryo)	28.00	102.00	?	?	Louisiana	Maceration
	ROM R7964	32.02	22.40	F	?	Florida	?
	MOR OST 1645 (embryo)	33.00	14.70	?	?	Louisiana	Maceration
	ROM R7966	34.70	28.30	M	?	Florida	?
	ROM R7965	38.83	24.00	M	?	Florida	?
	ROM R6251	42.99	31.00	?	?	South Carolina	?
	ROM R6252	43.10	29.80	?	?	South Carolina	?
	ROM R6253	43.33	19.80	?	?	South Carolina	?
	MOR OST 148	74.00	57.40 (e, M); 56.40 (e, F)	?	?	?	Dermeistid beetles
	ROM R8352	105.64	80.50	M	W	Florida	Dermeistid beetles
	ROM R8349	107.93	81.75	F	W	Florida	Dermeistid beetles
	ROM R8350	111.03	86.50	M	W	Florida	Dermeistid beetles
	MOR OST 820	119.00	90.50 (e, M); 89.90 (e, F)	?	?	?	?
	MOR OST 1028	121.00	91.90 (e, M); 91.30 (e, F)	?	?	?	Dermeistid beetles
	ROM R8354	139.19	100.00	M	W	Florida	Dermeistid beetles
	ROM R8355	147.97	113.00	M	W	Florida	Dermeistid beetles
Sub-adults	ROM R8345	172.27	129.00	M	W	Florida	Dermeistid beetles
	ROM R4405	175.82	138.43	M	D	Florida	Invert. Biota
	ROM R1698	195.74	?	?	?	Florida	?

Table 2.2 continued.

	ROM R8335	202.55	161.30	M	W	Louisiana	Dermestid beetles
	ROM R4418	215.29	166.37	M	D	Florida	Invert. Biota
	ROM R8332	226.94	176.50	M	W	Louisiana	Dermestid beetles
	ROM R8334	244.72	198.10	F	W	Louisiana	Dermestid beetles
	ROM R8322	247.44	191.80	F	W	Florida	Maceration
	ROM R4420	247.93	182.88	M	D	Florida	Invert. Biota
	MOR OST 1029	250.00	184.30 (e, M); 186.00 (e, F)	?	?	?	Dermestid beetles
	ROM R8347	252.43	191.30	F	W	Florida	Dermestid beetles
Sexually mature adults	ROM R8323	254.86 (e)	204.00	F	W	Florida	Dermestid beetles
	ROM R4421	266.54	208.28	M	W	Florida	Invert. Biota
	ROM R8331	277.00 (e)	215.90	F	W	Louisiana	Dermestid beetles
	ROM R8344	288.52	203.20	M	W	Florida	Dermestid beetles
	ROM R8336	330.00	243.80	M	W	Louisiana	Dermestid beetles
	MOR OST 156	340.00	247.40 (e, M); 251.40 (e, F)	?	?	?	?
	ROM R8342	343.00	247.00	F	W	Florida	Dermestid beetles
	ROM R4422	346.00	236.22	F	W	Florida	Invert. Biota
	ROM R4416	350.00	242.97	F	D	Florida	Invert. Biota
	ROM R4401	357.00	269.88	F	D	Florida	Invert. Biota
	ROM R8343	370.00 (e)	264.10	F	W	Florida	Dermestid beetles or Invert. Biota
Skeletally mature adults	ROM R8326	373.00	274.30	F	D	Louisiana	Dermestid beetles
	ROM R8327	375 (e)	276.90	F	D	Louisiana	Dermestid beetles
	MOR OST 155	380.00	275.30 (e)	M	W	?	?

Table 2.2 continued.

	ROM R4415	405.00	287.02	M	D	Florida	Invert. Biota
	ROM R8329	405.00	284.50	F	D	Louisiana	Dermestid beetles or Invert.Biota
	ROM R4411	445.00	330.20	M	D	Florida	Invert. Biota
	MOR OST 795	464.00	333.30 (e)	?	?	?	?
	ROM R8337	?(Braincase only)	360.70	M	W	Louisiana	Dermestid beetles or Invert.Biota
	ROM R8328	?(Braincase only)	375.90	M	D	Louisiana	Dermestid beetles or Invert.Biota
	ROM R8324	580.00	383.60	M	W	Florida	Dermestid beetles
	ROM R8333	?(Braincase only)	381.00	M	D	Louisiana	Dermestid beetles or Invert.Biota
?	ROM R51011	660.00	?	?	W	Canada (Pleistocene)	None

Table 2.3. List of sutures and synchondroses examined in this study.

Anatomical Group	Sutures/Synchondroses	<i>D. novaehollandiae</i>	<i>A. mississippiensis</i>
Facial sutures	nasal-mesethmoid	x	
	nasal-prefrontal	x	x
	nasal-frontal	x	x
	nasal-premaxilla	x	x
	frontal-mesethmoid	x	
	internasal		x
	nasal-maxilla		x
	maxilla-jugal		x
	maxilla-premaxilla		x
	maxilla-lachrymal		x
	prefrontal-lachrymal		x
	lachrymal-jugal		x
	frontal-prefrontal		x
	interpremaxillary		x
Cranial sutures	interfrontal	x	x
	frontal-parietal	x	x
	interparietal suture	x	x
	parietal-squamosal	x	x
	supraoccipital-parietal	x	x
	exoccipital-squamosal	x	x
	laterosphenoid-parietal	x	
	laterosphenoid-squamosal	x	
	frontal-postorbital		x
	postorbital-squamosal		x
Palatal sutures	pterygoid-vomer	x	
	vomer-palatine	x	
	maxilla-premaxilla	x	x
	premaxilla-vomer	x	
	intermaxillary		x
	maxilla-palatine		x
	interpalatine		x
Braincase synchondroses (or articulations)	exoccipital-supraoccipital	x	x
	exoccipital-basioccipital	x	x
	laterosphenoid-prootic		x
	laterosphenoid-basisphenoid		x
	laterosphenoid-ptyergoid		x
	laterosphenoid-quadrato		x
	basisphenoid-prootic		x
	basisphenoid-basioccipital	x	x
	basisphenoid-ptyergoid		x
	exoccipital-prootic		x
	exoccipital-basioccipital		x
	exoccipital-quadrato		x
	quadrato-ptyergoid		x

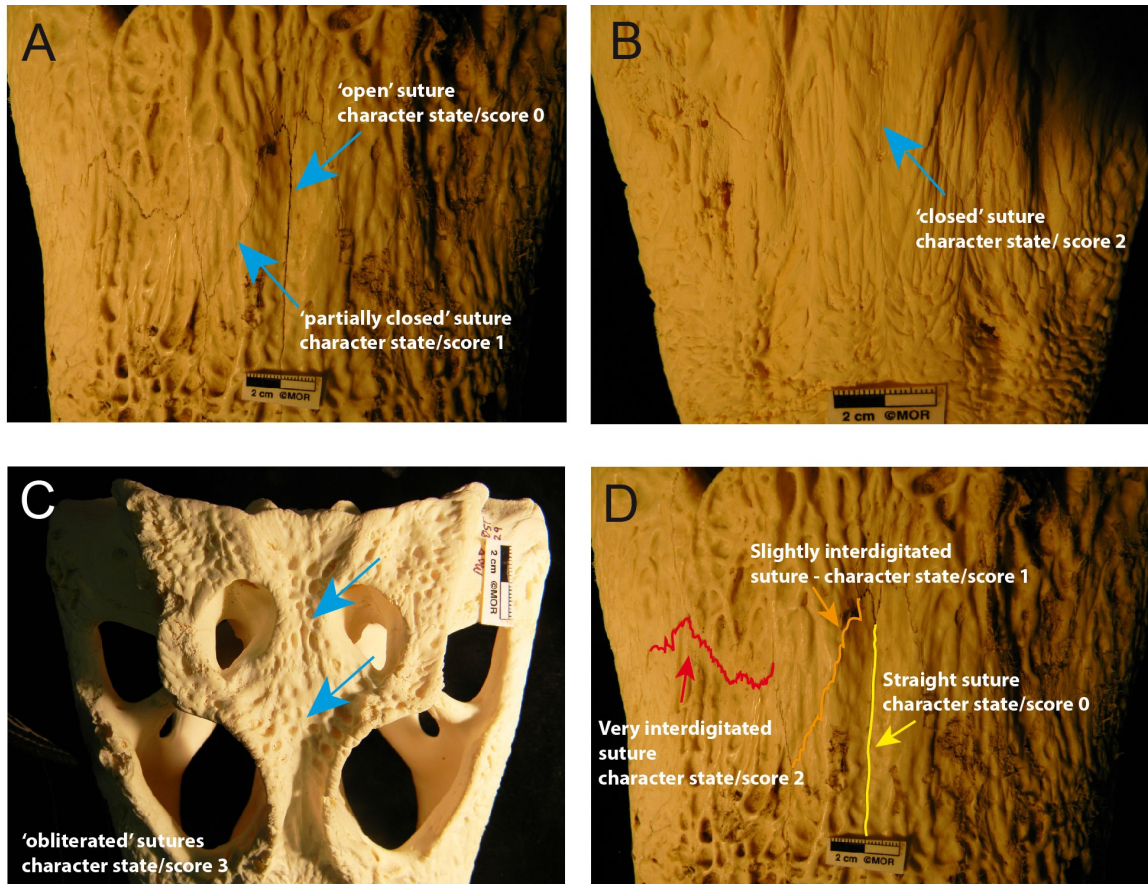


Figure 2.1. Character states and scores describing the degree of sutural closure (A-C) and interdigitation (D) in some American alligator skulls. A) The blue arrows show an open and a partially closed suture of MOR OST 155. B) The blue arrow shows a closed suture (with its suture line still visible) of MOR OST 1029. C) The blue arrows show two obliterated suture (with their suture lines morphologically invisible) in MOR OST 1029. D) The colored lines show the different degrees of interdigitation (or sutural shapes): a straight (yellow line), slightly interdigitated (orange line) and a very interdigitated suture (red line) in MOR OST 155. These interdigitation degrees are based on those exposed in (Herring, 1972).

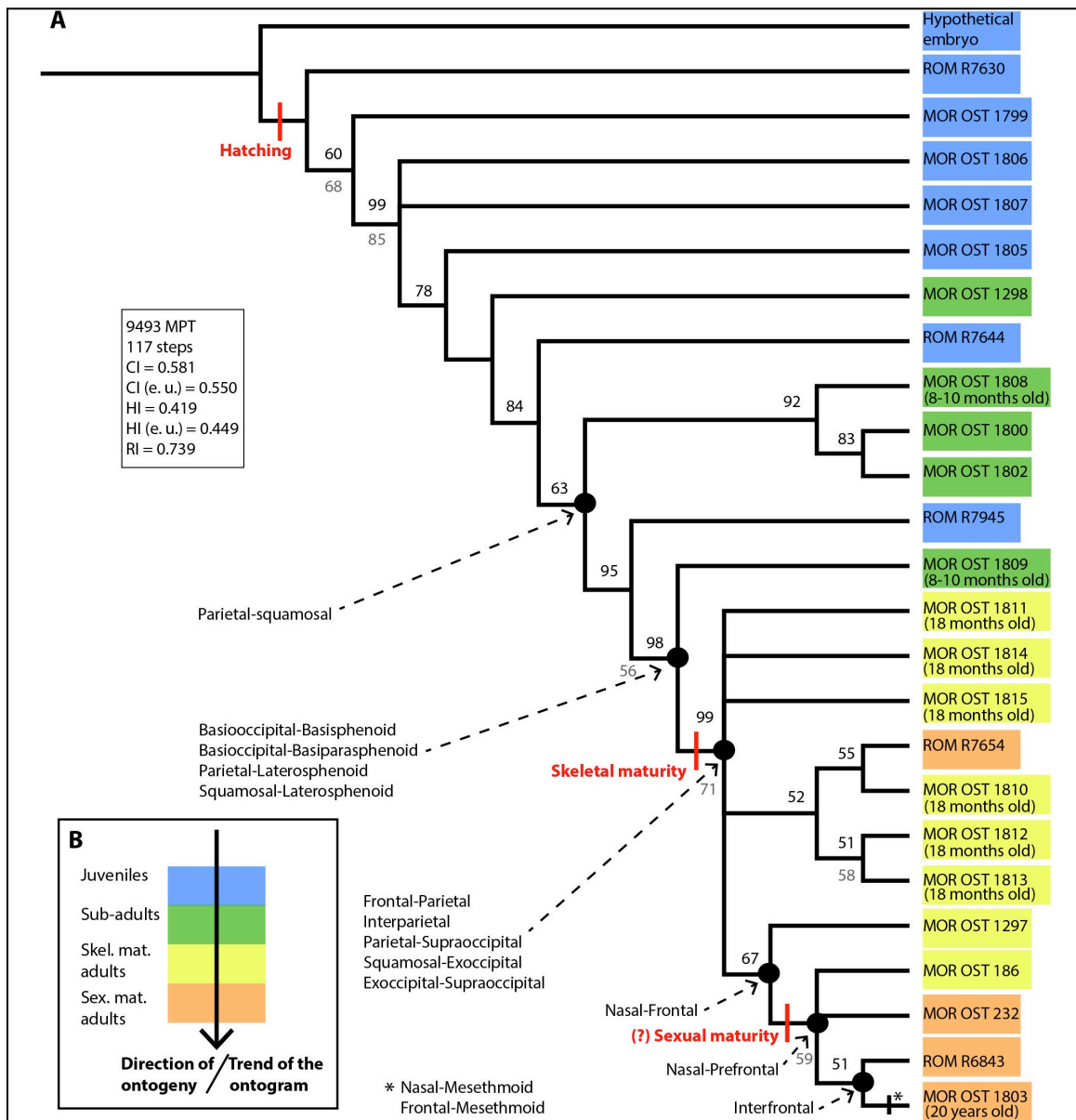


Figure 2.2. Ontogram of *D. novaehollandiae* (n=24). A) This ontogram is the 50% majority rule consensus tree for 9493 MPT. The onset of hatching, skeletal maturity and (approximate) sexual maturity are mapped on this diagram. Synontomorphies concerning the obliteration of some sutures (or group of sutures) are shown. Bootstrap support values are in grey below nodes, and the percent occurrence for nodes are in black above horizontal lines. B) Color code indicating the ontogenetic category of each specimen. This color is the same in A. Note that the 'trend' of the ontogram follows the direction of ontogeny in general. Abbreviations: CI, consistency index; e.u., excluding uninformative characters; HI, homoplasy index; MPT, most parsimonious trees; RI, retention index; Skel. mat., skeletally mature; Sex. mat., sexually mature.

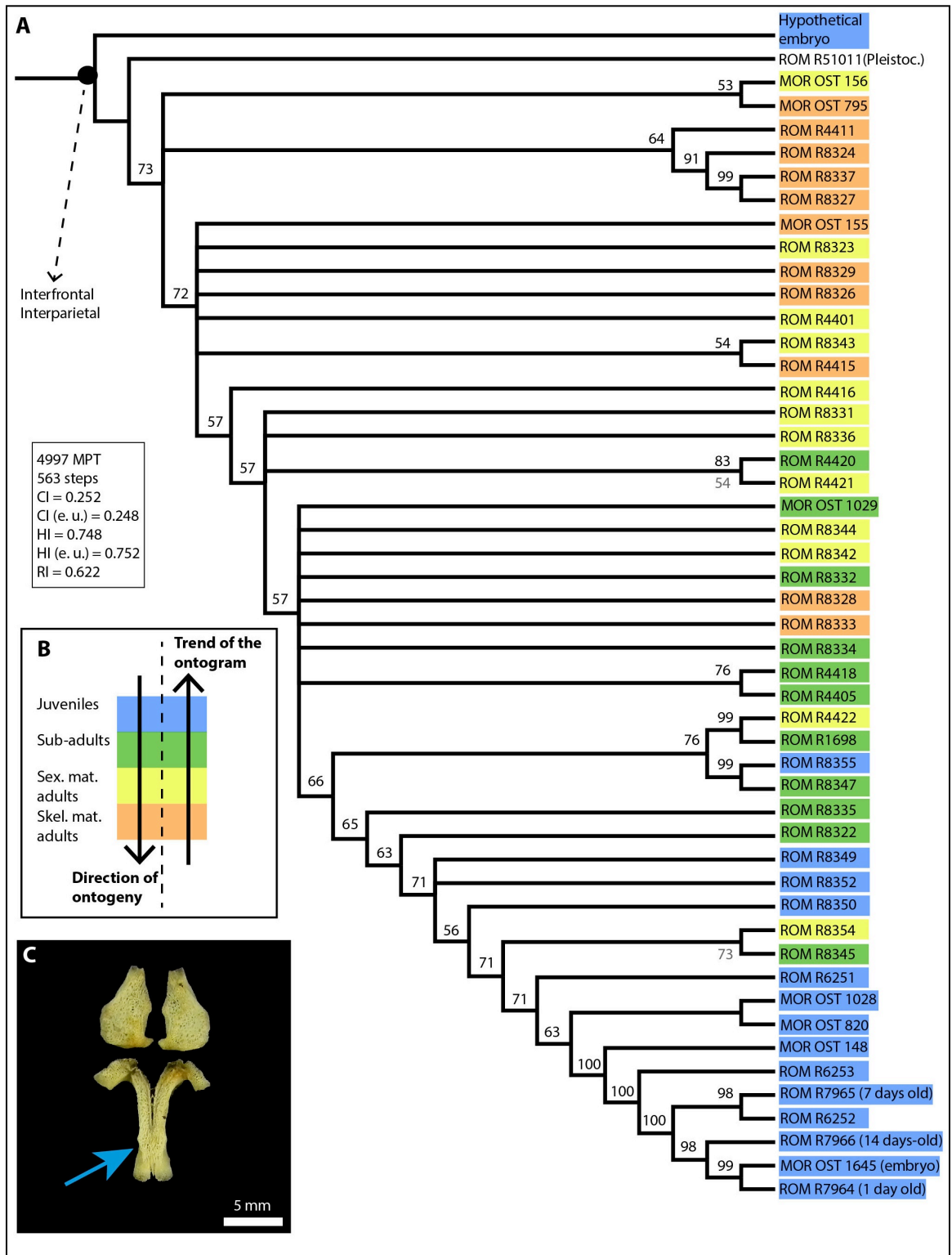


Figure 2.3. Ontogram of *A. mississippiensis* (n=49). A) This ontogram is the 50% majority rule consensus tree for 4997 MPT. The analysis only produced two synontomorphies concerning sutural obliterations (the interfrontal and interparietal

sutures). Bootstrap support values are in grey below nodes, and the percent occurrence for nodes are in black above horizontal lines. B) Color code indicating the ontogenetic category of each specimen in A. Note that in general, the ‘trend’ of the ontogram is opposite to the correct direction of ontogeny. C) Paired frontals (bottom) and parietals (top) of an unhatched embryo (MOR OST 1646). The blue arrow indicates an anterior-posterior gradient of fusion. Abbreviations: CI, consistency index; e.u., excluding uninformative characters; HI, homoplasy index; MPT, most parsimonious trees; Pleistoc., Pleistocene; RI, retention index; Skel. mat., skeletally mature; Sex. mat., sexually mature.

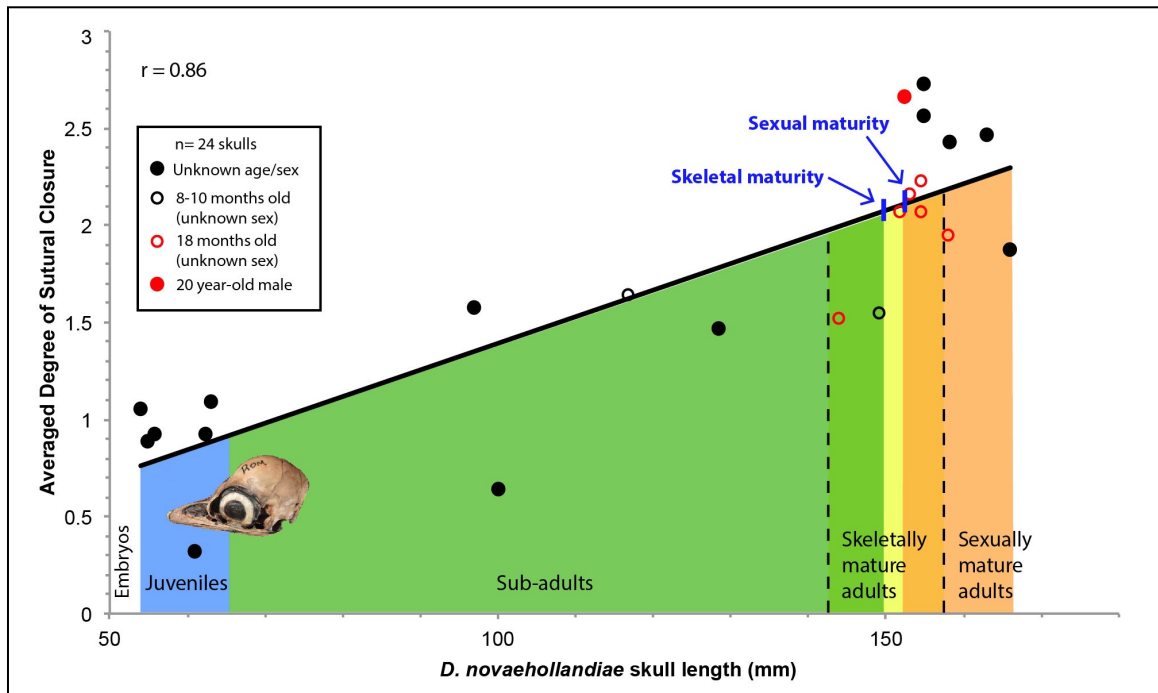


Figure 2.4. Linear relationship between skull length and the averaged degree of sutural closure in *D. novaehollandiae* (n= 24). A) This relationship has a person's coefficient of correlation (r) of 0.86. The approximate onsets of skeletal and sexual maturity are mapped on the trend line. Below the trend line are the different ontogenetic categories, indicated with the same color code as that in figure 2B. Each data point (i.e., each skull) is characterized by its age (see legend in black square). This relationship shows an obvious increase in the degree of sutural closure as ontogeny proceeds, in other words sutures become more closed.

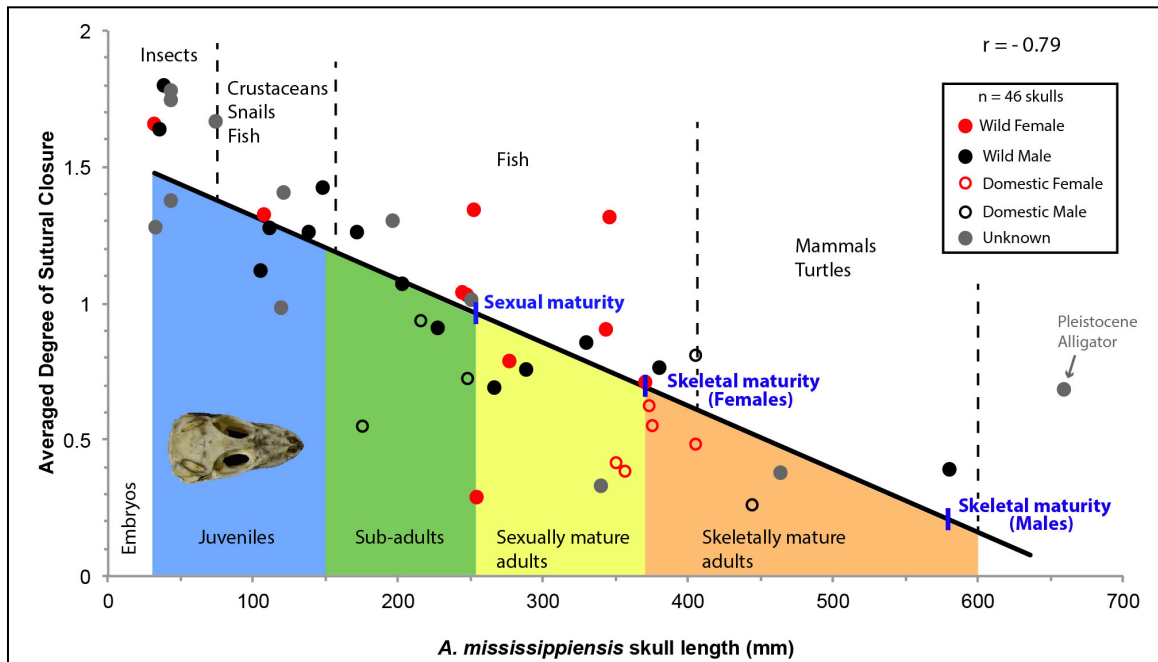


Figure 2.5. Linear relationship between skull length and the averaged degree of sutural closure in *A. mississippiensis* ($n = 46$). A) This relationship has a Pearson's coefficient of correlation (r) of -0.79 . The approximate onsets of sexual maturity and skeletal maturity for females and males are mapped on the trend line. Below the trend line are the different ontogenetic categories, indicated with the same color code as that in figure 3B. Above the trend line are exposed the estimated diet of these animals at the time of death, based on previous studies (see text). Each data point (i.e., each skull) is characterized by its sex and domestic or wild status (see legend in black square). This relationship shows an obvious decrease in the degree of sutural closure as ontogeny proceeds, in other words, sutures become more open.

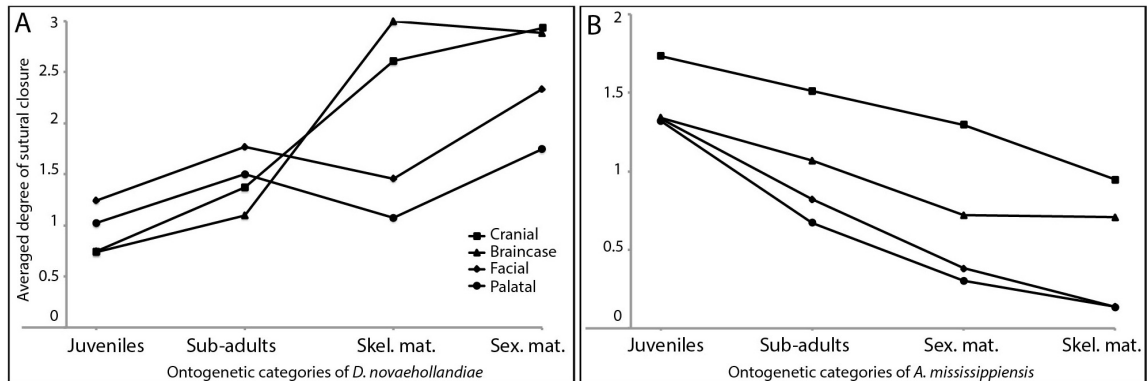


Figure 2.6. Relationship between ontogeny and the averaged degree of sutural closure in the four different anatomical groups of sutures in *D. novaehollandiae* (A) and *A. mississippiensis* (B). A) In the emus, the braincase synchondroses are the first to be completely obliterated (at skeletal maturity), while cranial sutures reach their highest degree of closure later, around the onset of sexual maturity. The least open sutures are the palatal sutures. The degree of closure of facial sutures is intermediate between these two extremes. B) In the alligators, cranial sutures are always more closed than the sutures of any other anatomical group. They are followed by the braincase synchondroses, facial sutures and finally palatal sutures. This 'hierarchy' of closure is similar in both emus and American alligators. Abbreviations: Skel mat., skeletally mature adults; Sex. mat., sexually mature adults.

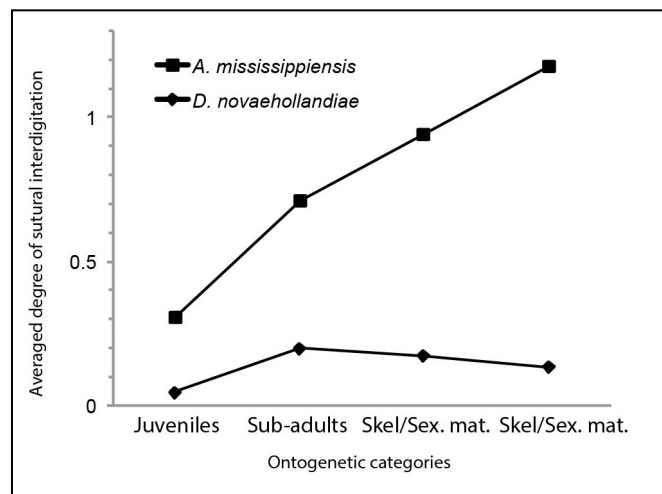


Figure 2.7. Relationship between ontogeny and the averaged degree of interdigitation in *D. novaehollandiae* and *A. mississippiensis*. In *A. mississippiensis*, the degree of interdigitation increases drastically as ontogeny proceeds. In emus, values are much lower (meaning that sutures are more straight than in the alligators overall) and they increase until sub-adulthood. They are followed by a decrease until sexual maturity, but it is an artifact of the coding used in the phylogenetic analysis. The 'real' trend should show an increase of interdigitation followed by a plateau after sub-adulthood in the emus.

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CHAPTER THREE

COMPARATIVE HISTOLOGY OF SOME CRANIOFACIAL SUTURES AND
SKULL-BASE SYNCHONDROSES IN NON-AVIAN DINOSAURS AND THEIR
EXTANT PHYLOGENETIC BRACKET

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Alida M. Bailleul

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

Co-Author: John R. Horner

Contributions: Provided funding and access to specimens. Assisted with study design, discussed implications of results and edited early versions of the manuscript.

Manuscript Information Page

Alida M. Bailleul, John R. Horner.

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Abstract

Sutures are fibrous tissues that unite the skull bones of vertebrates while the cartilaginous junctions between the endochondral bones of the skull-base are known as synchondroses. The degree of sutural and synchondroseal closure has been used for decades in paleontology to assess maturity in mammals and non-avian dinosaurs. Surprisingly, little is known about the biology of these articulations in extant archosaurs, birds and crocodilians. We use paleohistology to retrace the ontogenetic history of craniofacial sutures and skull-base synchondroses in the emu, the American alligator, hadrosaurs, pachycephalosaurs and ceratopsids. Comparisons with mammalian histology from the literature were made. Emus and mammals possess a sutural periosteum, but it disappears rapidly during ontogeny in American alligators. We identify seven types of sutural mineralized tissues in extant and extinct archosaurs and group them into four categories: periosteal tissues, acellular tissues, fibrous tissues and intratendinous tissues. Due to the presence of a periosteum in their sutures, emus and mammals possess periosteal tissues at their sutural borders. The mineralized sutural tissues of crocodilians and non-avian dinosaurs are more variable and can also develop via a form of necrosis for acellular tissues and metaplasia for fibrous and intratendinous tissues. We hypothesize that non-avian dinosaurs, like the American alligator, lacked a sutural periosteum and their primary mode of ossification involved the direct mineralization of craniofacial sutures.

Synchondroseal histology is relatively similar in archosaurs and mammals: they all present four different synchondroseal zones and their resting zone may be replaced by

fibrocartilage and/or aggregates of thick collagenous fibers (i.e., “asbestos transformation”). The only difference that is noteworthy is the ability of crocodilian (and perhaps dinosaurian) synchondroses to turn into sutures late during ontogeny. Our results suggest that synchondroseal cellular organization was relatively well conserved during evolution. However, the microstructural differences between the sutures of archosaurs and mammals are undeniable. This suggests that mammals should not be used as the extant analogues for non-avian dinosaurs in many paleontological matters, including maturity assessment using sutural closure as a proxy.

Introduction

Sutures are the un-ossified fibrous tissues that unite the membrane bones of the skulls of vertebrates (Herring, 2000; Kokich, 1986; Opperman, 2000), while the cartilaginous junctions between the endochondral bones of the skull-base are known as synchondroses (Opperman et al., 2005). Sutures and synchondroses are two different types of articulations, fibrous for the former (without a joint cavity) and cartilaginous for the latter (with a joint cavity) (Marieb, 2014). When these articulations are un-ossified (i.e., open), they allow the growth of the skull during ontogeny (Herring, 2000). They may ‘close’ by fusion of the sutural bone fronts in sutures, or by the replacement of cartilage by endochondral bone in synchondroses. Both have been extensively studied to answer a variety of biological questions, e.g., for the understanding of craniosynostosis in infants (i.e., premature fusion of the bones of the skull; Kokich, 1986; Opperman et al., 2005) or for applications in orthodontology (Kokich, 1976). In the field of paleontology,

these articulations have been used for decades as a proxy to assess the maturity of non-avian dinosaurs. These assessments are based on the observed trend in which vertebrate sutures and synchondroses are patent (i.e., open and un-ossified) early in development and progressively close as maturity is attained (see Cole et al., 2003; Herring, 2000). This mostly comes from observations of mammalian species (e.g., see Krogman, 1930; Marsh, 1887; Todd and Lyon, 1925). In a recent study (Chapter Two) concerning the sequence and timing of sutural closure in the skull of two members of the extant phylogenetic bracket (Witmer, 1995) of non-avian dinosaurs, the emu and the American alligator, this general trend was observed in the former, but not in the latter. In fact, in the American alligator almost all the sutures never fuse during ontogeny (Chapter Two). Since open sutures can sometimes act as shock absorbers, reducing the stress applied to the skull bones (e.g., Herring, 1972, 2000; Jaslow, 1989; Jaslow and Biewener, 1995; Rafferty et al., 2003), it was hypothesized that the pattern seen in American alligators was mostly mechanical and due to diet rather than ontogeny. As a consequence, it was suggested that the use of sutural closure as a proxy for maturity in non-avian dinosaurs should be carefully reconsidered. However, this examination was only based on morphological observations, and since data is lacking concerning the basic biology of archosaurian articulations in general, it is extremely important to start documenting and understanding them at a much finer scale, i.e., the histological level. Therefore, in the present study, the ontogeny of some sutures and synchondroses is characterized at the microscopic level in the extant phylogenetic bracket of the Dinosauria. This was investigated by means of histological analyses in two growth series of skulls: in the emu, *Dromaius*

novaehollandiae and in the American alligator, *Alligator mississippiensis* (for which morphological data was already collected, Chapter Two). Both cranial and facial sutures were analyzed, as well as some braincase synchondroses (Table 1). Paleohistological sections of these same structures were also made in some non-avian dinosaurs and a fossil crocodilian (Table 2). Finally, all these results were compared to what is available in the literature concerning the histology of these articulations in other vertebrates (which is strongly biased towards mammals; Herring 2000). This investigation was undertaken for two reasons: 1) as just mentioned, the way that sutures and synchondroses form and fuse has only been reported in mammals (e.g., Herring, 2000; Kokich, 1976; Opperman et al., 2005; Persson et al., 1978; Pritchard et al., 1956) and unfortunately, nothing is known in the Archosauria. Considering that the assumptions applied to non-avian dinosaurs are often based essentially on mammalian observations (e.g., sutural patency indicates juvenescence and sutural closure indicates maturity), it is important to understand how these articulations form and fuse in the extant phylogenetic bracket of non-avian dinosaurs. Even though sutures and synchondroses are present in all vertebrates, it is not implausible to hypothesize that archosaurian and mammalian articulations (as well as their associated mineralized tissues) have considerably different histologies, due to the phylogenetic distance between these two clades. 2) It is important to document how sutures with different morphological degrees of closure (e.g., open or obliterated) appear at the microscopic scale, because morphology and histology could show contradicting results. Indeed, even though closure may be evident morphologically, the fusion of sutures cannot be confirmed until they are investigated with histological techniques (e.g.,

Cole et al., 2003; Herring, 2000). We wanted to test whether the articulations that were morphologically established as obliterated in the emu (Chapter Two) are obliterated histologically as well. On the other hand, it is also important to verify if the articulations that were considered ‘opened’ morphologically in the American alligators (Chapter Two) are also open histologically.

Since the use of sutural and synchondroseal closure as a proxy for maturity in non-avian dinosaurs is very abundant in paleontology nowadays, and since data is lacking concerning these structures in general in any non-mammalian group of animals, the main goal of our study was to gain an understanding of their biology beyond the mammalian monopoly, more precisely in the Archosauria. Perhaps the types of bone formation and bone fusion in the sutures of archosaurs and mammals are not comparable. If this is the case, the maturity assessment method involving sutural closure (developed for and within Mammalia) should no longer be employed in the Archosauria. To frame methodology and analyses, a brief review of the basic biology and histology of sutures and synchondroses is necessary.

Biology and Histology of Sutures

Human anatomy textbooks and neontologists describe “sutures” as the fibrous connective tissues between two membrane bones (Fig. 1; Herring, 2000; Kokich, 1986; Marieb, 2014). The borders of the bone in contact with a suture have been described by various terms such as “juxta-sutural bone” (Enlow, 1990), “sutural bony margins” (Kokich, 1986) or “sutural bone fronts” (Opperman, 2000). In the present study, they will be called sutural bone or sutural mineralized tissues (Fig. 1). It is common in

paleontological studies to see a more global definition for the term “suture”, which often includes the cartilaginous articulations between endochondral bones (e.g., the neurocentral ‘sutures’ of the vertebrae, the ‘sutures’ of the skull base, those between the scapula and coracoid, or even in the epiphyses of long bones; see Cole et al., 2003). The proper biological and medical term for these cartilaginous articulations are “synchondroses” (Fig. 2; in the skull base and vertebrae) and “epiphyseal growth plates” (in limb bones). It is important that the distinction between these two terms is kept in the present study because the embryological origins and histologies of these articulations are completely different (e.g., synchondroses have articular cavities while sutures do not; compare figure 1 with figure 2).

While sutures are patent, they function as compensatory growth sites during craniofacial development (Opperman, 2000). In other words, they allow bone formation at the edges of the sutural bone fronts in response to their soft-tissue environment, which is mostly the expanding brain in mammals (Kokich, 1986). Bone formation (and resorption) at the sutural margins is not a primary growth force of the skull, but it is secondary since it occurs as a response (also known as an adaptive force, see Hall, 2005). The preservation of the overall shape and dimensions of bones during the growth of the skull (at least in mammals) is maintained by remodeling apposition and resorption of the periosteal and endosteal surfaces (Enlow, 1990). Sutural obliteration occurs by fusion of the bone fronts across the suture (Opperman, 2000), however obliteration is optional since sutures often never fuse throughout the ontogeny of many species (e.g., those of some carnivoran mammals; Goswami et al., 2013, some ruminants; Bärmann and Sanchez Villagra, 2012,

snakes; Herring, 2000; or American alligators; Chapter Two). After fusion, the ‘suture’ is called a synostosis (Marieb, 2014). It is commonly assumed that sutural obliteration marks the cessation of any growth. This assumption has been shown to be incorrect and growth is still possible after fusion of cranio-facial sutures (Cohen, 1993, 2000). Despite this, the relationship between closure (seen morphologically), fusion (seen histologically) and the overall growth of the skull remains ambiguous and is undoubtedly not simple. Studies concerning the precise microscopic structure of cranio-facial sutures through ontogeny remain rare and exclusively concern humans (e.g., Kokich, 1976; Persson and Thilander, 1977) and other mammals (mostly rats and rabbits: e.g., Moss, 1958; Persson, 1973; Pritchard et al., 1956).

The fibrous tissues in sutures are known as the sutural ligament. The major differences between facial and cranial sutures is that the former originate from the mesenchyme, while the latter differentiate in a preformed fibrous membrane (the ectomeninx) surrounding the brain (Pritchard et al., 1956). Sutures are composed of (1) the periosteum of the two separated bones, (each having a cambial layer containing collagenous fibers, osteoprogenitor cells and osteoblasts, and a rarely observed capsular layer composed mostly of collagenous fibers, small blood vessels, and fibroblasts) and (2) a middle layer that is non-osteogenic and composed mostly of collagenous fibers, mesenchymal cells and fibroblasts (Fig. 1; Pritchard et al., 1956). They are encapsulated in dense fibrous connective tissues called uniting layers (Fig. 1; and see Pritchard et al., 1956). Some authors have however noted that the differentiation between all these sutural layers is sometimes difficult, and that it is not uncommon to observe only one single

osteogenic layer throughout the whole suture (e.g., Persson, 1973, see its figure 1 p. 10 to compare all the sutural layers reported by different authors).

The middle layer is first called the “presumptive suture mesenchyme”, and then differentiates into a “suture” proper, more fibrous and more cellular (e.g., see Opperman, 2000). The periosteum is continuous with the ectomeninx (preformed in cranial sutures, but formed secondarily in facial sutures; Pritchard et al., 1956). Once cranial and facial sutures are fully formed, it is thought that they grow in the same way. However recent studies suspect that the different tissues underlying these two types of sutures (i.e., cartilage of the nasal capsules in the face *versus* the dura mater in the calvaria) might play different roles during their growth; Opperman, 2000).

Sutural bone is usually woven early in development, but is resorbed and replaced by lamellar bone later during ontogeny (Pritchard et al., 1956). Sutures have three distinct shapes: flat (or straight), interdigitated and beveled (or overlapping) (Herring, 1972, 2000). Through ontogeny it is common for sutures to start flat and to develop increasing degrees of interdigitations with age, with high degrees of interdigitations seen in locations that are heavily loaded (Herring, 2000). Interdigitations seem to be associated with compression and flat sutures with tensile stresses (see Herring, 1972 figure 10 p. 238; Herring and Mucci, 1991; Rafferty and Herring, 1999; Sun et al., 2004). After the bone fronts fuse, interdigitations disappear and the suture is often replaced by bone marrow and blood vessels (Pritchard et al., 1956). The mode of sutural fusion (i.e., interpreted from the type of bone/mineralized tissue bridging the suture) is thought to be mostly made via ‘normal’ intramembranous ossification (e.g., Kokich, 1976; Persson and

Thilander, 1977; Pritchard et al., 1956; Hinton et al., 1984) or via chondroid bone formation, an intermediate tissue between bone and cartilage (e.g., Goret-Nicaise et al., 1984, 1988; Manzanares et al., 1988; Rafferty and Herring, 1999). The latter has been reported as the “major vector of sutural growth” during early development (i.e., embryonic and early post-natal) but it is resorbed rapidly a few months after birth (Goret-Nicaise et al., 1988).

Finally, a type of cartilaginous tissue, called secondary cartilage, is sometimes found in the sutures of mammals. It arises after bone formation on membrane bones, and is restricted to fetal and/or juvenile specimens (e.g., Kokich, 1986; Persson, 1973; Vinkka-Puhakka, 1991). By definition, secondary cartilage cannot be found in synchondroses (*contra* Ikejiri, 2012) because the latter form in between endochondral bones, not membrane bones. In birds (Hall, 1967, 1968) and non-avian dinosaurs (Bailleul et al., 2012, 2013), it has been found at many jaw articulations, but not in the sutures *per se*. This suggests that it would be unlikely to find secondary cartilage in the specimens analyzed here (especially in specimens of relatively late ontogenetic stages, Tables 1 and 2).

Biology and Histology of Synchondroses

Unlike sutures, synchondroses are cartilaginous and have an intrinsic growth potential (recall that bone growth occurs in response to extrinsic factors in sutures; Opperman et al., 2005). At the histological level, synchondroses are unique: they can be described as two epiphyseal growth plates positioned back to back with one common resting zone and mirroring proliferative zones (where cellular divisions occur),

hypertrophied cartilage/calcification zones and ossification zones (Fig. 2; and see Cendekiawan et al., 2010; Opperman et al., 2005). As a consequence of these mirroring layers, synchondroseal growth is bidirectional (Fig. 2), as opposed to that of an epiphyseal growth plate which is unidirectional (e.g., see Barreto et al., 1993).

Unfortunately, even in mammals, little is known about the biology and histology of synchondroses (in both the cranial base and the vertebrae). Some authors argued that this is due to the common assumption that synchondroses function like the growth plates of long bones (Dixon and Gakunga, 1993; Opperman et al., 2005). Nevertheless, it has been shown in the fetal rat that synchondroses start out as “presumptive synchondroses” composed of undifferentiated mesenchymal cells (Dorenbos, 1973). At this time the adjacent cartilaginous models of some endochondral elements of the braincase are already formed (i.e., chondrocytes are already present in the basioccipital, but not in the presumptive basioccipital-basisphenoid synchondrosis; Dorenbos, 1973). Only at the end of embryonic development are the different cell layers of the synchondroses differentiated into chondrocytes and organized, only then can this union be called a true synchondrosis (Dorenbos, 1973). The post-natal ontogeny of the spheno-occipital synchondrosis of humans has also been studied (Thilander and Ingervall, 1973).

Obliteration of this synchondrosis is made via endochondral ossification. The hyaline cartilage of the synchondrosis can also be replaced by fibro-cartilage in some instances (Thilander and Ingervall, 1973).

Material and Methods

Specimens

The extant archosaurs consist of two growth series of six skulls each: of the emu, *D. novaehollandiae*, and the American alligator, *A. mississippiensis* (Table 1). All these skulls belong to the osteology collections of the Museum of the Rockies (MOR) and all were originally obtained from animals that had died of natural causes. Five of the emu specimens came from Montana Emu Ranch (MER, Kalispell, Montana). MOR OST 232 had been previously purchased online from a legal commercial website (Skulls Unlimited). Fleshy heads from MER were sent frozen to the MOR and then defleshed by a dermestid beetles colony (Deer Lodge, Montana). MOR OST 1801 was not defleshed and its soft tissues were included in the histological slides. The estimated age of these specimens is shown in Table 2. Only three specimens had a precise known age (MOR OST 1801, MOR OST 1802 and MOR OST 1803) provided directly by the emu rancher (D. Collis, MER). Specimens were placed into ontogenetic categories according to data available in the literature about the growth of this species (e.g., Minnaar and Minnaar, 1992; Davies, 2002). All these specimens had previously been examined for another study (Chapter Two).

Three of the American alligator skulls (MOR OST 1796, MOR OST 1795, MOR OST 1789) previously defleshed by dermestid beetles were also purchased online from legal commercial websites (Skulls Unlimited and Atlantic Coral Enterprise). The other three alligators heads were from the Rockefeller Wildlife Refuge (Louisiana) and their soft-tissues were kept for histological analyses (MOR OST 1647, MOR OST 1797, MOR

OST 1798). Their age (and estimated ontogenetic categories) were calculated/evaluated by using data previously published on the growth curve of this species, and using their geographic provenance and their skull length as a proxy (Chabreck and Joanen, 1979; Woodward et al., 1995). Note that all these emu and alligator skulls (not the heads) were prepared by dermestid beetles, because this is thought to be the best method to preserve bony articulations.

Most of the fossil specimens came from the collections of the MOR, and had been previously collected during various field seasons in Montana (Table 2). Some specimens belong to the University of California Museum of Paleontology (UCMP), the Tyrell Museum of Paleontology (TMP) and Sierra College in California (VRD). Fossil taxa include members of the Crocodylia and Ornithischia (Hadrosauridae, Ceratopsidae, Pachycephalosauridae). Their estimated ontogenetic stage is also shown (E. Freedman-Fowler, J. Scannella, personal communications; J. Horner, personal observation). They were all isolated bony elements containing sutures and do not come from articulated skulls.

Histological and Paleo-Histological Preparation

The same three sutures were systematically chosen and sectioned in the extant specimens. These include the fronto-parietal suture (cranial), the internasal suture (facial), and the exoccipital-basioccipital synchondrosis (in the skull base). In some fossil specimens, it was not possible to cut these specific sutures therefore the most adjacent sutures were sectioned (e.g., jugal-epijugal and interfrontal sutures; Table 2). All ground

sections were un-decalcified and have a final thickness of 80 microns to 120 microns (ground sections of the extant specimens being generally thinner than the fossils).

Preparation of Extant Specimens: Small fragments of bone possessing the sutures/synchondroses of interest were extracted from previously defleshed skulls using a dremel and a diamond blade. These fragments were then fixed in 10% buffered formalin solution for three to four days (with one solution change per day), then transferred to a solution of 70% Ethanol (EtOH) and serially dehydrated in graded solutions of 80%, 95% and finally 100% EtOH. The time spent in each of these solutions varied from 24 hours to three days depending on the size of the extracted fragments, but specimens were never in 100% EtOH for more than 24-48h (according to methods suggested by A. Lee, personal communication). The cranial and facial fragments of alligator bone took considerably longer than any of the emu fragments.

When the fleshy heads were relatively small, they were entirely fixed in formalin and the bony fragments with their associated soft-tissues were extracted and transferred to EtOH separately (MOR OST 1801 and MOR OST 1797). One fleshy head was too large to be fixed entirely in formalin so the fragments were extracted from it while it was still frozen (MOR OST 1798). The head was put back into the freezer after extraction. All the bony fragments that have associated soft-tissues (i.e., from the fleshy heads) were embedded in Poly-methyl-methacrylate (PMMA) because this resin preserves tissues better than resins generally used for paleohistological embedding (e.g., epoxy or polyester resins; A. Lee and E-T Lamm, personal communications). Almost all the bony fragments coming from dry skulls were embedded in epoxy resin in a vacuum chamber

for 5 minutes (Epothin 2™, Buehler™; see the embedding media of each specimen in Table 1). Prior to epoxy embedding, fragments were cleared in xylene following dehydration. After 2 days (time for the resin to set), they were treated in the same manner as small fossils (according to the techniques established for small fossil thin-sectioning by Lamm (2013, see following paragraph). Two sections were made for each suture/synchondrosis, leaving one slide unstained and the other one stained with Toluidine-blue (staining performed at the MOR). Three of those unstained slides were stained with Masson's trichrome at the Veterinary Diagnostic Laboratory in Bozeman, Montana. Slides coming from frozen heads gave more details about the un-mineralized parts of the sutures and synchondroses than those of the skulls defleshed by dermestid beetles, which showed some soft-tissue degradation. However, the latter slides still showed well-preserved structures at the bony sutural borders (and even in the sutures and synchondroses in some instances).

Preparation of Fossil Specimens: Molds and casts were made of all the bones before sectioning to retain morphological data. A few important casts were painted and restored into the real bone. Bones were embedded in either polyester (for large specimens) or epoxy resin (for small specimens, with Epothin 2™, Buehler™). Small specimens were sectioned with a Norton 5" or 7" diamond-edge blade on an Isomet precision saw (Buehler™). Thick sections (between 1.0 and 1.3 mm) were mounted on plastic (plexiglas) slides with cyanoacrylate glue. Large specimens were sectioned with a tile saw and the thick sections (from 2.5 to 3.5 mm) were mounted on glass slides with 2-ton epoxy resin (on the side previously frosted with aluminum powder). All mounted

thick-sections were then ground by hand on a Buehler Ecomet™ grinder with water and silicon carbide paper of decreasing grit sizes: 60, 120, 180, 320, 400, 600 and 800. For the small slides, the rougher papers (60, 120 and 180) were not used. Sections were then polished on the grinder with 800 grit paper and 5µm aluminum oxide powder (with the paper slightly wet). Finally, they were polished by hand with wet polishing cloths and more aluminum oxide powder (5µm then 1µm). Finished petrographic thin-sections were studied by light microscopy under normal and polarized light with a Nikon Optiphot-Pol polarizing microscope. Photographs were taken with a Nikon DS-Fi1 digital sight camera and the NIS Elements BR 4.13 software. Whole slide images (of the fossils only) were digitized on a Canon Canoscan 8800F flatbed scanner. One picture (Fig. 4H) was taken with a Jenoptik ProgRes C14Plus camera, with the software ProgRes Capture Pro on a Zeiss Axio Scope A1. Note that many of the petrographic thin-sections of the pachycephalosaurs were already made. They are published in Goodwin and Horner (2004) and Horner and Goodwin (2009).

In the following section, we describe both un-mineralized soft tissues (in the extant specimens only) and mineralized tissues (in the extant and fossil specimens). Soft-tissues were not preserved in the fossils, and the original structures are filled in with sediments, minerals and/or broken fragments of bone. The soft-tissues that are described include 1) the sutures and 2) the un-mineralized parts of the synchondroses (see Figs. 1 and 2). Descriptions of these structures are made in the extant specimens who were embedded with their soft tissues and are avoided in the dry skulls (due to some soft-tissue deterioration, unless preservation was exceptional or the dry specimens showed very

important features). The extant specimens will not be designated by their MOR OST numbers but as Emu 1 through 6 and Alligator 1 through 6 (see Table 1). The described mineralized tissues include 1) the sutural mineralized tissues and 2) the mineralized parts of the synchondroses (see Figs. 1 and 2). Emphasis will not be put on the non-sutural mineralized tissues, nor on those bordering the edges of the synchondroses unless they show unique characteristics.

Results

Cranial and Facial Sutures

Extant Archosaurs

Cranial Sutures: Figure 3 shows selected specimens of *D. novaehollandiae* stained with Toluidine blue (Emu 1, Fig. 3A; Emu 3, Figs. 3B, D, E; Emu 6, Figs. 3C, F). The suture is beveled and does not form any interdigitations throughout ontogeny. In Emu 1, the sutural borders are composed of woven bone with many simple vascular canals typical of an actively growing bone (Fig. 3A). Lamellar bone starts to form at the stage of Emu 3 and 4 (8 to 10 months) and the suture is still completely open during this stage (Fig. 3B). In Emu 5 and 6, the fronto-parietal suture is completely obliterated and the entire elements are composed of trabeculae of lamellar bone (Fig. 3C). No osteonal tissue is present even in these late stages of ontogeny. The suture is replaced by bony trabeculae and bone marrow spaces (Fig. 3F). In Emu 3, the suture is encapsulated in a uniting layer composed of a dense regular connective tissue (Fig. 3D, double arrows). A

periosteal cambial layer with numerous osteoblasts can be seen right next to the sutural bone (Fig. 3E, double arrows) and it appears to be continuous with the periosteum located on the ectocranial side of this element (Fig. 3E). The suture is also composed of a middle layer that is less cellular (Fig. 3E, white asterisk). Pritchard et al., (1956) reported a periosteal capsular layer surrounding the cambial layer in the sutures of some mammals, but it is not observed here in the emu or in any other suture of this sample.

The fronto-parietal suture in *A. mississippiensis* has a very different histology (Fig. 4). Selected specimens stained with Toluidine blue and Masson's trichrome are shown: Alligator 1 (Fig. 4A), Alligator 3 (Figs. 4B, D-E) and Alligator 6 (Figs. 4C, F-K). This suture stays open during ontogeny in the American alligator (i.e., the sutural mineralized tissues never meet; Fig. 4). This is in accordance with the morphological observations found in Chapter Two. Through ontogeny, interdigitations increase in number and in size and the sutural mineralized tissues become much more fibrous (Figs. 4A-C). Alligator 1 shows two thin struts of woven bone (the frontal on the top and the parietal on the bottom; Fig. 4A). In the suture, a loose irregular connective tissue can be seen (Fig. 4A) and it is not comparable to the dense fibrous tissue that was observed in the emu (Fig. 3). Perhaps this loose connective tissue represents a presumptive suture (i.e., an incompletely formed suture). In Alligator 3 and 4, the sutural bone is still woven with very little vascularization, but the non-sutural bone has a few primary and secondary osteons. The suture is now formed of a dense connective tissue (Figs. 4D, E; asterisks). Only one uniform layer can be discerned in the suture, with collagenous fibers and a few fibroblasts (see the elongated cell nuclei in blue; Figs. 4D, E, black asterisks). Unlike the

sutures of emus (Fig. 3E), the crocodilian sutures do not show any periosteal cambial layer. In some areas, fibers are thick and continuous between the sutural bone and the suture itself (Fig. 4E, arrow). In the most mature alligators (Alligator 5 and 6), two important features can be seen: 1) a very high fibrous content (Fig. 4C) and 2) an enigmatic sutural mineralized tissue (Fig. 4F). Due to the absence of periosteum in the sutures and the high fibrous content of the sutural mineralized tissues, it is difficult to characterize the fronto-parietal bones of these specimens as ‘regular’ intramembranous bone. Toluidine blue reveals this enigmatic sutural mineralized tissue with a light purple stain that clearly demarcates it from the darker woven bone (Figs. 4F, G). It also possesses simple vascular canals (Fig. 4F, arrows). At higher magnification, the entire tissue lacks the characteristics of regular bone (Fig. 4G). The vascular canal has irregular internal borders and the tissues surrounding this canal show can be differentiated into cellular, acellular, fibrous and non-fibrous zones. The cellular zones show cellular lacunae resembling plump osteocytes with canaliculi. At higher magnification (Fig. 4H) acellular zones have globular structures with arc-shaped borders (black arrows) and dark spaces of many different shapes (yellow arrows). While these dark spaces could potentially be cellular lacunae, they do not have the appearance of regular osteocyte lacunae. In an adjacent section of the same suture, Masson’s trichrome reveals that this tissue is present almost everywhere along the sutural borders (Fig. 4I; black arrows). It does not stain like the core of the interdigitations (see the clear junctions labeled with white arrows). Higher magnification reveals that this tissue stains with blue and red mottles (Fig. 4J), while the core of the interdigitations only stain red. Unfortunately we

have not been able to identify the meaning of these differences in staining. In rare instances, some Howship's lacunae can be seen which indicate that it is a mineralized tissue (Fig. 4K).

Facial Sutures: The internasal suture of the emu stained with Toluidine blue is shown in figure 5 (Emu 3, Figs. 5A, C-E and Emu 6, Fig. 5B). The cartilage of the nasal capsule is stained purple (Fig. 5A). As opposed to the fronto-parietal suture of the same species, this suture never fuses during ontogeny (i.e., it is still open in Emu 6, a 20 year-old male; Fig. 5B). Through ontogeny, both the sutural bone and the non-sutural bone transition from being woven with simple vascular canals (Figs. 5C-D) to a compacta completely reworked with secondary osteons (Fig. 5B). The internasal suture is composed of different layers: (1) a thick cambial layer with numerous osteoblasts, continuous with the periosteum located more ectocranially (see the nuclei stained in blue, double white arrows), (2) and a middle layer (white asterisk). The latter is a vascularized loose irregular connective tissue (see veins and arteries, black arrows) (Fig. 5C). A mineralized tissue with cartilage-like cell lacunae embedded in a bone matrix was seen in the sutural areas of Emu 3 (Fig. 5E). It contains the features of chondroid bone, a tissue that has already been reported in the sutural areas of the chick, some mammals and a hadrosaur (e.g., see Bailleul et al., in press, Chapter Six).

The ontogeny of the internasal suture of the American alligator is shown in figure 6. It is shown in Alligator 1 (Fig. 6A), Alligator 3 (Figs. 6B, D, E), Alligator 4 (Fig. 6F) and Alligator 6 (Figs. 6C, G). It is extremely similar to that of the fronto-parietal suture of this same species (Fig. 4). In Alligator 1, the suture is beveled and the sutural bone is

composed of two small struts (Fig. 6A). The suture is composed of two layers: a dense connective tissue closest to the sutural bone, and a middle layer that looks similar to the loose connective tissue reported previously (Fig. 4A). It is unclear if the layer closest to the sutural bone represents a periosteum. Through ontogeny, the interdigitations increase in number and size while the sutural bone becomes much more fibrous (Figs. 6B-C). In the suture of Alligator 3, the central loose connective tissue layer has disappeared and a uniform layer made of a dense fibrous connective tissue with fibroblasts can be seen (see the elongated cell nuclei stained in blue, Figs. 6D, E). Once again, this suture appears to be lacking a periosteum. The collagen fibers can be either parallel or perpendicular to the suture (Fig. 6D, black arrow and Fig. 6E). The ectocranial part of this suture in Alligator 4 is filled with numerous circular structures resembling nerves (Fig. 6F, black arrow). A neurovascular bundle is found within a depression on the ectocranial side of this same bone and perhaps these nerves could be part of a neuronal network. In the most mature alligators, the sutural mineralized tissues are extremely fibrous and show dark lamellae that are parallel to the suture and separated from each other by brighter-colored zones (Fig. 6G). This organization is similar to lamellar-zone bone (Francillon-Vieillot et al., 1990), but these brighter zones do not appear to be lines of arrested growth (LAGs).

Fossil Archosaurs/Non-Avian Dinosaurs

Cranial Sutures: Crocodilian: The fronto-postorbital suture of a fossil crocodilian (MOR 552) is shown in figure 7 (red arrows). Like the fronto-parietal and the internasal sutures of modern American alligators, this suture is open and interdigitated (Figs. 7A,

B). The fibrous suture is not preserved and is filled in by minerals (Fig. 7B). As it was observed in the most mature alligators (Fig. 6G), the mineralized sutural tissues are composed of alternating lamellae resembling lamellar-zonal bone (Francillon-Vieillot et al., 1990, Fig. 7C). However, as it was observed in the modern alligators, the limit between each lamella does not appear to represent a complete arrest of growth, but only a slowing of growth (compare with Fig. 7E that shows clear LAGs, white arrows). Polarized light indicates that these lamellae are made of woven tissues (Fig. 7D). All of this evidence suggests a relatively rapid growth of the sutural mineralized tissues compared to non-sutural tissues.

Hadrosaurs: Four hadrosaur fronto-parietal bones were sectioned but only two of them are shown here, a juvenile *Brachylophosaurus*, (Figs. 8A-D) and an adult *Gryposaurus* (Figs. 8E-H) as the remainder were diagenetically altered too greatly to permit histological analysis (see Table 2). In *Brachylophosaurus* (MOR 1071) and *Gryposaurus* (MOR 2573), the frontoparietal suture is completely open and filled in with minerals (e.g., Fig. 8C). It is interdigitated (Figs. 8A, E, red arrows), but the interdigitations are not as sharp as the ones present in the modern and fossil crocodilians described previously (Figs. 4, 6, 7). In *Brachylophosaurus*, part of the parietal-postorbital suture can also be seen (black arrow). The sutural mineralized tissues found in these two specimens are identical: the sutural borders are composed of a tissue that looks extremely fibrous and cellular at low magnification (Figs. 8B, F). A compacta composed of secondary osteons can be seen bordering this tissue (Fig. 8B). The latter forms entire mineralized struts in the parietal-postorbital suture of *Brachylophosaurus* (Fig. 8C). At

higher magnification, this tissue shows globular structures (Figs. 8D, H, white arrows) separated from each other by dark spaces of different shapes, many of which are arcuate-shaped (Figs. 8D, H, blue arrows). This organization is similar to that found in modern alligators (Fig. 4H).

Pachycephalosaurs: The cranial sutures of some Pachycephalosauridae are shown in figures 9 and 10 including the fronto-parietal sutures of a juvenile *Stegoceras* (UCMP 130049, Figs. 9A, C-D), a juvenile Pachycephalosauridae indet (TMP 1974.10.74, Figs. 9B, E-F), the interfrontal suture in a Pachycephalosauridae indet (UCMP 154919, Figs. 9G-J), and the parietal-postorbital sutures of a Pachycephalosauridae indet (MOR 2915, Figs. 10A-F), and *Pachycephalosaurus* (VRD 13, Figs. 10G-K). Two more specimens were sectioned but they are not shown here because their histology is similar to other specimens (for UCMP 128383), or because they were diagenetically altered beyond reasonable limits for histological analysis (for MOR 2555). The two small fronto-parietal domes show obliterated sutures on the ectocranial side, but open sutures on the endocranial side (Figs. 9A-C, red arrows). On the endocranial side the suture is slightly interdigitated (e.g., Fig. 9C), but ectocranially it is completely obliterated and replaced by a mineralized tissue displaying the characteristics of intramembranous bone (Fig. 9D). In TMP 1974.10.74, the majority of the mineralized sutural borders are composed of a highly fibrous tissue with cellular and acellular zones (Fig. 9E). This same tissue bridges the suture in some areas (Fig. 9F).

In the interfrontal suture of UCMP 154919 (Fig. 9G, red arrows) the sutural borders are slightly undulating (Fig. 9H). This suture is open and the sutural borders are

formed of another type of fibrous tissue (compare Figs. 9E with 9H). At higher magnification one of these struts shows a central acellular core with fibers surrounded by a tissue containing numerous dark spaces. These dark spaces are too wide to be fibers cut in a transverse plane. They also do not look like osteocyte lacunae since they are irregularly shaped and do not present canaliculi. Two options are possible: they are the cellular lacunae of another cell type (e.g., fibroblasts or fibrocytes or they are osteocyte lacunae that were mineralized and underwent ‘acellularization’. Acellularization of cellular bone has been described in teleosts and this process involves osteocytes becoming pyknotic (or necrotic) and subsequently mineralizing by “intracellular calcification” (Moss, 1961, 1963; Meunier, 1989). The fact that these cellular lacunae (1) have different sizes and shapes and (2) that some tissues are completely acellular in many ‘non-sutural’ areas (see Fig. 9J, but also Goodwin and Horner, 2004) suggest that a similar acellularization process is occurring here. Perhaps newly formed sutural mineralized tissues are cellular but they undergo intracellular calcification later during ontogeny (i.e., once relocated to the ‘non-sutural’ areas, Fig. 9J).

The parietal-postorbital suture of MOR 2915 is straight (red arrows, Fig. 10A). The mineralized struts composing the sutural borders have a different orientation to that of the non-sutural mineralized tissues (i.e., they are perpendicular to the suture, Fig. 10A). Higher magnification shows that this suture is obliterated and replaced by vascular and/or marrow spaces (Figs. 10B-C). Three different types of tissues bridge the suture in this specimen: an acellular fibrous tissue (Figs. 10C-D), a cellular fibrous tissue (Fig. 10E, similar to that present in Figs. 9E, I), and an extremely fibrous tissue (Fig. 9F). The

organization of this last tissue (i.e., with bony lamellae parallel to the suture) looks rather similar to that found in the most mature alligator (Fig. 6G) and fossil crocodile (Fig. 7C) specimens.

In the last pachycephalosaur specimen (an adult *Pachycephalosaurus*), the parietal-postorbital suture is open on the ectocranial side and closed on the endocranial side (Figs. 10G-H, J). It is straight ectocranially but becomes slightly interdigitated endocranially (Fig. 10G). On the ectocranial side, the mineralized sutural borders are formed of bright acellular bands directly bordering the suture (Fig. 10I, arrow) and a fibrous tissue more internally (Fig. 10I). These fibers are similar to the ones just described (Fig. 9E, F, 10F). On the endocranial part, the suture is synostosed and replaced by this this bright acellular tissue (Figs. 10J-K, blue arrows). Once more, it appears that some sort of acellularization occurred since higher magnification reveals cellular lacunae with different shapes, perhaps all undergoing different levels of intracellular calcification (Fig. 10K): some areas have numerous large cell lacunae (white arrows), other only show a few irregular lacunae (green arrows), and finally some are completely acellular (blue arrows). In this adult specimen, it appears that the parietal-postorbital suture was replaced by this acellular tissue.

Facial Sutures: Triceratops: The interpremaxillary and the nasal-premaxillary sutures were sectioned in an adult *Triceratops* (MOR 8661, Fig. 11B, red arrows). An isolated nasal of a younger specimen (MOR 2587) is shown for anatomical purposes (Fig. 11A). The nasal-premaxillary suture in MOR 8661 is completely synostosed and replaced by trabeculae of intramembranous bone (perhaps endosteal bone) and bone

marrow/vascular spaces (Fig. 11C). The interpremaxillary suture is also synostosed, but its former position is indicated by a large canal along its whole length. The function of this canal is unclear, but it undoubtedly belongs to the morphologically observed tube-like structure on the ventral side of these elements (Fig. 11E). Even though this ‘tube’ obscured the sutural histology of this specimen, it appears that sutural closure in the interpremaxillary and the nasal-premaxillary sutures of *Triceratops* was made via intramembranous ossification.

The jugal-epijugal and jugal-quadratojugal sutures of two adult *Triceratops* are shown in figure 12 (UCMP 174838, Figs. 12A, C-F; and MOR 2570, Figs. 12B, G-H). These sutures are open in UCMP 174838 (Fig. 12A) and MOR 2570 (Fig. 12B) except for the lateral side of the jugal-epijugal suture of UCMP 174838 (Fig. 12A). They do not show any interdigitations. In UCMP 174838, two types of sutural mineralized tissues can be found. First, a tissue with highly disorganized fibers (Fig. 12C) is seen that looks exactly like the tissue found at the periosteal borders of this same element (e.g., see the periosteal borders of the epijugal, Fig. 12D). This fibrous tissue presents the characteristics of the “metaplastic bone” that has been found in the parietal frill of this same species (Horner and Lamm, 2011, see their figures 3C and 6B-C, and see discussion for further elaboration). It was also involved in the sutural obliteration of the lateral part of the jugal-epijugal suture of this specimen (data not shown). The second mineralized sutural tissue that was found shows mineralized lamellae parallel to the suture (Fig. 12E, white arrows). At higher magnification, this arrangement into lamellae is less visible (Fig. 12F) and instead it is composed of numerous black spaces resembling cellular

lacunae (Fig. 12F, white arrows). These lacunae however do not look like regular osteocyte lacunae (compare with the osteocytes of the adjacent lamellar bone, blue arrows, Fig. 12F), and yet they are different from the other enigmatic cellular lacunae observed in UCMP 154919 (Fig. 9I). Horner and Lamm (2011) reported these same structures and hypothesized that they are not osteocyte lacunae, but rather are spaces fitted between bundles of collagen fibers cut in a transverse direction (see their figure 8E, and discussion for further elaboration). In MOR 2570, a third sutural mineralized tissue was found (Figs. 12G-H). It presents the general characteristics of the acellular tissue previously described in the pachycephalosaurs (Figs. 9-10) with areas containing irregularly shaped cellular lacunae (white arrows, Fig. 12H) and other completely acellular areas.

Synchondroses of the Skull Base

Extant Archosaurs: The basioccipital-exoccipital synchondrosis is shown in a growth series consisting of Emu 1 (Figs. 13A, D), Emu 3 (Figs. 13B, E-G), Emu 4 (Figs. 13H-I) and Emu 6 (Fig. 13C) stained with Toluidine blue. The bone stains blue, the hyaline cartilage light purple and the calcified cartilage dark purple. The synchondrosis is straight in Emu 1 (Fig. 13A), slightly undulating in Emu 3 (Fig. 13B) and completely ossified in Emu 6 (Fig. 13C). In this 20 year-old emu the synchondroseal cartilage has been eroded and replaced by endochondral ossification (Fig. 13C). Emu 3 and 4 show that ossification starts from the lateral side of the synchondrosis (Figs. 13B, E, yellow arrows).

In the youngest emu the differentiation between the resting and the proliferative zone is difficult, but in Emu 3 and 4 all the different synchondroseal zones are easily recognizable (Fig. 13F; except in the most lateral parts of the synchondrosis, where no cellular organization into resting and proliferating zones can be seen, Figs. 13H-I). In Emu 3 and 4 the proliferative zone is composed of 6 to 12 cells oriented into columns (white arrows, Fig. 13G). According to Trueta and Trias (1961), the more numerous the cells are in this layer, the higher the growth rate is. The number of hypertrophic cartilage cells is usually 10 to 15 (Fig. 13F). It appears that the unmineralized hyaline cartilage of the resting zone becomes fibrocartilage and/or undergoes ‘asbestos’ transformation (i.e., thick collagen fiber aggregates, Kuhnel, 2003; Thilander and Ingervall, 1973) (Figs. 13G-I, yellow arrows). Note that the sections of Emu 4 are exquisitely preserved (see the cellular divisions, white arrows, Figs. 13H-I) even though they were obtained from a dry skull.

The same synchondrosis stained with Toluidine blue is shown in Alligator 1 (Fig. 14A), Alligator 3 (Figs. 14B-D, E), Alligator 4 (Fig. 14E) and Alligator 6 (Figs. 14F-H). In Alligator 1, the basioccipital-exoccipital synchondrosis is not formed yet (red arrow, Fig. 14A), which makes it a presumptive synchondrosis. In Alligator 3, 4 and 5 the synchondrosis is slightly undulating (Figs. 14B-C, E) and it becomes interdigitated in Alligator 6 (Fig. 14F). The different synchondroseal zones are easily identifiable in Alligator 3, 4 and 5 and are similar to those reported by Ikejiri (2012) in the vertebral column of this species (Fig. 14D). The number of cells in the proliferative and the hypertrophic zones of the alligator are very low compared to those of the emu averaging

two to three (Fig. 14D, white arrows) and four to eight cells (Fig. 14D), respectively. In Alligator 6 (a dry skull) the unmineralized hyaline cartilage seems to have been replaced with fibrocartilage (Fig. 14G, yellow arrow). The hypertrophied cartilage zone is still present, but the resting and proliferative zones are no longer visible in the fibrocartilage. The most astonishing characteristic featured in this specimen is the apparent transformation of the most medial and lateral parts of this synchondrosis into a suture-like structure (see the medial part, Fig. 14H). Even though the soft tissues have been slightly deteriorated in this dry skull, the most internal part of the synchondrosis appears to be made of fibrocartilage (yellow arrow, Fig. 14H), but the most external part shows thick bundles of collagen fibers bridging the two bone fronts (red arrow, Fig. 14H). No cartilage can be seen in this fibrous area (Fig. 14H, red arrow). To our knowledge, this type of transformation or replacement (i.e., from a synchondrosis to a suture) has not been reported before.

Fossil Archosaurs/Non-Avian Dinosaurs

Crocodylian: The basioccipital-exoccipital and the basisphenoid-basioccipital synchondroses (see red and green arrows) in the fossil crocodylian (MOR 552) are shown in figure 15 (Figs. 15A-G). The basioccipital-exoccipital synchondrosis shows two mirroring layers of calcified cartilage (Fig. 15C, white arrows) and a gap between them filled with minerals (Fig. 15C, double black arrows). These two cartilaginous layers were undoubtedly the hypertrophied cartilage zones and the gap used to host the unmineralized resting and proliferative zones. Laterally, the calcified cartilage zones end abruptly (see

the limit between cartilage and bone, yellow arrows, Fig. 15D) and eventually no trace of hypertrophied cartilage can be seen in the synchondrosis (Fig. 15E). This suggests that, just as it was observed in Alligator 6 (Fig. 14H), the lateral margin turned into or was replaced by a suture. We hypothesize that the gap between the two elements was filled with fibers and not by unmineralized cartilage (Fig. 15E). The entire basisphenoid-basioccipital synchondrosis shares the same histology as that presented in figure 15E where no calcified cartilage was found anywhere at its borders (e.g., Fig. 15F). These observations are very enigmatic, but we hypothesize that the entire basisphenoid-basioccipital synchondrosis had been ‘replaced’ by a fibrous suture (see discussion for further elaboration). The borders of this same articulation are composed of a mineralized tissue not resembling endochondral bone or intramembranous bone (Fig. 15G). It shows globular structures (white arrows, Fig. 15G) comparable to the ones previously found in the modern alligators (Fig. 4H) and the hadrosaurs (Figs. 8D, H). These globular structures resemble chondrocytes a low magnification, but higher magnification reveals that they are not (see discussion for further description).

Hadrosaurs: Some hadrosaur synchondroses were also sectioned but their preservation was very poor (*Hypacrosaurus* MOR 548, data not shown; and *Prosaurolophus*, MOR 447, Figs. 15H-I). The basisphenoid-basioccipital synchondrosis of *Prosaurolophus* is shown (red arrows, Fig. 15H), but the entire element is diagenetically altered beyond histological resolution and only a few areas show relatively decent preservation (Fig. 15I). The two hypertrophic zones can be seen as well as an empty middle zone, which was undoubtedly the unmineralized resting and proliferative

zones (double arrows, Fig. 15I). Since the two hadrosaur specimens were diagenetically altered, we cannot extract much data from the synchondroses in this group of non-avian dinosaurs.

Triceratops: The ontogeny of the basioccipital-exoccipital synchondrosis was interpreted from the analyses of three specimens of *Triceratops*: a juvenile in MOR 1110 (Figs. 16A, D), and two sub-adults in MOR 8657 (Figs. 16B, E-F) and MOR 8658 (Figs. 16C, G). In the juvenile, the occipital condyle is not fused yet (only one of the two exoccipitals is shown here). The elements are composed of cancellous lamellar trabeculae resembling normal endochondral bone (perhaps endosteal bone) with islands of calcified cartilage that are remnants of their primary cartilage model (Fig. 16D, black arrows). The periosteal borders of these two bones were completely obscured by a black mineral (possibly hematite, D. Varricchio, personal communication) and therefore the synchondrosis was not observable. In MOR 8657 and MOR 8658 the occipital condyles are partially fused (Figs. 16B-C). The histology of these condyles is the same so they are described together. The basioccipital-exoccipital and the exoccipital-exoccipital synchondroses are open on the outside of the condyles (Figs. 16B-C, red arrows and Figs. 16E, G), but they become completely obliterated deeper within the cortex, made of a dense compacta formed of secondary osteons (Fig. 16F). All the synchondroses transition into cracks (Figs. 16B-C, possibly post-mortem cracks). These two condyles do not show any remnant of calcified cartilage (whether synchondroseal or from the primary cartilage models, e.g., Figs. 16E, G). This suggests that parts of these synchondroses were

transformed into fibrous sutures, perhaps in a similar fashion to that observed in the extant and fossil crocodilians (Figs. 14-15).

The basisphenoid-basioccipital synchondrosis of a *Triceratops* (MOR 8659) was also sectioned, but it was completely obliterated and replaced by trabeculae of lamellar bone. No calcified cartilage was found anywhere in this element. The overall histology of MOR 8659 resembles that presented in figures 11 and 16. Therefore we do not find it necessary to show these sections in the present study.

Morphological vs. Histological Degree of Closure

As mentioned in the introduction, it is often assumed that if a suture is open morphologically (i.e., if the sutural borders do not meet or if the suture line is still visible on the skull) then the suture is open internally (histologically) as well. Likewise, if the suture line is obliterated morphologically, it is often assumed that it is the case histologically. Our results show that these assumptions are generally correct. Indeed, most of the time, when sutures/synchondroses are morphologically open they are also open histologically. This is the case for all the open sutures/synchondroses of emus (e.g., compare Fig. 17A with Fig. 3A), all the open sutures/synchondroses of American alligators and the fossil crocodilian (e.g., compare Fig. 17B with Fig 4), and the open fronto-parietal suture of *Gryposaurus* and *Brachylophosaurus* (e.g, compare Fig. 17C with Fig. 8A).

Many of the morphologically obliterated sutures are obliterated histologically as well. This is the case for all the fused sutures/synchondroses of emus (e.g., compare Fig.

17D with Fig. 3C), the interpremaxillary and nasal-premaxillary sutures of *Triceratops* (compare Fig. 11E with Fig. 11B), and the basisphenoid-basioccipital synchondroses of *Triceratops* (MOR 8659). However, in two specimens histology and morphology show contradicting results. This is the case for the occipital condyles of two *Triceratops* (MOR 8657 and MOR 8658) which show open synchondroses morphologically (Fig. 17E), but obliterated synchondroses more internally (Fig. 16). Moreover, since sutures and synchondroses have two openings (e.g., an endocranial and an ectocranial side), two types of morphologies are indicated by the same articulation in some instances. This is the case in some pachycephalosaurs (Figs. 9A-B, 10G) and in the skull base of emus (Fig. 13B). The jugal-epijugal suture of *Triceratops* (UCMP 174838) show an obliterated suture on its lateral side (Fig. 17F), but due to taphonomical alterations it is impossible to identify the degree of patency of this suture on its medial side. Histological analyses show that this side is open (Fig. 12A, left red arrow).

Discussion

For decades, the degree of sutural and synchondroseal closure has been used intuitively to assess the maturity of fossil vertebrates, including mammals (e.g., Black et al., 2010) and non-avian dinosaurs (e.g., Bakker and Williams, 1988). While many aspects of these articulations are extremely well studied in Mammalia, data is lacking morphologically and histologically in archosaurs. In this section, we summarize and categorize the archosaurian sutural and synchondroseal tissues that were found in the samples. We also compare them to those that have been previously reported in mammals

(since sutural and synchondroseal histology is essentially known in this taxon, it is the only point of comparison available in this present study). We discuss and hypothesize the modes of ossification by which they might have been formed.

Comparative Sutural Histology: Archosauria *vs.* Mammalia

A total of seven sutural mineralized tissues were found in our samples and they are summarized in Table 3. They are sub-divided into four groups: periosteal tissues, acellular tissues, fibrous tissues and intratendinous tissues (Table 3).

Periosteal Tissues: We report two types of periosteal tissues in the sutural borders of archosaurs: **intramembranous bone** and **chondroid bone**. These two types of tissues are known to be deposited by the osteogenic cells originating from the periosteum (e.g., Lengelé, 1997; Marieb, 2014) hence the term ‘periosteal tissues’.

Intramembranous bone is reported in the sutural areas of archosaurs. Its identification was based on comparisons with the ‘standard’ appearance of mammalian intramembranous bone and/or on the presence of a periosteum (i.e., the sutural cambial layer). This mineralized tissue was found in the sutural areas of emus (e.g., Figs. 3E, 5C), pachycephalosaurs (e.g., Fig. 9D) and *Triceratops* (e.g., Figs. 11 and 16). It was involved in sutural obliteration in these three taxa. However, since intramembranous ossification is plesiomorphic and is the main mode of formation of the membrane bones of vertebrates, we make the safe assumption that at least at some point during their ontogeny, intramembranous bone was a constituent of the mineralized sutural borders of all the

archosaurs of our sample (even though this type of bone and/or a periosteum was not directly observed, see Table. 3).

Chondroid bone was solely found in the emu and only in the internasal sutural areas of an actively growing specimen (Fig. 5E). Chondroid bone had previously been reported in the sutural areas of mammals, chick embryos (Lengel  et al., 1996) and hadrosaurs (Chapter Six). It was also found in the embryonic skull of *A. mississippiensis* (Vickaryous and Hall, 2008). While it is highly plausible that American alligators have chondroid bone in their sutural areas, we did not confidently identify this tissue in our samples. Like intramembranous ossification, it appears that chondroid bone is plesiomorphic within the vertebrate tree. Indeed, it was reported in species of many different clades in the Vertebrata: mammals, birds, hadrosaurs and fossil agnathans (see more complete review in Chapter Six). In the present study, we report the existence of this tissue in yet another species of birds. It is hypothesized that the absence of chondroid bone in all of the fossilized specimens is perhaps due to their relatively late ontogenetic stages and/or to the absence of a sutural periosteum.

These two periosteal tissues have been previously reported in mammals (e.g., see Cohen, 2000; Hinton et al., 1984; Kokich, 1976; Pritchard et al., 1956; Persson, 1973; Persson and Thilander, 1977; Persson et al., 1978 for intramembranous bone; and Goret-Nicaise et al., 1988; Lengel  et al., 1990, 1996; Manzanares et al., 1988; Sun et al., 2004 for chondroid bone). Secondary cartilage, another periosteal tissue is also commonly found in the sutures of mammals (e.g., Bloore et al., 1969; Friede, 1973; Hinton et al., 1984; Kokich, 1986; Manzanares et al., 1988; Pritchard et al., 1956; Persson, 1973;

Sitsen, 1933; Vinkka-Puhakka, 1991), but it was not found in any suture or sutural area in the archosaurs of the present study (Table. 3).

The next three categories of tissues that are exposed have not been reported in mammals, and in fact, many of them have not been observed in any extant vertebrate. Understanding this, caution was taken when hypothesizing and deducting their modes of formation.

Acellular Tissues: Acellular tissues are often found in the skeleton of teleosts (e.g., Ekanayake and Hall, 2008; Moss, 1961, 1963; Meunier, 1989), but are uncommon in the skeletons of tetrapods. It is therefore counterintuitive to observe acellular tissues with such a high abundance in marginocephalians (pachycephalosaurs and ceratopsids). An **acellular fibrous tissue** was found that obliterated the parietal-postorbital suture of a pachycephalosaurid (Figs. 10C-D). It shows irregular and ‘globular’ mineralized borders that contain radiating ‘fibers’. This acellular tissue and its ‘fibers’ will not be discussed further because they will be the subject of another study. An acellular non-fibrous tissue was found bordering the sutures of *Pachycephalosaurus* (Fig. 10I, white arrow) and *Triceratops* (Figs. 12G-H). It was also involved in the complete synostosis/obliteration of a *Pachycephalosaurus* suture (Figs. 10J-K). These tissues have previously been reported in pachycephalosaurids by Goodwin and Horner (2004) and Horner and Goodwin (2009), but not directly in sutural areas. As mentioned in the results section, due to the different shapes and sizes that osteocyte lacunae can have in the vicinity of this tissue, we hypothesized that it started out as a cellular tissue and then progressively underwent acellularization (with cellular pyknosis and intracellular mineralization, Moss, 1961), hence we propose the term ‘**acellular necrotic tissue**’. A “bionecrotic” mineralized

tissue has been reported in the sutures of humans and rabbits, but in these cases, it was much less abundant and appeared as free- nodules in the suture or as masses attached to short spicules extending from the sutural borders (see figure 7 in Persson et al., 1978). It is not comparable to the potential necrotic sutural tissue found in marginocephalians. The function and advantage (if there are any) of this acellularization are unknown, but a potential cause of this process could be mechanical. This could be analogous to the acellularization of mammalian periodontal ligaments that may occur when the ligaments receive stresses above a certain threshold causing their fibroblasts undergo apoptosis, resulting in a ligament with completely acellular zones. This process is called ‘hyalinization’ (e.g., see Jónsdóttir et al., 2011). Even though this example does not relate directly to a mineralized tissue it shows the possibility that some mechanical stresses in the sutures (or sutural mineralized borders) may be the cause of acellularization. This is a plausible hypothesis considering that sutures are known to be shock absorbers and protect the non-sutural mineralized tissues from fracture (e.g., Jaslow and Biewener, 1995). Further investigation of this acellular necrotic tissue is underway and will be part of a future study.

Fibrous Tissues: We identified two different types of sutural mineralized ‘fibrous’ tissues. The first type shows numerous fibers that are all perpendicular to the suture. It was found in the modern alligators (Fig. 6G) and the pachycephalosaurids (Figs. 9E, 10I). In the latter taxa it was also found obliterating the sutures (Figs. 9F, 10F). In some instances these mineralized tissues are composed of lamellae that are all parallel to the suture (Figs. 6G and 10F). These lamellae undoubtedly represent the earlier sutural

borders. We hypothesize that these fibers were collagenous fibers initially located in the sutures that became incorporated in the mineralized sutural tissues. Similar fibers were observed in the fronto-parietal suture of the American alligator (Fig. 4D, white arrow, Fig. 4E, black arrow). This type of mineralized tissue is referred to as a ‘**sutural-fiber rich tissue**’. In some instances, the sutural fibers are so numerous that it is not clear if the tissue is cellular or acellular. The second type of fibrous tissue was found exclusively in the sutural areas of *Triceratops* (Fig. 12C). Its fibers are also very numerous, but unlike the previous fibrous tissue they are disorganized and not always found perpendicular to the suture (Fig. 12C). This type of tissue has already been reported and named “**metaplastic bone**” in the osteoderms of ankylosaurs (Scheyer and Sander, 2004) and stegosaurs (Main et al., 2005). Metaplasia is the direct transformation of soft-tissues *in situ* without the intervention of a periosteum (Vickaryous and Hall, 2008). The term “metaplastic bone” was created by Haines and Mohuidin (1968) to describe a hard skeletal tissue formed in the absence of osteoblasts. Vickaryous and Hall (2008) reported that the osteoderms of *A. mississippiensis* form partially via metaplasia, which suggested that the previous assessments of Main et al., (2005) and Scheyer and Sander (2004) were correct. However, Vickaryous and Hall (2008) only examined decalcified sections, and it is still unclear if their metaplastic tissue corresponds to the “metaplastic bone” observed previously by Scheyer and Sander (2004) and Main et al., (2005) in thick un-decalcified sections. More recently, this same “metaplastic bone” was found very abundantly in the parietal frill of *Triceratops* (Horner and Lamm, 2011). Two lines of evidence suggest that the ‘sutural-fiber rich tissue’ and the “metaplastic bone” found in our sample were indeed

formed by metaplasia: (1) they do not present osteocyte lacunae with canaliculi and (2) they are in direct contact with soft-tissues (i.e., the suture). Moreover, unlike mammalian sutures that present a periosteal cambial layer containing numerous osteoblasts (Fig. 1), the sutures of American alligators appear to lack a periosteum (they are formed of a uniform layer with fibers and a few fibroblasts, Figs. 4D-E, 6D-E). It is therefore not implausible to hypothesize that some archosaurian sutural tissues mineralize directly from the soft-tissues of their sutures. As mentioned above, since fibrous tissues presenting these same characteristics in un-decalcified sections of extant animals have never reported, it is yet impossible to confirm this hypothesis.

In the last category of sutural mineralized tissues (intratendinous tissues), we provide considerably stronger evidence that some metaplastic transformations were involved during the formation of these tissues.

Intratendinous Tissues: This last category is characterized by mineralized tissues that show globular structures separated by arcuate-shaped spaces. They were found in the modern alligators (Fig. 4H), the fossil crocodilian (Fig. 15G) and two species of hadrosaurs (Figs. 8D, G-H). The histology of these ‘globular’ tissues is virtually identical to that of the ossified tendons in hadrosaurs (see Adams and Organ, 2005) and extant birds (Fig. 18). Avian mineralized tendons do not form via normal intramembranous ossification, but via metaplastic transformations (Horner et al., in press). This mode of ossification was previously designated as “intratendinous ossification” (Rooney, 1994; Berge and Storer, 1995), hence the term ‘**intratendinous tissues**’ used here. Figure 18 is modified from Horner et al., (in press) and shows the

microstructure of a mineralized tendon of *Bubo virginianus* (Great horned owl) stained with Toluidine blue. Mineralized tendons are manifested as vascular canals (black arrow) with indistinct boundaries (red arrows). These types of canals do not have the regular characteristics of Haversian systems (Fig. 18A) and were therefore named ‘secondary reconstruction’ by Horner et al. (in press). The collagen fiber fascicles forming the ‘young’ un-mineralized tendons calcify *in situ*. This process produces globular structures in un-decalcified sections (i.e., the mineralized collagen fiber fascicles, yellow arrows) separated from each other by dark spaces of different shapes (white arrows). When these shapes are arcuate, they represent the endotenons of the fascicles while the other irregular spaces may host osteogenic cells (Horner et al., in press). No evidence of osteocyte lacunae and/or canaliculi are observed in avian tendons and therefore it was hypothesized that some non-osteogenic cells transformed into and played the role of osteoblasts (perhaps a population of fibroblasts transforming directly into osteoblasts, M. Vickaryous personal communication in Horner et al., in press).

The histological similarities between mineralized avian tendons and the sutural mineralized tissues of *A. mississippiensis* (Fig. 4H), *Brachylophosaurus* (Fig. 8D) and *Gryposaurus* (Fig. 8H) are undeniable. Additionally, it appears that large fiber fascicles in avian mineralized tendons are able to undergo destruction or a rearrangement into smaller fiber bundles (e.g., Fig. 18B, red arrows). The globular structures shown by the fossil crocodilian that resembled chondrocytes at first sight (Fig. 15G) are very similar to these ‘rearranging’ fiber bundles when observed at higher magnification. Based on the histological similarities with avian mineralized tendons known to be formed by

metaplasia, we hypothesize that the intratendinous tissues found in the American alligator, the fossil crocodilian and the hadrosaurs formed via metaplasia and undoubtedly involved the direct mineralization of their sutures in the absence of a sutural periosteum.

Another ceratopsian tissue presents some unusual dark spaces that do not have the morphology of osteocyte lacunae (Fig. 12F). As mentioned earlier, this same type of tissue was found in the parietal frill of *Triceratops* and it was hypothesized that the dark structures are spaces fitted between fiber fascicles (Horner and Lamm, 2011). Due to the lack of a known extant analogue presenting these structures, it is still unclear if this ceratopsian tissue (Fig. 12F) is a form of intratendinous tissue as well.

Comparative Synchondroseal Histology: Archosauria vs. Mammalia

This discussion is essentially based on the synchondroses of the extant archosaurs since the fossils had incompletely preserved synchondroses (only their calcified parts). Archosaurian and mammalian synchondroses are very similar. They all show four synchondroseal zones (resting, proliferative, calcification and ossification zones). The number of cells in the proliferative zones in 8 to 10 months old emus (6 to 12 cells; Fig. 13G) is similar to that reported in 4 day-old rats (8 to 12 cells; Roberts and Blackwood, 1983). This is much higher than what was found in American alligators (2 to 3 cells, Fig. 14D). These differences likely relate to physiological regimes (i.e., extant mammals and birds are endotherms, while alligators are ectotherms and grow much more slowly, Lee and Werning, 2008). The fates of synchondroseal hyaline cartilage seem to be the same in

mammals and archosaurs as well: it can be replaced by fibrocartilage, undergo ‘asbestos’ transformation (Fig. 13G and see Cendekiawan et al., 2010 and Thilander and Ingervall, 1973) or be replaced by endochondral ossification (Fig. 13E and see Opperman et al., 2005). Endochondral ossification was not observed, however, in American alligators since their synchondroses never obliterate (i.e., there is still cartilage in the most mature alligator, Fig. 14G). This matches our previously reported morphological results (Chapter Two). The fact that the histological characteristics of mammalian and archosaurian synchondroses are very similar suggests a conservation of synchondroseal cellular organization through evolution. Our results suggest that synchondroseal architecture is more conserved than sutural architecture (see the spectrum of different tissues in Table. 3).

Only one important dissimilarity was found between archosaurian and mammalian synchondroses. During the late phases of ontogeny, it appears that the medial and lateral parts of the basioccipital-exoccipital synchondrosis of *A. mississippiensis* can turn into a suture (Fig. 14H). This was found in the exact same synchondrosis in the fossil crocodilian, suggesting that it is a crocodilian characteristic (Figs. 15D-E). More surprisingly, the entire basisphenoid-basioccipital synchondrosis of this fossil lacks calcified cartilage and has the appearance of a suture (Fig. 15F). This suggests that even though some articulations are embryonically ‘designed’ to be synchondroses, they may be able to show the histological characteristics of a suture (late during ontogeny). In a similar fashion the synchondroses of the occipital condyle in the *Triceratops* adults did not show any trace of calcified cartilage and their most external openings also had the

appearance of sutures (Fig. 16). This suggests that the ability of synchondroses to show the morphological characteristics of sutures is perhaps also shared by some non-avian dinosaurs. The mode of formation of these ‘sutures’ is not clear; it could involve neoplasia or a form of metaplasia (perhaps the synchondroseal chondrocytes transforming into fibroblasts, followed by the secretion of sutural fibers).

Further histological investigations of synchondroses in the skull base of extant crocodilians must be made in order to answer these questions.

Morphology vs. Histology

We attempted to provide a preliminary assessment of the relationship between the morphological and the histological degree of sutural closure. Our results show that the histological degree of closure matches its associated morphology relatively well for both extant and fossil archosaurs at a qualitative level. Our results confirm that the morphological observations previously found in the skulls of *D. novaehollandiae* and *A. mississippiensis* are also congruent with observations at the histological level (Chapter Two). In other words, all of the morphologically obliterated sutures and synchondroses of emus are obliterated histologically, and all the open sutures and synchondroses of American alligators are open histologically (with fibers in the sutures and fibrocartilage in the synchondroses regardless of specimen age).

It appears that the sutures of American alligator stay open during ontogeny via some mechanisms that inhibit fusion. These mechanisms do not involve resorption of the sutural borders like it was reported after the initial stages of fusion in the cranio-facial sutures of rabbits (Persson and Thilander, 1977; Persson et al., 1978). Perhaps instead

some transcription factors present in the sutures (such as Twist1 or Jagged1, see Morris-Kay and Wilkie, 2005) and/or some mechanical signals inhibit sutural fusion.

Some of our results show morphologies and histologies that are opposite (see results section), and when one suture presents two different morphologies at opposite ends, the accurate degree of closure of a suture can only be known via histological analyses. This confirms what previous authors have suggested (Brochu, 1996; Cole et al., 2003; Herring, 2000; Irmis, 2007). This preliminary examination is however imperfect due to the methods that we employed: each suture and each synchondrosis was sampled at two specific points along its whole length (i.e., two cuts per articulation). Therefore, the histological sections only show the degree of closure in specific points, and do not necessarily reflect the rest of the articulation. Cohen (1993) stated that obliteration begins at one point and spreads along the suture, without any way to predict where the point of fusion will start. According to this statement, serial sections along the entire length of sutures are necessary to assess their correct and complete degree of closure. Moreover, our methods are qualitative and not quantitative. Persson and Thilander (1978) proposed that the use of an “obliteration index”, i.e. the ratio between the fused suture length and the entire suture length, could be used to quantitatively define closure. While this ratio is much more informative than our qualitative methods, it is only meaningful if it is calculated in each serial section along the whole length of a suture. Other techniques have also been employed in the past to visualize sutures, such as X-ray computed tomography and micro-tomography (e.g., Schott et al., 2011). These methods have the great advantage of being rapid, but *a priori* in that their end results do not show the details provided by

paleohistological analyses (e.g., they cannot provide a precise obliteration index). In order to improve our preliminary assessment, a comparison between results obtained by histological, computed tomography and micro-tomography analyses should be made in order to find the best method to clearly and relatively rapidly visualize sutures and synchondroses in both extant and fossil archosaurs.

Sutural Histology: Implications for Maturity Assessment in Non-avian Dinosaurs

In summary, we showed that the sutures of mammals and archosaurs grow and fuse with different mechanisms: the sutural mineralized tissues of archosaurs and mammals are completely different and the diversity of archosaurian tissues is higher than that of mammalian tissues (seven tissues for archosaurs and three for mammals, Table. 3). All the tissues found in the sutural areas of mammals are deposited via a periosteum, while the tissues of archosaurs involve other modes of ossification (a form of bionecrosis and metaplastic transformations). It appears that metaplasia was the prevalent mode of ossification in the sutural areas of archosaurs. This echoes the findings of Horner et al., (in press), who suggest that metaplastic transformations are more abundant than previously thought in the soft-tissues of non-avian dinosaurs. It was hypothesized that these transformations are more rapid and more efficient than regular intramembranous ossification, since they start from pre-formed precursor tissues (i.e., the sutures in this case). It would appear that mammals are not capable of such extensive metaplastic transformations.

Enigmatically, the sutures of emus are more similar to those of mammals than those of other archosaurs. Emus also show the lowest diversity of sutural mineralized tissues in this sample (with only two tissues, Table. 3). We hypothesize that this low histological diversity will also be observed in other species of extant birds. This may be due to the increase of avian growth rate that occurred during evolution (Padian et al., 2001). In other words, since extant birds attain skeletal maturity so rapidly (usually in less than one year, Lee and Werning, 2008) they probably do not have sufficient time to develop many different tissue types (e.g., tissues that were present in their archosaurian ancestors and that appear late during ontogeny). Histological analyses with both decalcified and un-decalcified sections of other extant archosaurs have to be made in order to fully understand the process by which non-periosteal archosaurian sutural tissues form.

The degree of sutural closure is a method that has been used to assess maturity in non-avian dinosaurs, even though it has archaic foundations based on mammalian observations (e.g., see Dwight, 1890; Krogman, 1930; Marsh, 1887; Todd and Lyon, 1925) and has never been tested in the extant phylogenetic bracket of the Dinosauria (except in Chapter Two). Our hypothesis was that while this method may provide accurate maturity assessment in fossil mammals, this might not be the case in fossil archosaurs. We have shown the considerable differences in the patterns and timings of sutural fusion between extant mammals and archosaurs (Chapter Two), and in the present study, we confirm that those differences are also present at the microscopic scale. As of today, it is not possible to understand what our histological findings mean at the

morphological level and therefore they do not have a direct impact on the method of maturity assessment using sutural closure *per se* (since this method is only based on morphology). However, our results simply exemplify the fact that this method developed within Mammalia should no longer be applied directly to the Dinosauria (nor Archosauria). We urge caution in the excessive use of mammals as the extant analogues for non-avian dinosaurs for other general matters in paleontology as well (e.g., behavioral or functional).

Potential Implications for the Evolution of Craniosynostosis

As mentioned earlier, craniosynostosis is the premature fusion of the bones of the skulls of humans (Cohen, 2000). It is a birth defect that can be caused by mutations of transcription factors that regulate osteoblast proliferation, differentiation, apoptosis and migration at the sutural borders (Opperman, 2000). In vertebrates sutural patency is the norm, but what is not fully understood is sutural fusion (Susan Herring, personal communication in Cohen, 2000). Gaengler (2000) hypothesized that tooth attachment pathologies seen in humans are reverted plesiomorphic conditions in amniotes, due to a “phylogenetic memory”, and normal tissues of the past can explain pathological tissues of the present. Perhaps investigating the different types of sutural mineralized tissues in non-mammalian vertebrates (including fossil archosaurs) might help give insights in the evolution of craniosynostosis and better understand its occurrence in humans nowadays. This investigation may also encourage experimental studies on non-mammalian species.

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Table 3.1. List of the extant specimens sectioned in the present study. The same articulations were systematically chosen and sectioned in each extant specimens: 1) the fronto-parietal suture (cranial), 2) the internasal suture (facial) and 3) the basioccipital-exoccipital synchondrosis (skull-base). Ages and ontogenetic categories were estimated based on skull length and on the literature available on the growth trajectories of these species (for the emu, Minnaar and Minnaar, 1992 and Davies, 2002; and for the American alligators, Chabreck and Joanen, 1979 and Woodward et al., 1995). Almost all of these specimens were previously analyzed morphologically and placed into these different ontogenetic categories in another study (see Chapter Two). Asterisks indicate that age data (and sex data in one case only) were known. Sexual dimorphism was taken into account when assessing the age/categories of American alligators. Note that Alligator 6 can only be a male (based on Chabreck and Joanen, 1979). Abbreviations: D, domestic; W, wild.

Taxon	Species	Specimen number	Other numerotation	Skull length (cm)	Provenance/D or W	Estimated age	Ontogenetic category	Skull/Head & embedding media
Aves	<i>Dromaius novaehollandiae</i>	MOR OST 1799	Emu 1	5.5	Montana/D	few weeks	hatchling	dry skull/Epoxy
		MOR OST 1800	Emu 2	9.7	Montana/D	few weeks	juvenile	dry skull/Epoxy
		MOR OST 1801	Emu 3	~12.8	Montana/D	8 to 10 months*	juvenile	head/PMMA
		MOR OST 1802	Emu 4	12.8	Montana/D	8 to 10 months*	juvenile	dry skull/Epoxy
		MOR OST 232	Emu 5	15.8	?	> 18 months	sexually/skeletally mature	dry skull/Epoxy
		MOR OST 1803	Emu 6	15.2	Montana/D	20 years-old (male)*	sexually/skeletally mature	dry skull/PMMA
Crocodylia	<i>Alligator mississippiensis</i>	MOR OST 1647	Alligator 1	2.54	Louisiana/?	a few days	hatchling	head/PMMA
		MOR OST 1796	Alligator 2	4.19	Florida/D	a few weeks	juvenile	dry skull/Epoxy
		MOR OST 1797	Alligator 3	15.5	Louisiana/?	4-5 years	sub-adult	head/PMMA
		MOR OST 1798	Alligator 4	28.5	Louisiana/W	9-12 years	sexually mature	head/PMMA
		MOR OST 1795	Alligator 5	35.5	Louisiana/W	10 years if male 21-23 years if female	sexually mature if male skeletally mature if female	dry skull/Epoxy
		MOR OST 1789	Alligator 6	42.6	Louisiana/W	15 years (male)	sexually mature	dry skull/Epoxy

Table 3.2. List of the fossil specimens and the articulations sectioned in the present study. The asterisks indicate the petrographic thin-sections that show diagenetically altered structures and for this reason, they are not shown in the present study.

Taxon	Genus	Geological Formation	Specimen number	Estimated Ontogenetic stage	Sutures/Synchondroses
Crocodylia	Crocodylia (indet.)	Two Medicine	MOR 552	?	Fronto-postorbital (Cranial)
					Basioccipital-exoccipital (Cranial base)
					Basisphenoid-basioccipital (Cranial Base)
Hadrosauria	<i>Brachylophosaurus</i>	Judith River	MOR 1071	juvenile	Fronto-parietal (Cranial)
	<i>Hypacrosaurus</i>	Two Medicine	MOR 548*	hatchling	Supraoccipital-exoccipital (Cranial Base)
			MOR 548	hatchling	
	<i>Gryposaurus</i>	Two Medicine	MOR 553M*	adult-not fully grown	Fronto-parietal (Cranial)
		Judith River	MOR 2573	adult-not fully grown	
	<i>Prosaurolophus</i>	Two Medicine	MOR 447*	sub-adult	Basisphenoid-basioccipital (Cranial Base)
Ceratopsia	<i>Triceratops</i>	Hell Creek	MOR 2587	sub-adult	Internasal (Facial)
			MOR 8661	at least an adult-not fully grown	Nasal-premaxilla (Facial)
					Interpremaxilla (Facial)
			MOR 2570	young adult	Jugal-epijugal (Facial)
			UCMP 174838	at least an adult-not fully grown	Jugal-quadratojugal (Facial)
			MOR 1110	juvenile	Basioccipital-exoccipital (Cranial Base)
			MOR 8657	at least sub-adult (young adult?)	
			MOR 8658	at least sub-adult	
			MOR 8659	at least a juvenile	Basisphenoid-basioccipital (Cranial Base)

Table 3.2 continued.

Pachycephalo sauria	<i>Stegoceras</i>	Judith River	UCMP 130049	juvenile	Fronto-parietal (Cranial)
	<i>Pachycephalosauridae</i> <i>indet.</i>	Judith River	MOR 2555*	?	
	<i>Pachycephalosaurus</i>	Hell Creek	UCMP 128383	juvenile	
	<i>Pachycephalosauridae</i> <i>indet.</i>	Judith River	TMP 1974.10.74	?	
		Hell Creek	MOR 2915	?	Parietal-postorbital (Cranial)
		Hell Creek	UCMP 154919	?	Interfrontal (Cranial)
	<i>Pachycephalosaurus</i>	Hell Creek	VRD 13	adult-not fully grown	Parietal-postorbital (Cranial)

Table 3.3. Summary of the sutural mineralized tissues found in archosaurs and mammals. This summary is based on the results of the present study and those of other previously published investigations. The asterisks indicate that the tissues were involved in sutural obliteration (either directly observed in the present study, or reported in the literature).

Sutural mineralized tissues		Taxon
Periosteal tissues	1) Intramembranous bone*	Mammals (e.g., Persson et al., 1978)
		<i>D. novaehollandiae</i> (Figs. 3, 5C)
		<i>A. mississippiensis</i> (not directly observed)
		Pachycephalosauridae (Fig. 9D)
		<i>Triceratops</i> (Figs. 11, 16)
		Crocodylian (fossil) (not directly observed)
		Hadrosauridae (not directly observed)
	2) Chondroid bone*	Mammals (e.g., Manzanares et al., 1988)
		<i>D. novaehollandiae</i> (Fig. 5E)
		<i>H. stebingeri</i> (Hadrosauridae) (Chapter Six)
	3) Sutural secondary cartilage	Mammals (e.g., Vinkka-Puhakka, 1991)
Acellular tissues	4) Acellular/fibrous tissue*	Pachycephalosauridae (Figs. 10C, D)
	5) Acellular necrotic tissue*	Pachycephalosauridae (Figs. 10I-K)
		<i>Triceratops</i> (Figs. 12G-H)
Fibrous tissues	6) Sutural fiber-rich tissue*	<i>A. mississippiensis</i> (Figs. 4D-E, 6G)
		Pachycephalosauridae (Fig. 10F, I)
	7) "Metaplastic bone" *	<i>Triceratops</i> (Fig. 12C)
Intratendinous tissues	8) Intratendinous mineralized tissue	<i>A. mississippiensis</i> (Fig. 4H)
		Hadrosauridae (Figs. 8D, H)
		Crocodylian (fossil) (Fig. 15G)
		? <i>Triceratops</i> (? Fig. 12F)

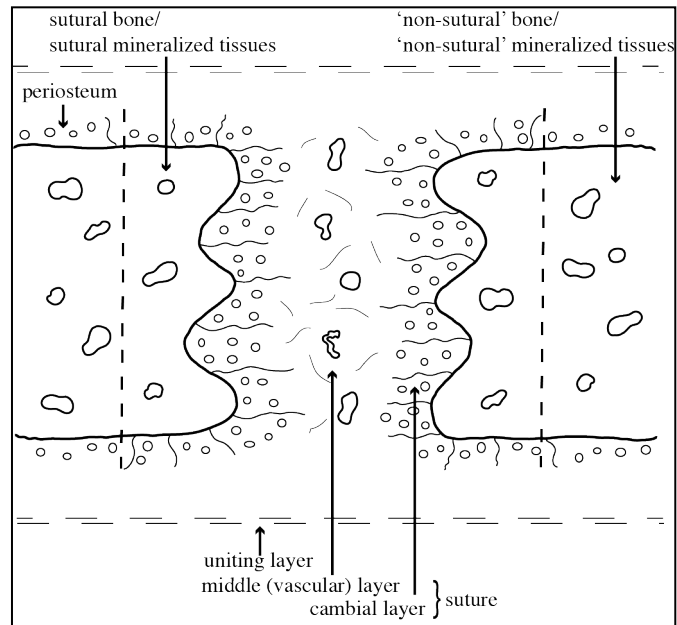


Figure 3.1. Schematic representation of a mammalian suture. Sutures are composed of a cambial layer with numerous osteoblasts and a vascular middle layer (non-osteogenic). The sutural cambial layer is continuous with the periosteum of the bones. In the present study, the mineralized tissues directly bordering the sutures are referred to as sutural bone or sutural mineralized tissues. The mineralized tissues that are more distant from the sutural borders are referred to as 'non-sutural' bone (or 'non-sutural' mineralized tissues). Skull bones and their sutures are united together by the uniting layers, dense regular connective tissues mostly composed of collagen fibers. This figure is a re-interpretation of the drawings of Pritchard et al., (1956) and Persson (1973).

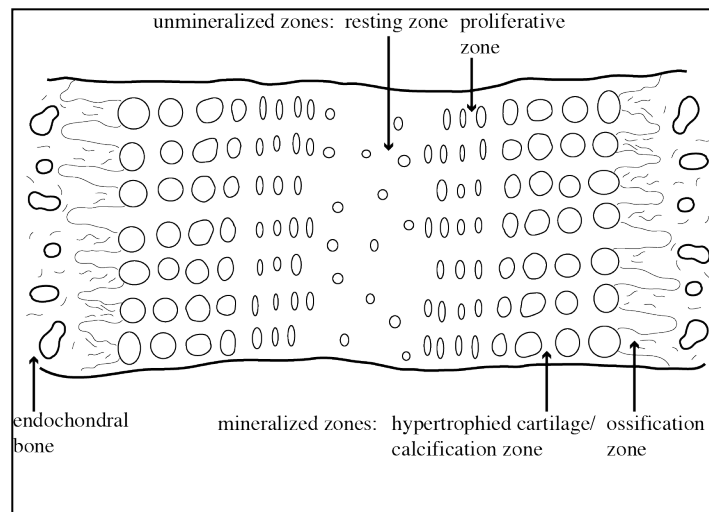


Figure 3.2. Schematic representation of a mammalian synchondrosis. Synchondroses are composed of different cellular zones: once central resting zone and mirroring proliferative, calcification and ossification zones. Due to this back-to-back organization, synchondroseal growth is bidirectional. The resting and proliferative zones are unmineralized (formed of hyaline cartilage) and the calcification/hypertrophied cartilage and ossification zones are mineralized. Endochondral bone is present on the two borders of synchondroses. This figure is a re-interpretation of a schematic representation in Opperman et al., (2005).

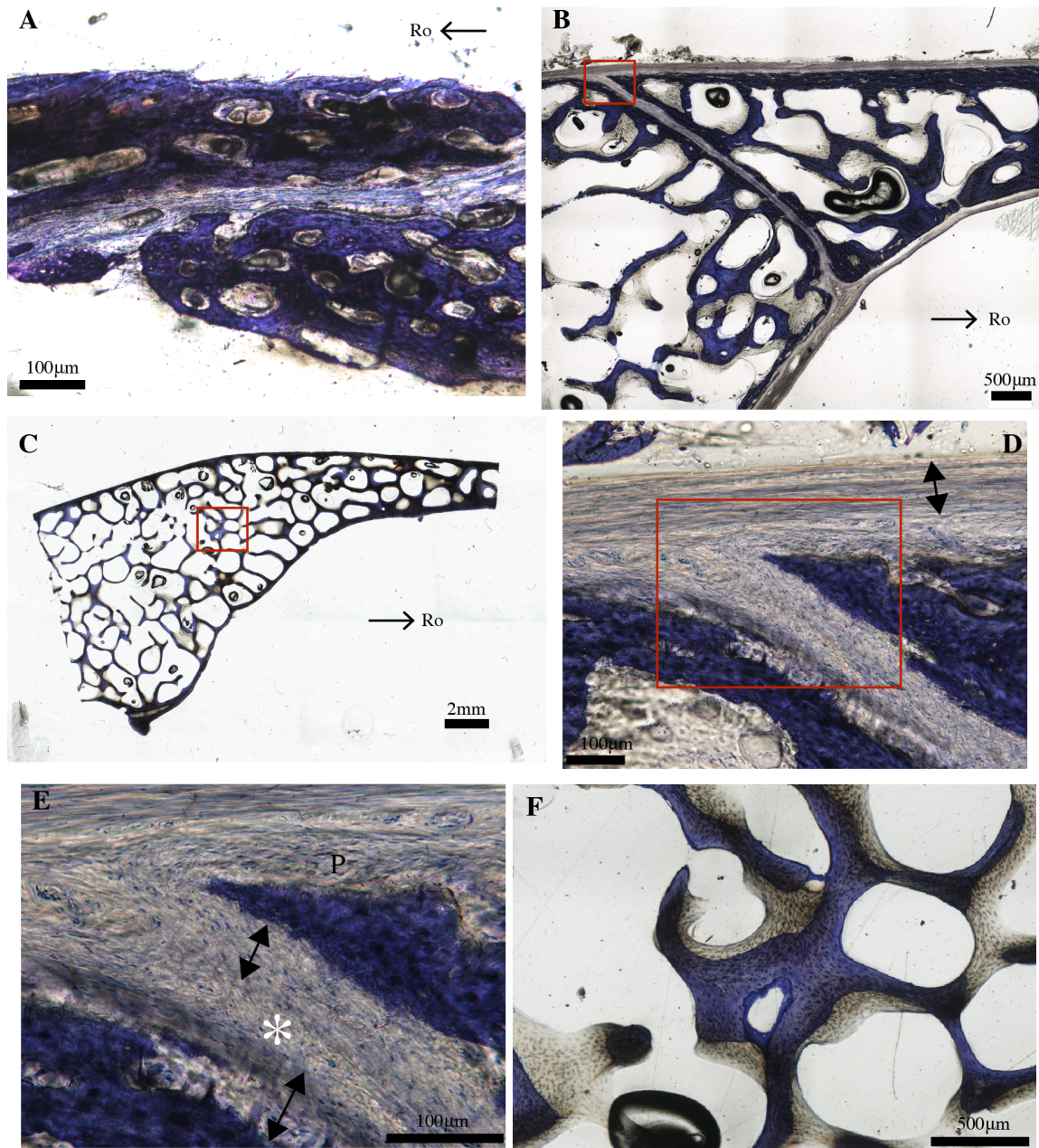


Figure 3.3. Parasagittal sections of the fronto-parietal suture of emus stained with Toluidine-blue. A, fronto-parietal suture of Emu 1. B, fronto-parietal suture of Emu 3. C, whole section of the fronto-parietal suture of Emu 6. D, close-up of the red box in B. The double black arrows show the uniting layer. Bone and cell nuclei are stained deep blue. E, close-up of the red box in D, showing the suture divided into two cambial layers (double black arrows) and one middle layer (white asterisk). The cambial layers are continuous with the periosteum. F, close-up of the red box in C, showing trabeculae of lamellar bone. Abbreviations: P, periosteum; Ro, rostral.

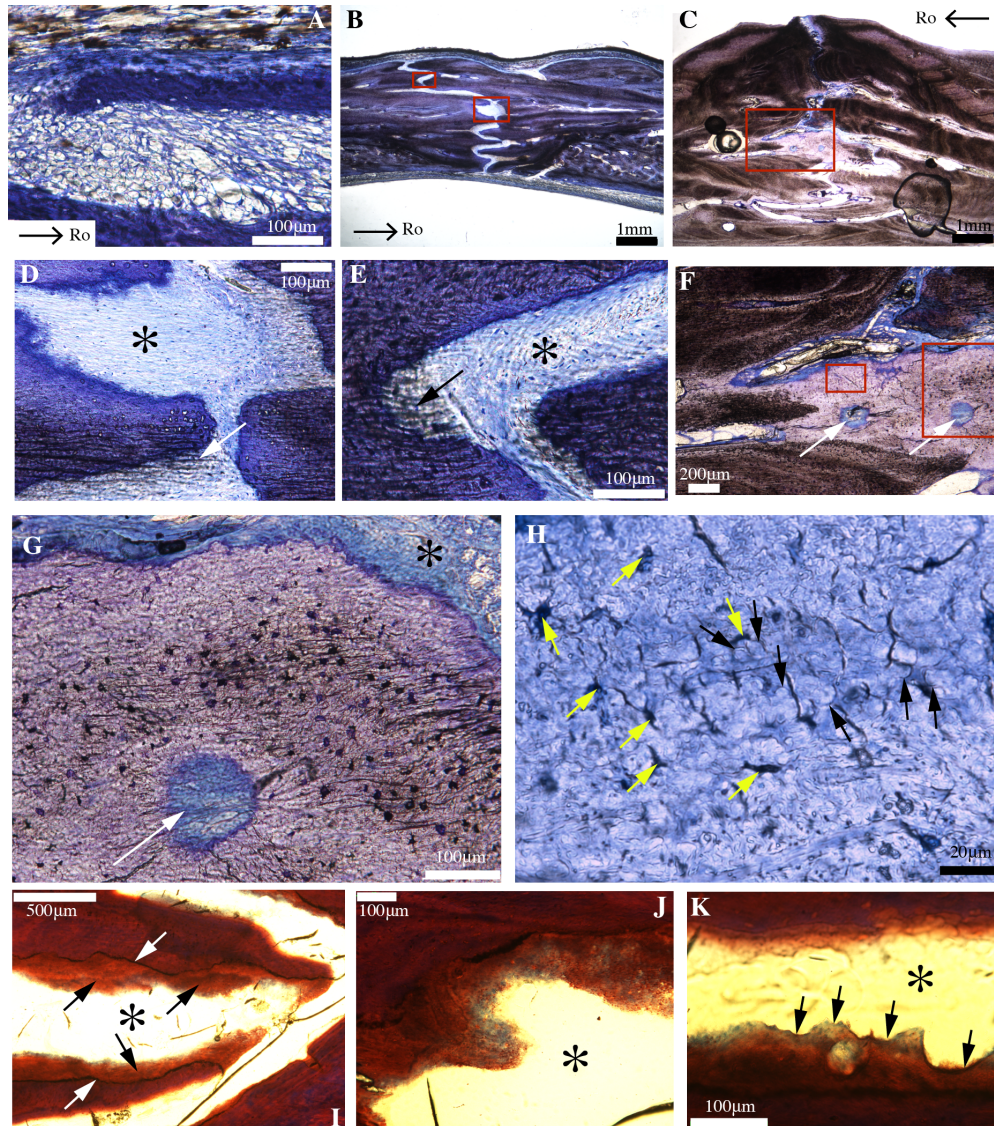


Figure 3.4. Parasagittal sections of the fronto-parietal suture of American alligators stained with Toluidine-blue (A-H) and Masson's trichrome (I-K). A, fronto-parietal suture of Alligator 1. It appears to be formed of mesenchyme. B, fronto-parietal suture of Alligator 3. C, ectocranial part of the fronto-parietal suture of Alligator 6. D, close-up of the lower red box in B. Thick collagen fiber bundles are continuous between the bone and the suture (white arrow). The black asterisk shows a uniform dense fibrous tissue (with blue fibroblast nuclei). This suture lacks a periosteum. E, close-up of the upper red box in B. Once more, thick collagenous fibers can be seen (black arrow), as well as a uniform sutural layer (black asterisk). F, close-up of the red box in C. A light purple tissue is found right at the sutural borders. It possesses two vascular canals (white arrows). G, close-up of the right red box in F, showing a vascular canal (white arrow) bordered by different zones: cellular, acellular, fibrous and non-fibrous. The cellular zones show cellular lacunae resembling plump osteocytes with canaliculi. H, close-up of the left red box in F. In areas that appear acellular, this tissue shows globular structures (black

arrows) and dark spaces of various shapes (yellow arrows). These spaces do not have the appearance of regular osteocyte lacunae and they show no evidence of canaliculi. I, adjacent section of this same suture in Alligator 6 (black asterisk) stained with Masson's trichrome. Interdigitations are 'covered' by this same tissue (black arrows). The core of the interdigitations do not stain like this tissue (see the clear limits indicated by the white arrows). J, close-up of this tissue from another area in this same section. It shows shades of blue and red, while the core of the interdigitations only stains red. The suture is indicated by the black asterisk. K, another area in this section shows that this tissue is mineralized since it shows Howship's lacunae (black arrows). The suture is indicated by the black asterisk. Abbreviations: same as previous figure.

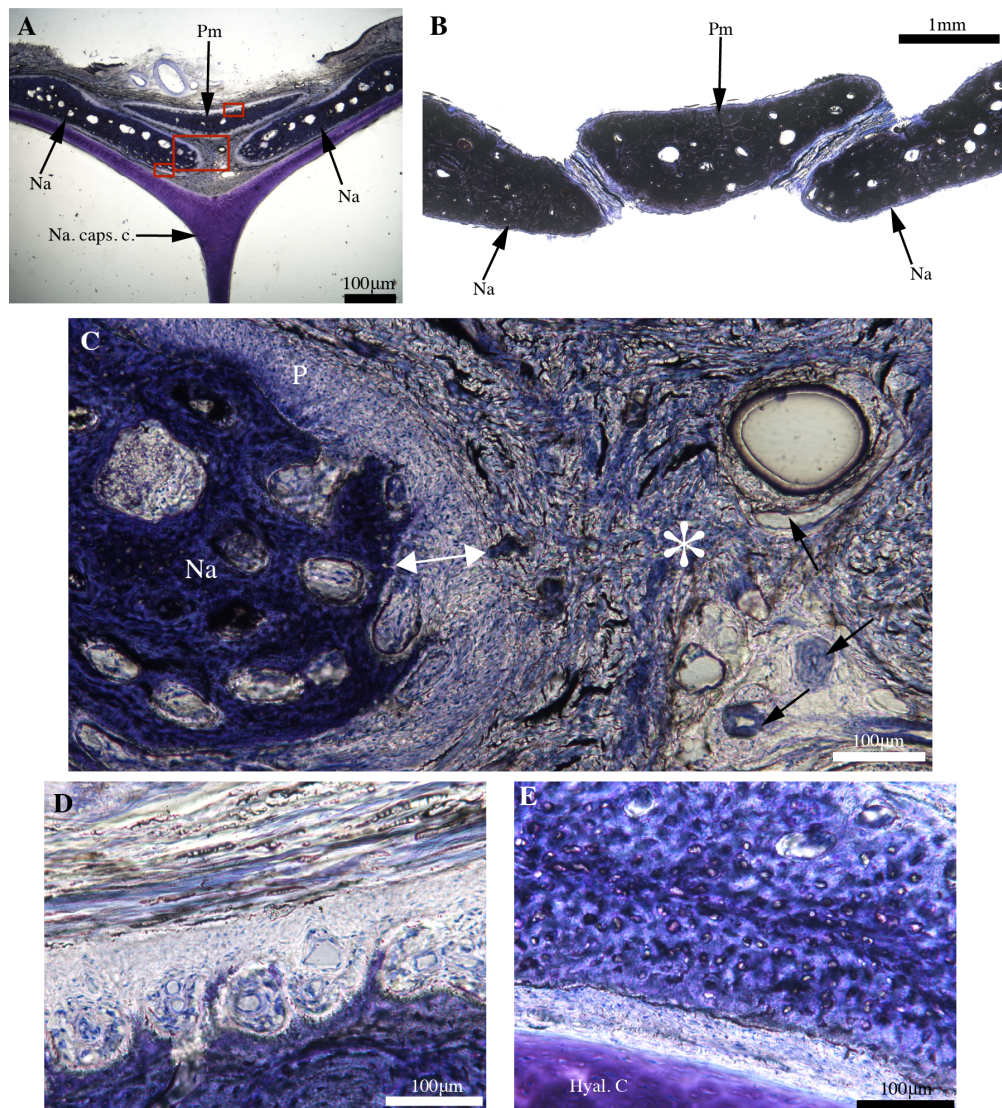


Figure 3.5. Cross-sections of the internasal and nasal-premaxilla sutures of emus stained with Toluidine blue. A, internasal and nasal-premaxilla sutures of Emu 3. The bone stains

deep blue and the cartilage of the nasal capsules is purple. B, internasal and nasal-premaxilla sutures of Emu 6. C, close-up of the middle red box in A. The suture is composed of a cambial layer (double white arrows) and a middle layer (white asterisk) formed of loose irregular connective tissues and some vessels (black arrows). D, close-up of the right box in A showing an actively growing bone and the formation of vascular canals. E, close-up of the left red box in A, showing chondroid bone (up) and hyaline cartilage (down, in purple). Cells of chondroid bone are cartilage-like (round) but are embedded in a bone matrix.

Abbreviations: Hyal. C, hyaline cartilage; Na, nasal; Na. caps. c., Nasal capsule cartilage; Pm, premaxilla.

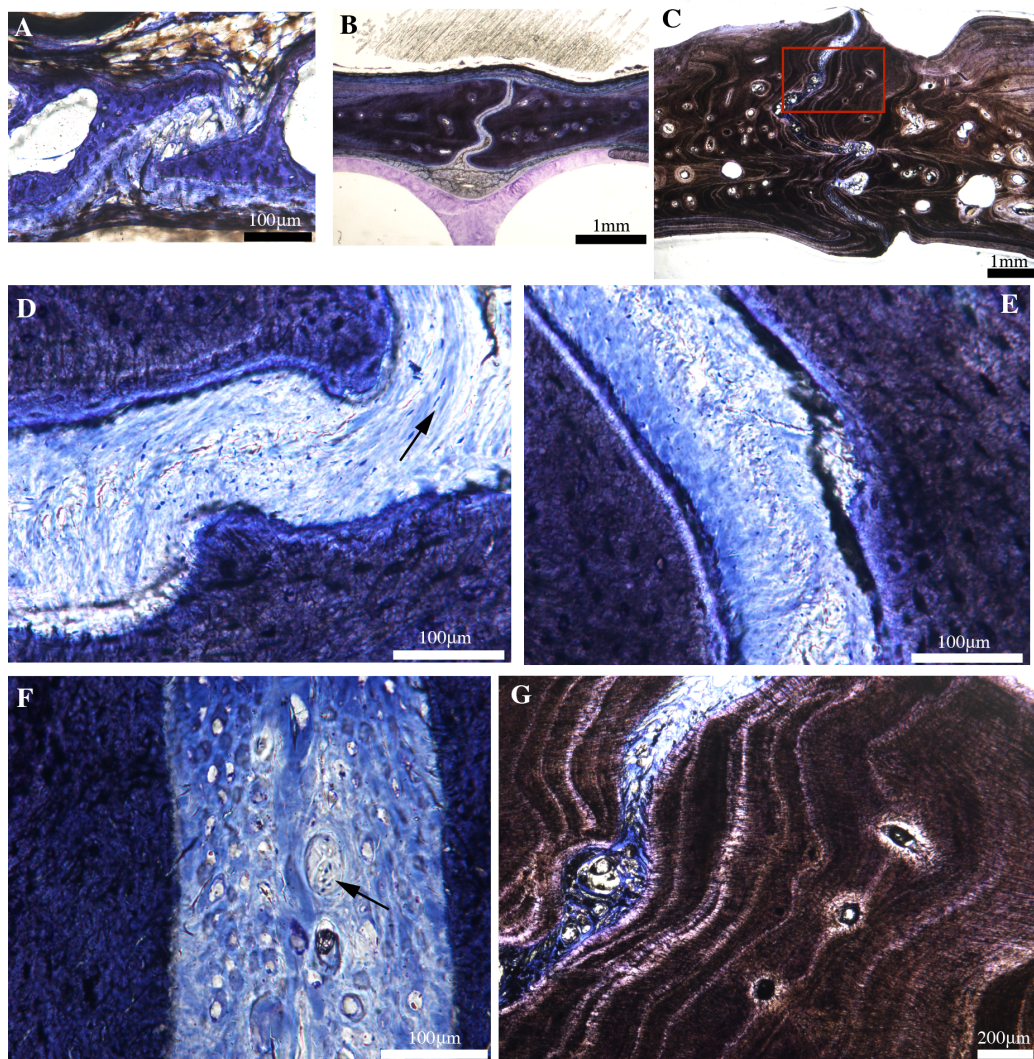


Figure 3.6. Cross-sections of the internasal suture of American alligators stained with Toluidine-blue. A, internasal suture of Alligator 1. B, internasal suture of Alligator 3. C, internasal suture of Alligator 6. D, close-up of the suture in B, showing one uniform

fibrous layer. Fibers can be oriented perpendicular or parallel to the sutural borders (black arrow). This suture lacks a periosteal cambial layer. E, close-up of another area in the suture in B, showing fibers that are perpendicular to the sutural borders. The suture is composed of a uniform dense fibrous connective tissue with fibroblast nuclei stained in blue. Again it lacks a periosteum. F, ectocranial part of the internasal suture of Alligator 4. The suture appears to be filled in with many nerves (black arrow). G, close-up of the red box in C. The sutural borders are composed of lamellae of fibrous bone, all parallel to the suture. This resembles lamellar-zonal bone

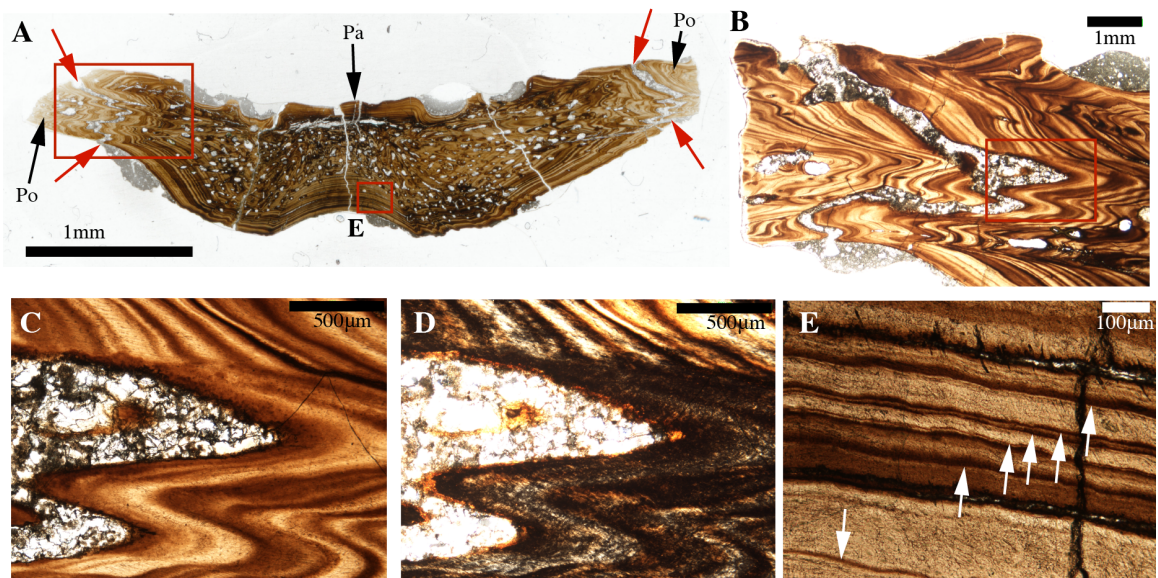


Figure 3.7. Paleohistological cross-sections in the parietal-postorbital sutures of a fossil crocodilian. A, whole cross-section of the parietal-postorbital bones of MOR 552. The ectocranial and endocranial parts of the parietal-postorbital sutures are indicated by the red arrows. B, close-up of the left red box in A. C, close-up of the red box in B. The sutural borders are composed of bone lamellae that are all parallel to the suture. D, close-up of the red box in B shown under polarized light. Polarized light indicates that this tissue is woven. E, close-up of the right red box in A, showing lamellar-zonal bone. The white arrows indicate lines of arrested growth. Abbreviations: Pa, parietal, Po, postorbital.

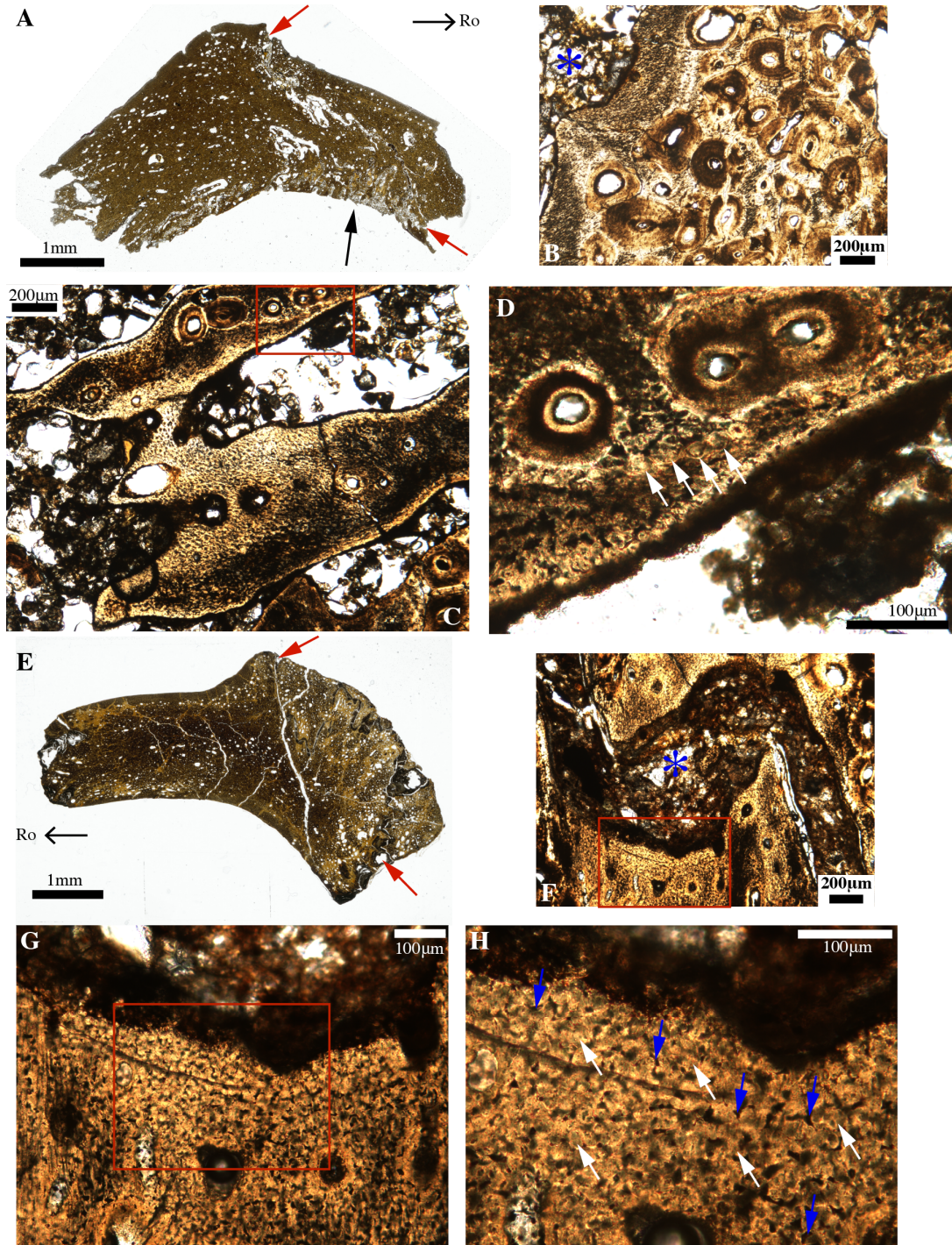


Figure 3.8. Paleohistological cross-sections in the fronto-parietal sutures of *Brachylophosaurus* (A-D) and *Gryposaurus* (E-H). A, whole section of MOR 1071. The red arrows indicate the ectocranial and endocranial sides of the fronto-parietal suture. The parietal-postorbital suture is indicated by the black arrow. B, close-up of the fronto-parietal suture in A. The sutural borders are composed of a tissue that appears highly fibrous. Many secondary osteons are adjacent to this tissue. The suture is indicated by the

blue asterisk. C, close-up of the same suture in another area showing interdigitations entirely composed of this same fibrous tissue. D, close-up of the red box in C. This tissue shows some globular structures (white arrows). E, whole section of MOR 2573. The red arrows indicate the ectocranial and endocranial sides of the fronto-parietal suture. F, close-up of this suture (indicated by the blue asterisk). G, close-up of the red box in F. H, close-up of the red box in G, showing globular structures (white arrows) separated from each other by spaces of different shapes (including arcuate shapes; blue arrows). Abbreviations: same as previous figures.

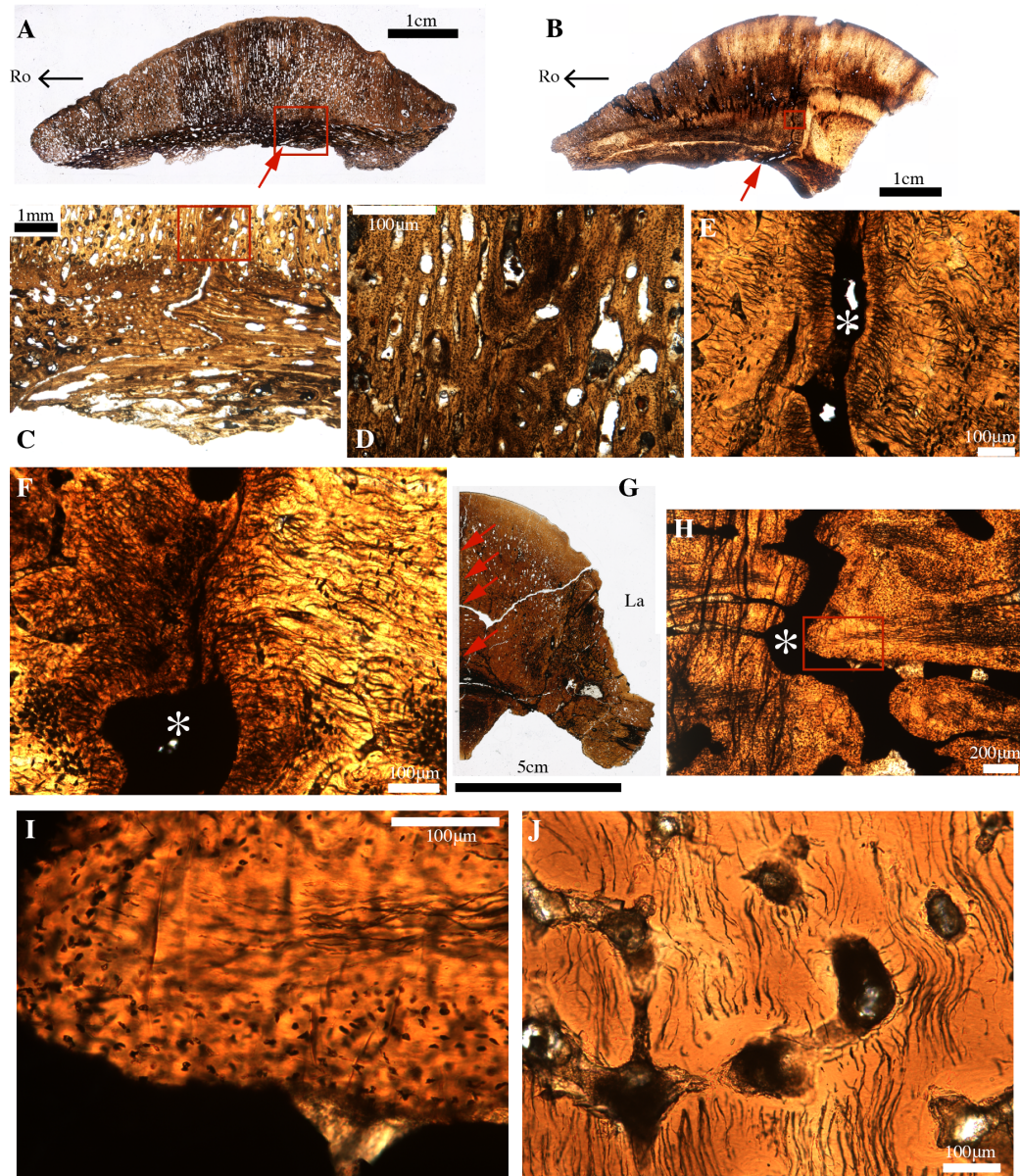


Figure 3.9. Paleohistological parasagittal sections (A-F) and cross-sections (G-J) in the cranial domes of some pachycephalosaurids. A, whole section of the fronto-parietal dome

of *Stegoceras* (UCMP 130049). The endocranial part of the suture is indicated by the red arrow. The ectocranial part of this suture is obliterated. B, whole section of the fronto-parietal dome of a *Pachycephalosauridae* indet (TMP 1974.10.74). The endocranial part of the suture is indicated by the red arrow. The ectocranial part of this suture is obliterated. C, close-up of the red box in A. D, close-up of the red box in C. E, close-up of the red box in B. The suture is indicated by the white asterisk. F, close-up of another area in B, showing an obliterated suture with two fusing fronts composed of a highly fibrous tissue. The white asterisk indicates the suture. G, whole section of the frontals of a *Pachycephalosauridae* indet (UCMP 154919). The interfrontal suture is indicated by the red arrows. H, close-up of the interfrontal suture in G. I, close-up of the red box in H. The interdigitation is composed of an acellular fibrous tissue in the center and a cellular tissue in the periphery. J, section showing acellular bone from a 'non-sutural' area. Abbreviations: La, lateral; Ro, rostral.

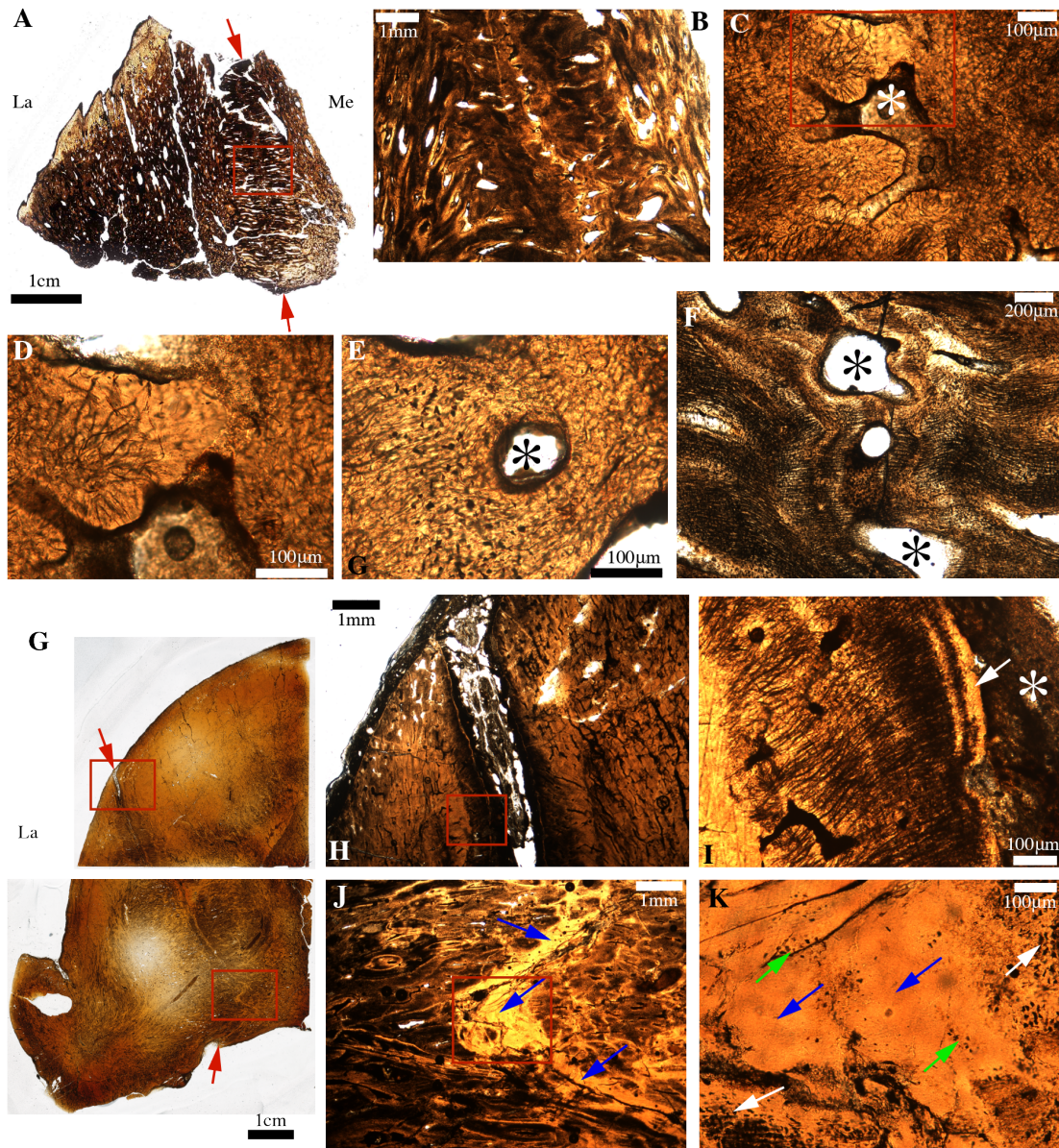


Figure 3.10. Paleohistological cross-sections in the parietal-postorbital sutures of some Pachycephalosaurids. A, whole section of a broken dome fragment of a Pachycephalosauridae indet (MOR 2915). The ectocranial and endocranial sides of the parietal-postorbital suture are indicated by the red arrows. B, close-up of the red box in A. C, close-up of B showing an obliterated suture. The fused bony fronts are fibrous and acellular. The white asterisk indicates what might be bone marrow and/or vascularized spaces. D, close-up of the red box in C. E, close-up of another obliterated area of this same suture. Here, the tissue looks fibrous and cellular. The black asterisk indicates what might be bone marrow and/or vascularized spaces. F, another area of this same suture where the sutural fronts are extremely fibrous and parallel to the sutural borders. The black asterisk indicates what might be bone marrow and/or vascularized spaces. G, whole sections of half of a *Pachycephalosaur* dome (VRD 13). The ectocranial and

endocranial sides of the parietal-postorbital suture are indicated by the red arrows. H, close-up of the upper red box in G. The ectocranial part of this suture is open. I, close-up of the red box in H. The sutural borders are composed of an acellular tissue (white arrow). The suture is indicated by the white asterisk. J, close-up of the lower red box in G, showing an interdigitated suture completely obliterated by a bright yellow acellular tissue (blue arrows). K, close-up of the red box in J. At higher magnification, this tissue has zones with regular and numerous osteocyte lacunae (white arrows), zones with lacunae that are less numerous and less regular (green arrows) and acellular areas (blue arrows). Abbreviations: La, lateral; Me, medial.

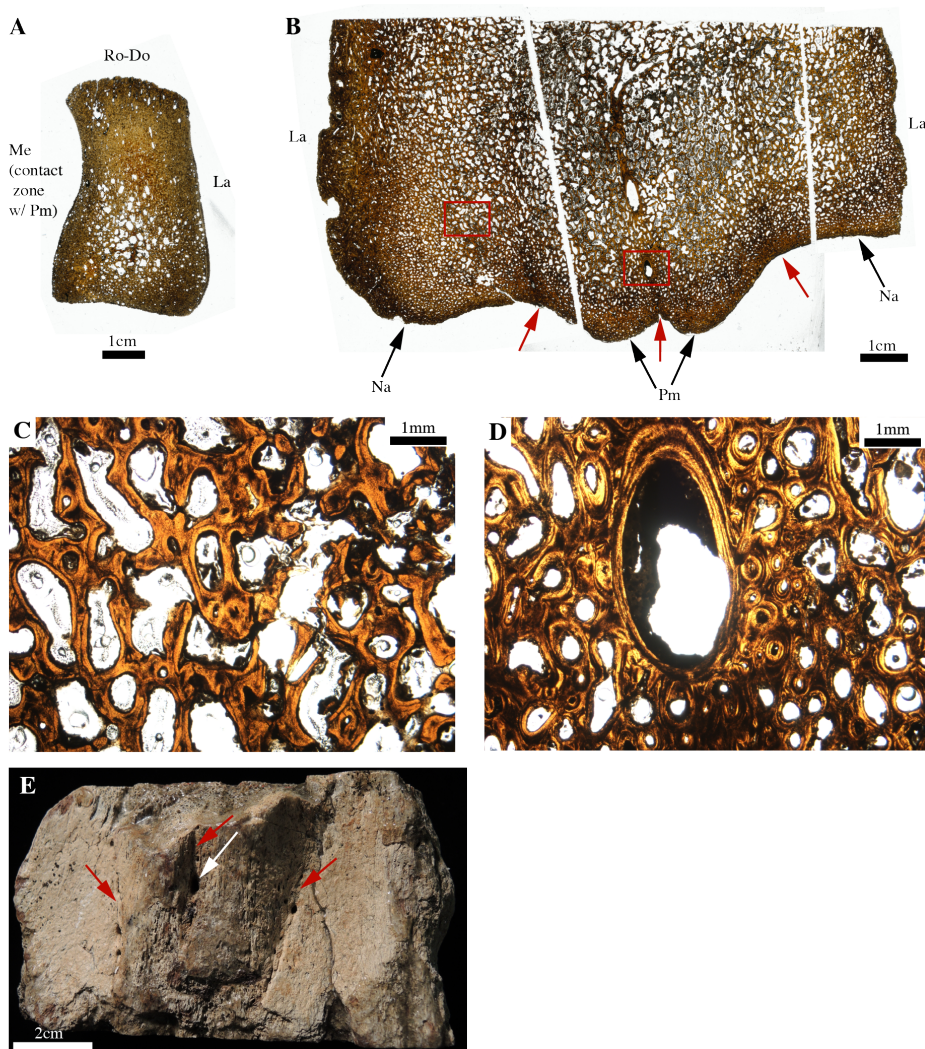


Figure 3.11. Paleohistological cross-sections of an isolated nasal (A) and of the fused nasals and premaxillae of *Triceratops* (B-E). A, whole section of an unfused nasal (MOR 2587). B, whole sections of the ventral part of fused premaxillae and nasals (MOR 8661). The ventral part of the two nasal-premaxilla and the interpremaxillary sutures are indicated by the red arrows. C, close-up of the left red box in B. It shows no trace of the

suture. It is obliterated and replaced by lamellar bone trabeculae, and vascular and/or bone marrow spaces. D, close-up of the right red box in B, showing a canal present at the interpremaxillary suture. The suture is also obliterated here. E, photograph of the morphology of these elements in ventral view. The canal is indicated by the white arrow. The three sutures are obliterated morphologically but their general locations are indicated by the red arrows. Abbreviations: La, Lateral; Me, medial; Na, nasal; Pm, premaxilla; Ro-Do, rostro-dorsal.

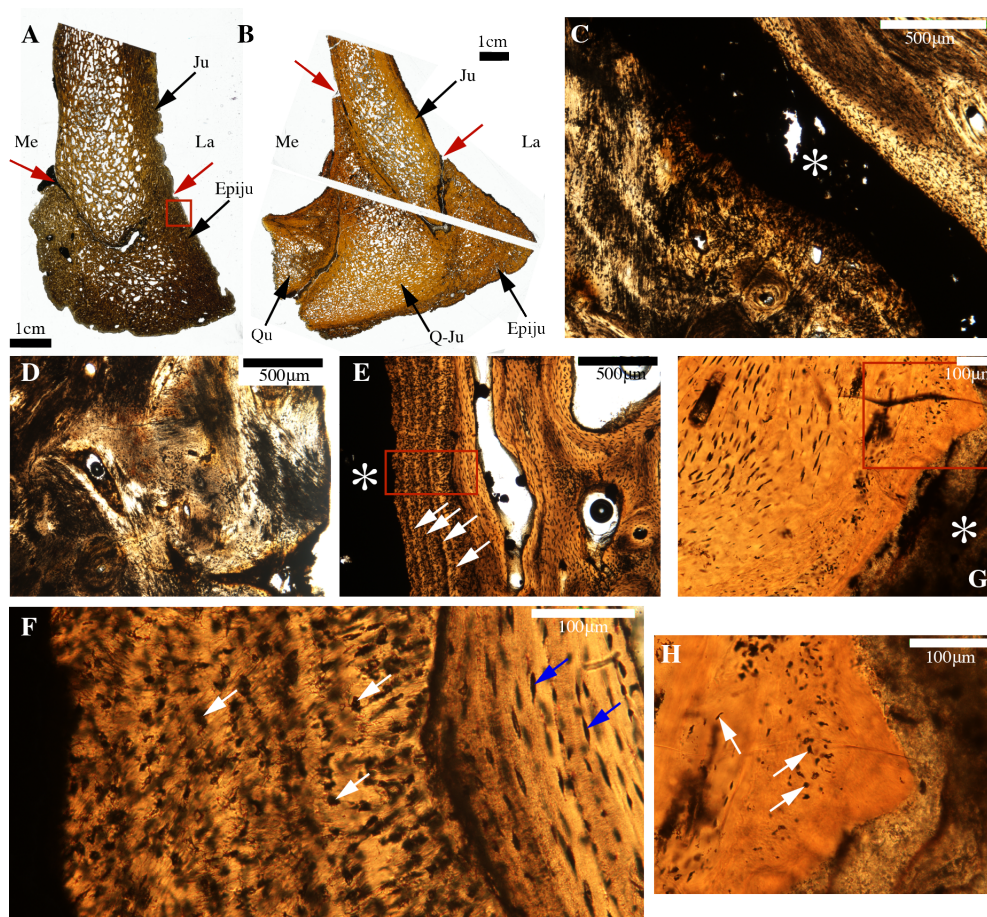


Figure 3.12. Paleohistological cross-sections of the jugal-epijugal and the jugal-quadratojugal sutures of *Triceratops*. A, whole section of UCMP 174838. The lateral and medial parts of the suture are indicated by the red arrows. B, whole sections of MOR 2570. The lateral and medial parts of the suture are indicated by the red arrows. C, close-up of the suture in A. The sutural borders on the lower part of the picture are composed of a very fibrous tissue. The suture is indicated by the white asterisk. D, close-up of the red box in A. The same fibrous tissue as that presented in C is also found at many external borders. It has previously been designated as “metaplastic bone”. E, close-up of another part of this same suture, showing lamellar bone on the right and some lamellae that are parallel to the suture (white arrows). The suture is indicated by the white asterisk. F, close-up of the red box in E, showing the internal lamellar bone with regular osteocyte

lacunae (blue arrows) and a tissue composed of irregular dark spaces (white arrows) near the suture. G, close-up of the suture in B. The sutural borders are composed of an acellular tissue. The white asterisk indicates the suture. H, close-up of the red box in G. The white arrows indicate cellular lacunae of various shapes and sizes. Abbreviations: same as previous figures, Epiju: epijugal; Ju, jugal; Q-Ju, quadratojugal; Qu, quadrate.

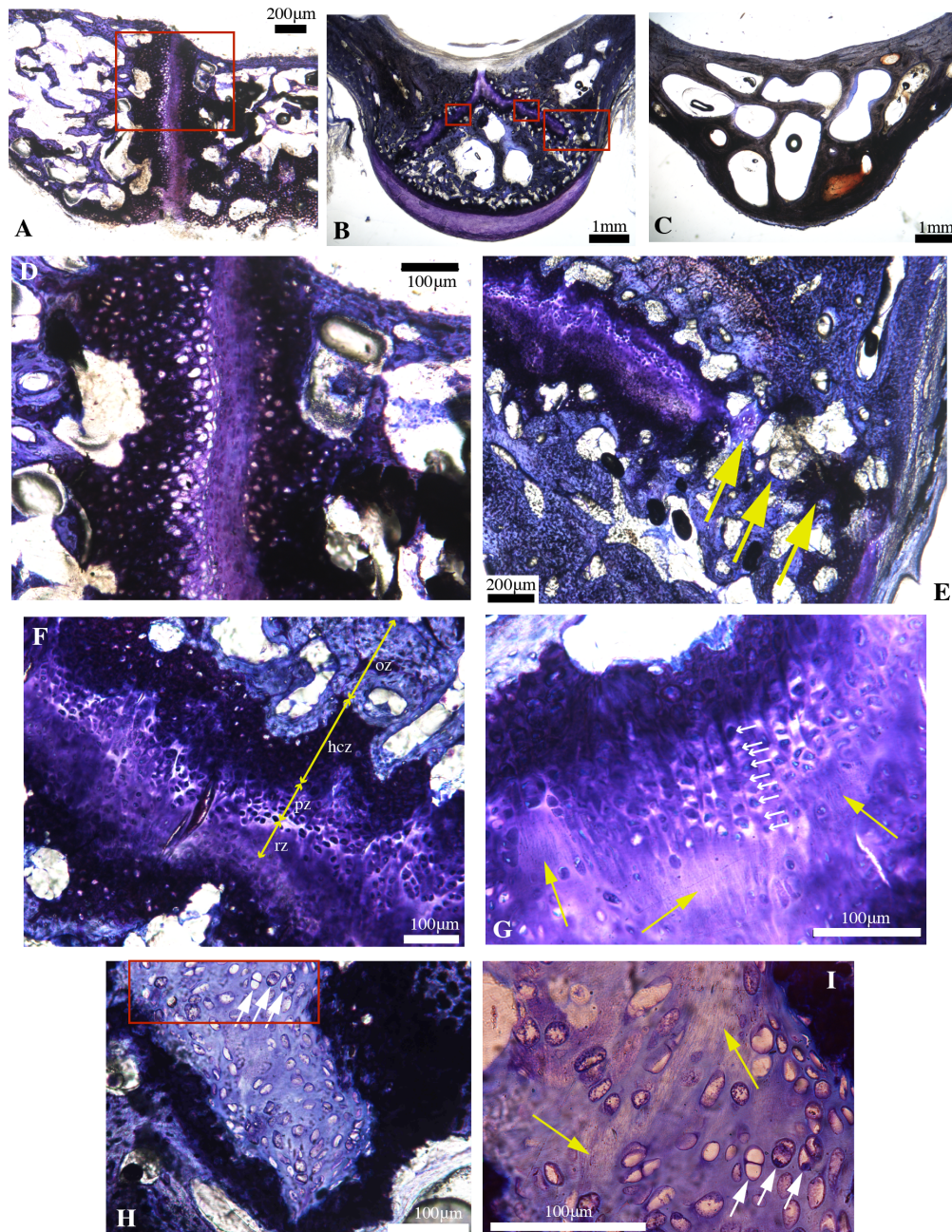


Figure 3.13. Cross-sections in the basioccipital-exoccipital synchondrosis of emus stained with Toluidine blue. A, section of the left basioccipital-exoccipital synchondrosis of Emu

1. The bone stains blue, the hyaline cartilage light purple and the calcified cartilage dark purple. B, whole section of the occipital condyle of Emu 3. C, whole section of the occipital condyle of Emu 6. D, close-up of the red box in A. E, close-up of the right box in B. The yellow arrows indicate where the synchondrosis is being replaced by bone via endochondral ossification. F, close-up of the middle red box in B. The resting zone can be seen in the middle, as well as the mirroring proliferative zones, hypertrophied cartilage zones and ossification zones (double yellow arrows). G, close-up of the left red box in A. The white arrows indicate proliferative chondroblasts. The yellow arrows indicate some collagen fiber aggregates (“asbestos transformation”) in the hyaline resting zone. H, most lateral part of the right basioccipital-exoccipital synchondrosis of Emu 4. This lateral edge shows a disorganization of the chondrocytes (i.e., no organization into resting and proliferative zones). Even though this section comes from a dry skull, the hyaline cartilage is still very well preserved and some cellular divisions can be seen (white arrows). I, close-up of the red box in H. Cellular divisions are indicated by white arrows. The yellow arrows indicate collagen fiber aggregates (“asbestos transformation”). Abbreviations: hcz, hypertrophied cartilage zone; oz, ossification zone; pz, proliferative zone; rz, resting zone.

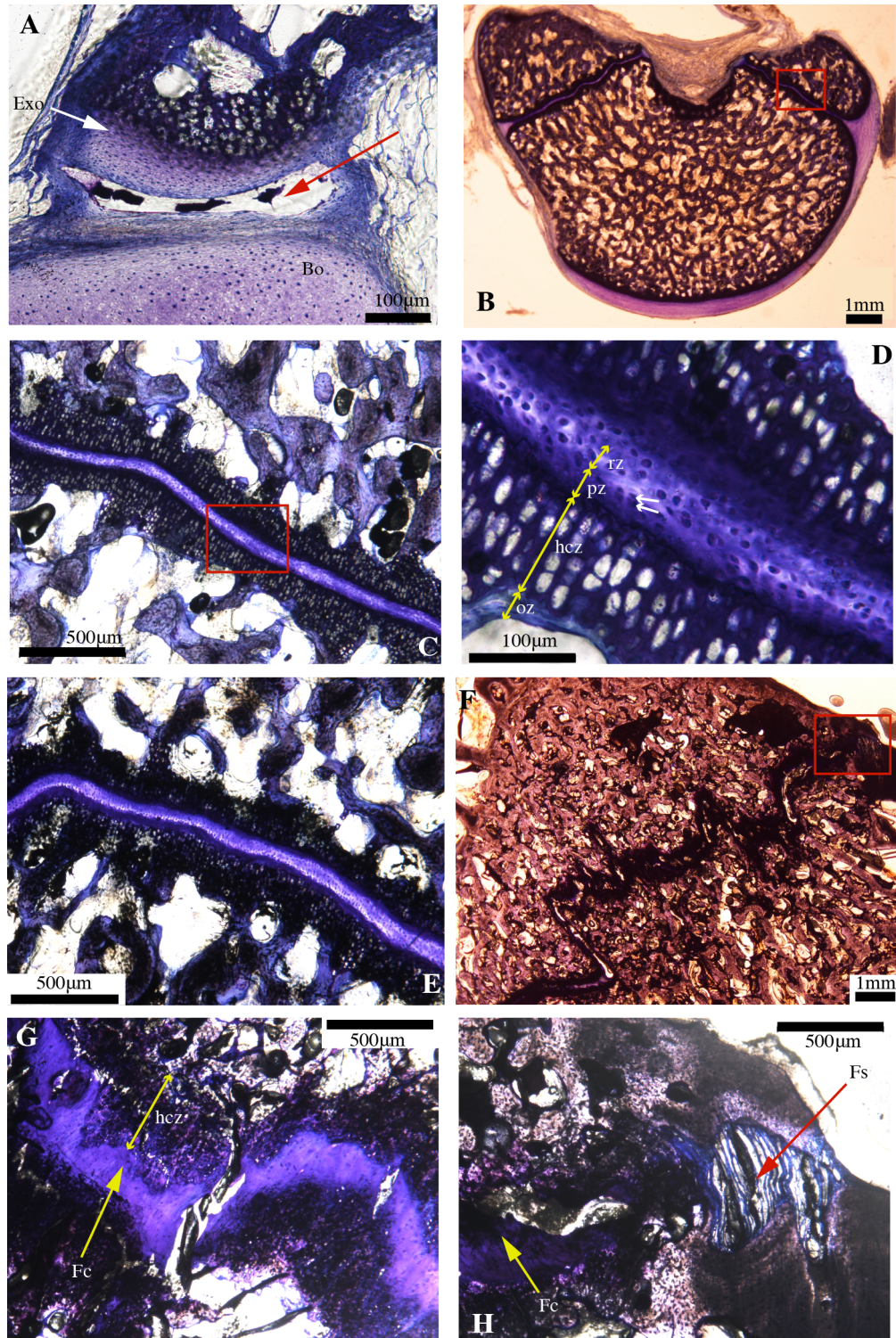


Figure 3.14. Cross-sections in the basioccipital-exoccipital synchondrosis in American alligators stained with Toluidine blue. A, right 'presumptive' basioccipital-exoccipital synchondrosis of Alligator 1. This synchondrosis is not formed yet (see empty space indicated by the red arrow). B, whole section showing the occipital condyle of Alligator

3. C, close-up of the red box in B. D, close-up of the red box in C, showing the middle resting zone and the mirroring proliferative zones, hypertrophied cartilage zones and ossification zones (double yellow arrows). The proliferative zone is composed of two to three cells (white arrows). E, right basioccipital-exoccipital synchondrosis of Alligator 4. F, left basioccipital-exoccipital synchondrosis of Alligator 6. This synchondrosis is interdigitated. G, close-up of the right basioccipital-exoccipital synchondrosis of Alligator 6. The hyaline cartilage has been replaced by fibrocartilage (yellow arrow). The mirroring hypertrophied cartilage zones are still present (double yellow arrows). H, close-up of the red box in F. The most internal part of the synchondrosis is composed of fibrocartilage (yellow arrow), while the most external (medial) part is composed of a fibrous tissue (red arrow), giving this portion the appearance of a suture. Abbreviations: same as the previous figure; Bo, basioccipital; Exo, exoccipital; Fc, fibrocartilage; Fs, fibrous suture.

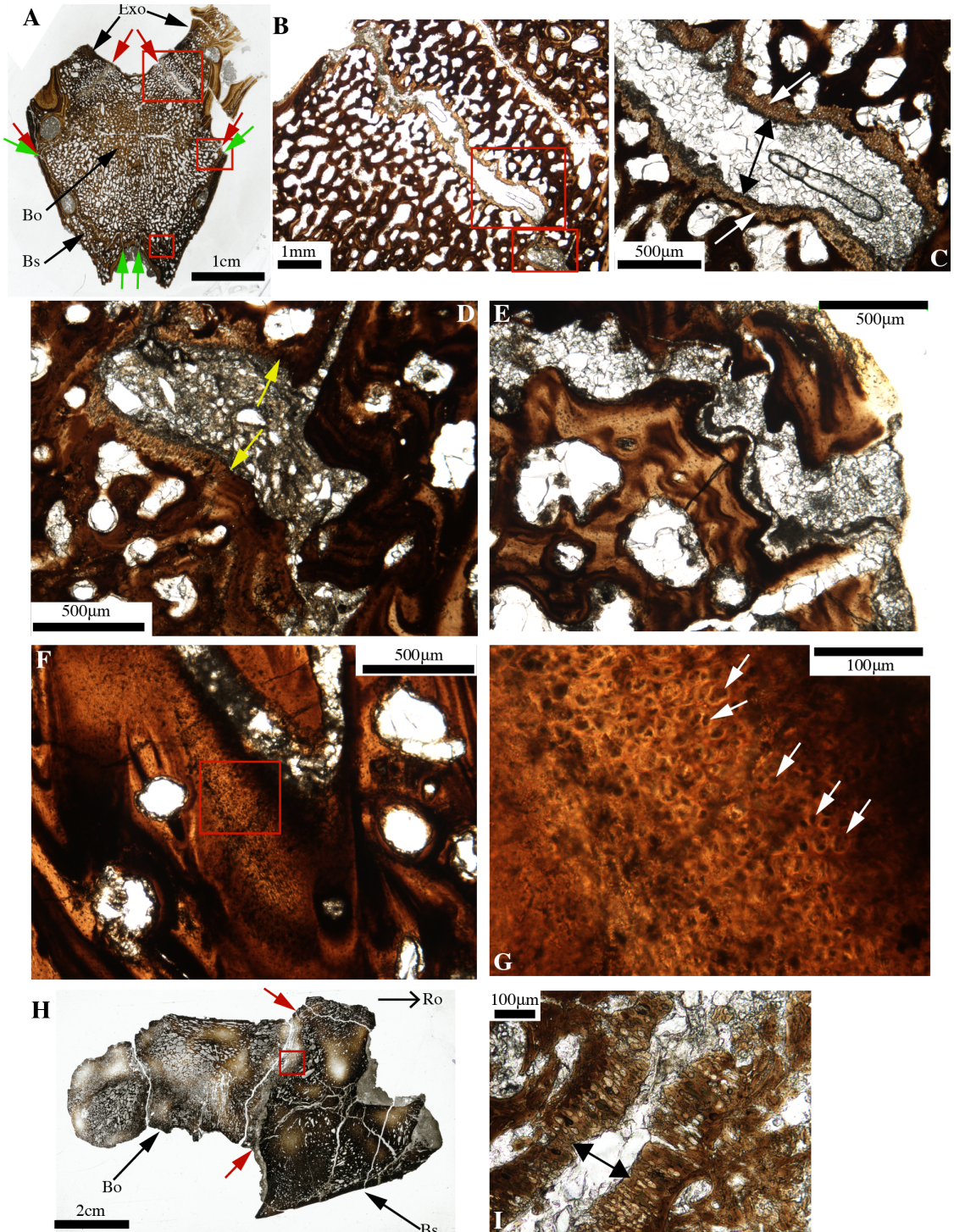


Figure 3.15. Paleohistological cross-sections of the basioccipital-exoccipital-basisphenoid complex of a fossil crocodilian (A-G) and parasagittal sections of the basisphenoid-basioccipital complex of *Prosaurollophus* (H-I). A, whole section of MOR 552. The basioccipital-exoccipital synchondroses are shown by the red arrows. The basisphenoid-basioccipital synchondroses are shown by the green arrows. B, close-up of the upper red

box in A. C, close-up of the upper red box in B, with a middle zone filled in with minerals (double black arrows) and two mirroring hypertrophied cartilage zones (white arrows). D, close-up of the lower red box in B. The hypertrophied cartilage of the synchondrosis disappears abruptly (yellow arrows) and the remainder of the 'synchondrosis' appears to be a 'suture'. E, close-up of the right red box in A, showing a histology comparable to that of a suture. F, close-up of the lower red box in A. In this basioccipital-basisphenoid junction, no hypertrophied calcified cartilage can be seen, which suggests again that it could be a suture. G, close-up of the red box in F. The sutural borders are composed of a tissue with globular structures (white arrows). H, whole section of the basisphenoid-basioccipital bones of MOR 447. The two ends of the basioccipital-basisphenoid synchondrosis are indicated by the red arrows. I, close-up of the red box in H. The middle resting and proliferative zones are not preserved (double black arrows). Two mirroring hypertrophied cartilage zones can be seen. Abbreviations: same as previous figure; Bs, basisphenoid.

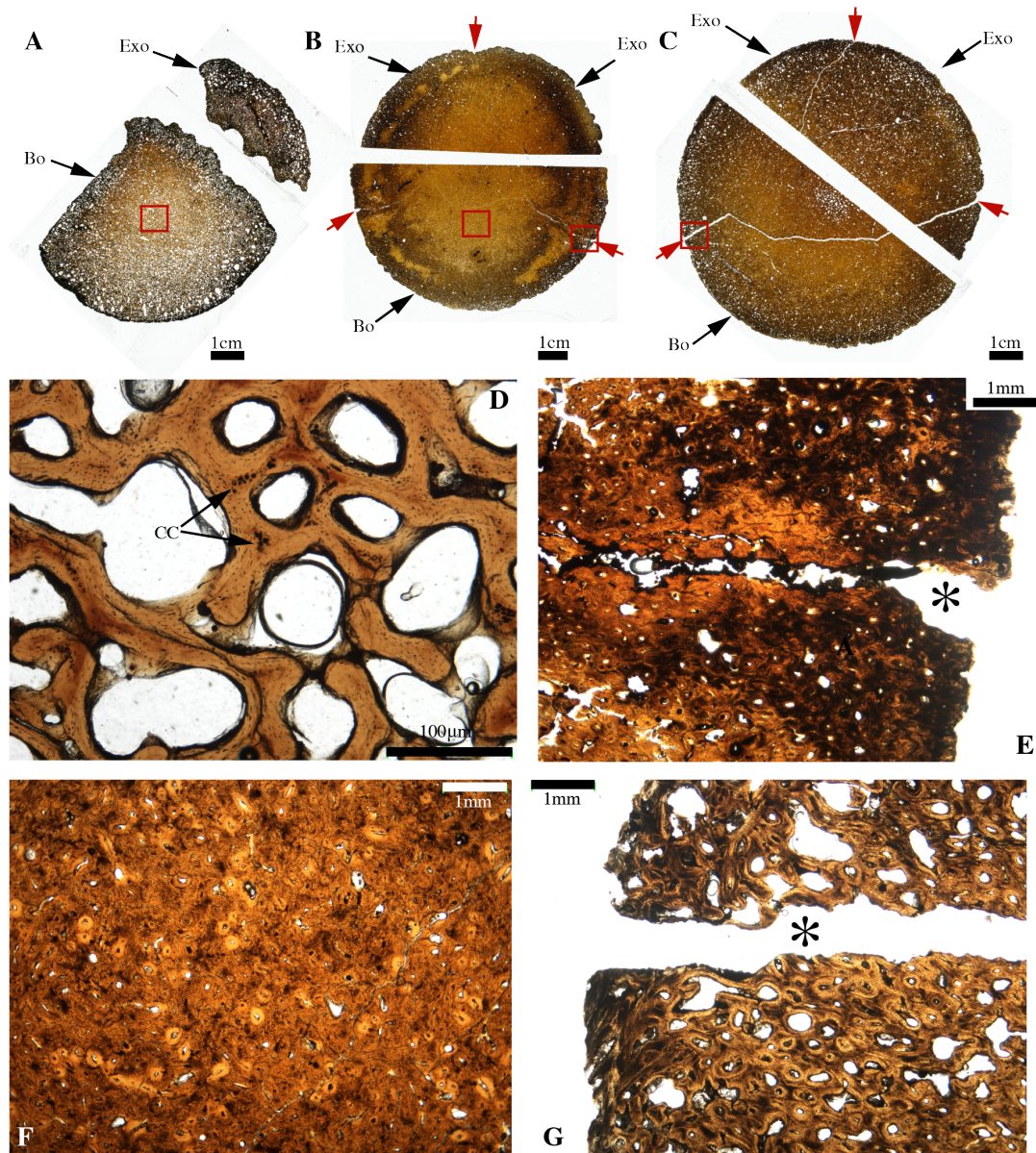


Figure 3.16. Paleohistological cross-sections of the occipital condyles of *Triceratops*. A, whole sections of the unfused occipital condyle of MOR 1110. B, whole sections of the fused occipital condyle of MOR 8657. C, whole sections of the fused occipital condyle of MOR 8658. Synchondroses are indicated by the red arrows in B and C. D, close-up of the red box in A. Trabeculae of lamellar bone can be seen, as well as some islands of calcified cartilage, remnants of the primary cartilage model (black arrows). E, close-up of the right red box in B. The right basioccipital-exoccipital synchondrosis is open externally (black asterisk) and does not present any calcified cartilage. It presents the histological characteristics of a suture. F, close-up of the left red box in B. The bone is a compacta with numerous secondary osteons. G, close-up of the red box in C. It presents the same histological characteristics as E. Abbreviations: same as previous figures, CC, calcified cartilage.

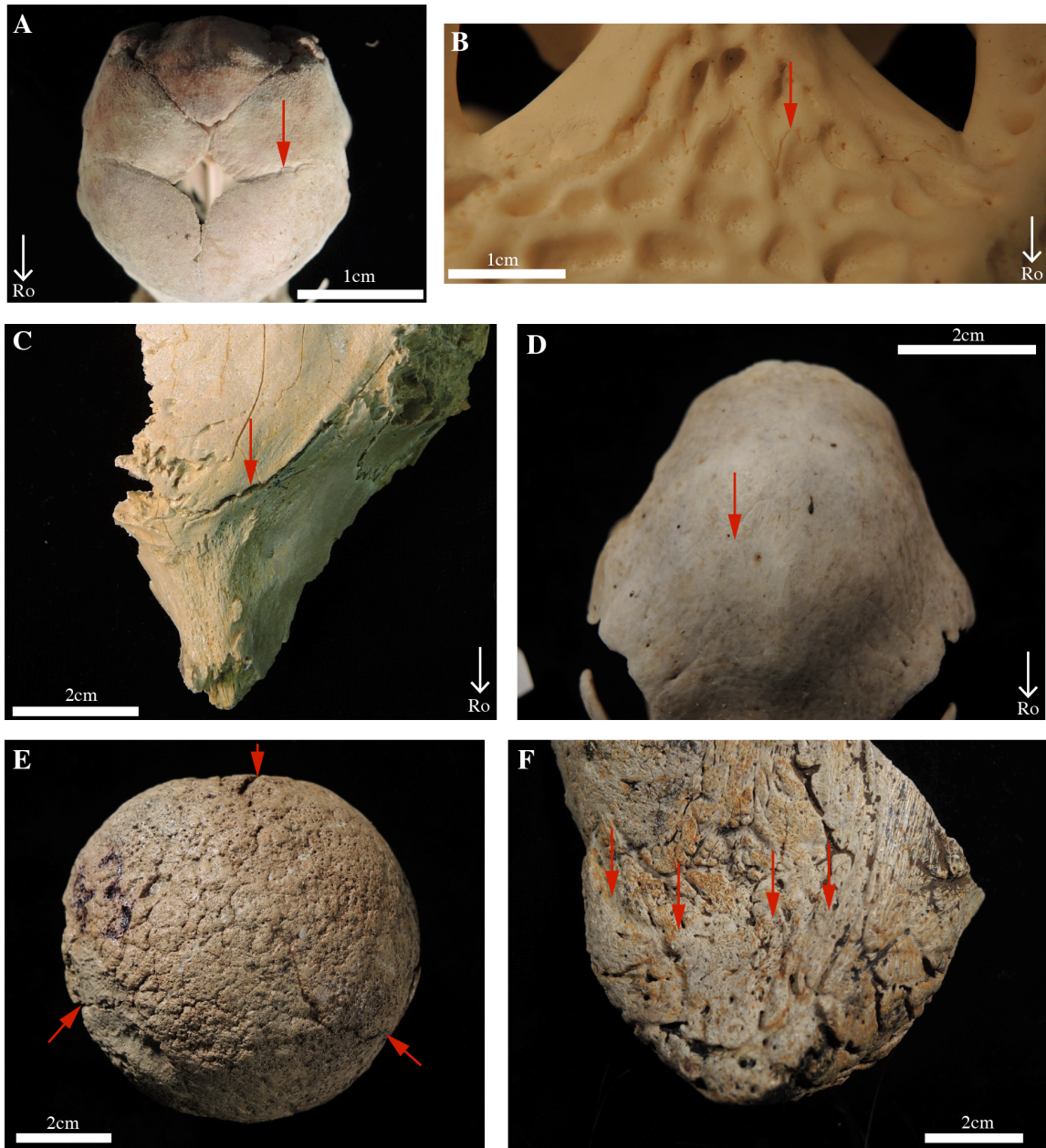


Figure 3.17. Morphological characteristics of the sutures and synchondroses of extant and fossil archosaurs. A, right fronto-parietal suture of Emu 1 in dorsal view (red arrow). It presents an 'open' morphology (with a suture line is still visible). B, open fronto-parietal suture of Alligator 5 in dorsal view (red arrow). C, open fronto-parietal suture of MOR 1071 (*Brachylophosaurus*) in dorsal view (red arrow). D, obliterated fronto-parietal suture of Emu 6 in dorsal view (red arrow). E, open synchondroses in the occipital condyle of MOR 8657 (*Triceratops*) in caudal view (red arrows). F, obliterated jugal-epijugal suture of UCMP 173848 (*Triceratops*) in lateral view (red arrows).

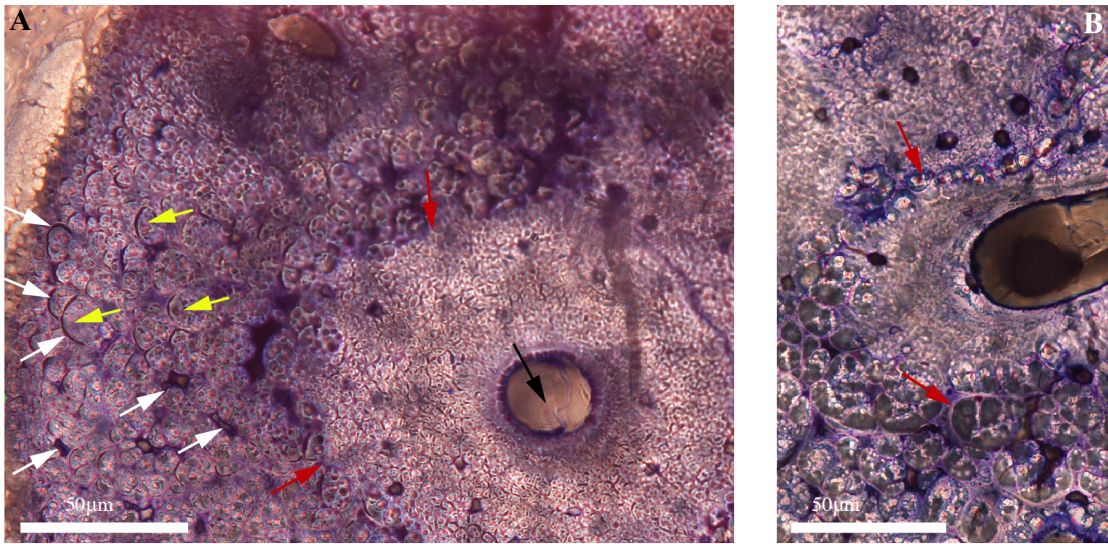


Figure 3.18. Cross-sections of a mineralized tendon from *M. extensor carpi radialis* of *Bubo virginianus* (Great Horned Owl). A, part of a mineralized tendon of MOR.H.2001-10R. A vascular canal can be seen (black arrow). It does not show the typical characteristics of a primary osteon or a haversian system and therefore was named “secondary reconstruction” (SR) by Horner et al., (in press). The boundary between this SR and the rest of the tissue is ragged and un-distinct (red arrows). It appears to be made of small collagen fiber bundles and fascicles tightly packed together. Further away from this SR, the tissue is composed of larger fiber fascicles (yellow arrows), separated from each other by spaces of different shapes, many of which are arc-shaped (white arrows). These arc-shaped spaces are the endotenons of the fiber fascicles. No regular osteocyte lacunae (nor canaliculi) can be seen anywhere in this tendon. B, section of another area in this same tendon showing the boundary of a SR where large fascicles are deconstructed into smaller fascicles (red arrows). This figure is modified from Horner et al., (in press.)

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CHAPTER FOUR

FIRST EVIDENCE OF DINOSAURIAN SECONDARY CARTILAGE IN THE POST-HATCHING SKULL OF *HYPACROSAURUS STEBINGERI* (DINOSAURIA, ORNITHISCHIA)

Contribution of Authors and Co-Authors

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

Co-Author: John R. Horner

Contributions: Provided funding and access to specimens. Conceived the study, performed experiments, analyzed data, interpreted results and commented on the manuscript.

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Abstract

Bone and calcified cartilage can be fossilized and preserved for hundreds of millions of years. While primary cartilage is fairly well studied in extant and fossilized organisms, nothing is known about secondary cartilage in fossils. In extant birds, secondary cartilage arises after bone formation during embryonic life at articulations, sutures and muscular attachments in order to accommodate mechanical stress. Considering the phylogenetic inclusion of birds within the Dinosauria, we hypothesized a dinosaurian origin for this “avian” tissue. Therefore, histological thin sectioning was used to investigate secondary chondrogenesis in disarticulated craniofacial elements of several post-hatching specimens of the non-avian dinosaur *Hypacrosaurus stebingeri* (Ornithischia, Lambeosaurinae). Secondary cartilage was found on three membrane bones directly involved with masticatory function: (1) as nodules on the dorso-caudal face of a surangular; and (2) on the bucco-caudal face of a maxilla; and (3) between teeth as islets in the alveolar processes of a dentary. Secondary chondrogenesis at these sites is consistent with the locations of secondary cartilage in extant birds and with the induction of the cartilage by different mechanical factors - stress generated by the articulation of the quadrate, stress of a ligamentous or muscular insertion, and stress of tooth formation. Thus, our study reveals the first evidence of “avian” secondary cartilage in a non-avian dinosaur. It pushes the origin of this “avian” tissue deep into dinosaurian ancestry, suggesting the creation of the more appropriate term “dinosaurian” secondary cartilage.

Introduction

Like bone microstructure, calcified cartilage can be fossilized and preserved for hundreds of millions of years (Bardack and Zangerl, 1971; Chinsamy-Turan, 2005; Scheyer, 2007). Indeed, primary cartilage has been widely found and documented in fossils (see review in Ricqlès 1975). However, another type of calcified cartilage, less studied than primary cartilage and known as secondary cartilage (because it arises after bone formation), has only been described in extant species and never been reported in a fossil so far. In extant birds, “avian” secondary cartilage is found on skull and jawbones and plays an important role in resisting mechanical stress from embryonic development up to adulthood (Figs. 1A, C). Considering the phylogenetic inclusion of birds within the Dinosauria (Gauthier, 1986), we hypothesized a dinosaurian origin for this “avian” tissue. Therefore, we investigated secondary chondrogenesis by means of histological thin sectioning in disarticulated craniofacial elements of several post-hatching specimens of the non-avian dinosaur *Hypacrosaurus stebingeri* (Ornithischia, Lambeosaurinae), from the Upper Cretaceous (Campanian) Two Medicine Formation of Montana (Horner and Currie, 1994).

Early ontogenetic stages are the most suitable to study chondrogenesis, therefore these elements are appropriate to our investigation. They represent the youngest non-avian dinosaur skulls ever studied from a histological perspective. A comparison with primary chondrogenesis could be and was undertaken as well. The main aim of the study was to investigate the proposed dinosaurian origin of “avian” secondary cartilage. The

presence of this “avian” tissue in a non-avian dinosaur would push its origin deep into the dinosaurian ancestry, and further cement the dinosaurian origin of birds.

Modes of Skeletal Formation in Vertebrates

Endochondral bones start out with **primary** cartilage. They are located in the postcranium (e.g., limb bones, vertebrae and ribs) and the neurocranium (i.e., the cartilaginous skull). The latter is composed of the cranial base (Hall, 2005) and the sensory capsules (otic, optic and nasal capsules; see Couly et al., 1993 for a complete list of endochondral bones in chick skulls). Bone arises (from osteogenic cells brought in by blood vessels) at the center (diaphysis) of the primary cartilaginous models of long bones from which it spreads. In many groups, secondary centers of ossification arise at the ends (epiphyses) of the cartilage models. Cartilage is replaced by bone at growth plates. More precisely, chondrocytes undergo hypertrophy and cellular apoptosis, and vascular invasion brings in osteogenic cells that lay down new bone matrix. This leaves cartilage only at the extremities as the articular cartilage of the joints. The shape of the final bone is laid down in the cartilage model.

Membrane bones, however, ossify directly through the process of intramembranous ossification (Hall, 1997, 2005) without a primary cartilaginous model (Bloom and Fawcett, 1986; Hall, 1971a). Membrane bones form the cranial vault (that protects the brain), the face, and the bone(s) of the jaws (Enlow, 1990). The only membrane bones found in the post-cranium are the paired clavicles. Although membrane bones ossify directly without a primary cartilage model, in some species cartilage can

arise secondarily, on pre-existing membrane bones, and is therefore called **secondary** cartilage (Hall, 1968a, 2005; Tran and Hall, 1989).

“Avian” Secondary Cartilage

Secondary cartilage arises because of the ability of periosteal cells to respond to mechanical influences by switching their differentiation from osteogenesis to chondrogenesis (Hall, 2000). The molecular basis of secondary cartilage formation is well known (Buxton et al., 2003). The organic phase of secondary cartilage presents simultaneously type I and type II collagens, while primary cartilage only secretes type II collagen (Hall, 2005). In histological sections, secondary cartilage presents a smaller amount of extracellular matrix than primary cartilage (Hall, 2005), but the identification of the former is based essentially and more accurately on its location (i.e., on the articular surface of a pre-existing membrane bone). In addition to this, the organization of the chondrocytes can also give a clue: while primary cartilage is usually organized into long straight tubes at epiphyseal growth plates (Barreto et al., 1993), secondary cartilage lacks this linear organization, with its chondrocytes organized randomly in nodules (Enlow, 1990; and see the Results section). Being induced and maintained by mechanical influences, secondary cartilages provide important regional adaptive growth (Enlow, 1990) and accommodate stress and strain during normal development (Hall, 1967, 1971b, 2000; Hall and Jacobson, 1975; Murray, 1963; Murray and Smiles, 1965), but also during fracture repair (Hall and Jacobson, 1975).

In birds, secondary cartilage is present in the growing craniofacial skeleton (Hall, 1967, 1971b, 2000; Hall and Jacobson, 1975; Lengelé et al., 1990, 1996a, 1996b;

Murray, 1963; Figs. 1A, C) and the growing clavicle (Hall, 1986). “Avian” secondary cartilages are initiated (and maintained) as nodules at sites subject to intermittent pressure, such as articulations, sutures and points of insertions of ligaments or masticatory muscles (Hall, 2000). Secondary cartilages arise during embryonic life and persist after hatching. In adults almost all of the secondary cartilages are resorbed and their place taken by newly formed endochondral bone. The remaining chondrocytes (in the superficial layers) become the chondrocytes of an articular fibrocartilage (Hall, 1967, 1971b). This makes embryos and the newly hatched the most suitable stages to study secondary chondrogenesis in birds.

However, secondary cartilage is not unique to birds. It has been widely sought among extant vertebrates and has also been found in two other groups (Hall, 2000): teleosts and mammals (Appendix K). It has not been reported in lissamphibians or in non-avian sauropsids, despite extensive examination of embryos and attempts to induce such cartilage experimentally (Hall and Hanken, 1985; Irwin and Ferguson, 1986; Vickaryous and Hall, 2008). Instead, these animals accommodate stress by forming syndesmoses (i.e., a dense fibrous connective tissue; e.g., see Hall, 2005; Vickaryous and Hall, 2008) at the junction of their membrane bones. The most parsimonious interpretation is that the secondary cartilages displayed by the Teleostei, Mammalia, and Aves are not homologous and arose independently (Hall, 2000). Therefore, within the Archosauria, secondary cartilage (or the dual ability of periosteal cells to form chondro- and osteoblasts) is unique to birds and seems to carry a phylogenetic signal.

Results

Secondary cartilage was found in three locations: on the dorso-caudal face of a surangular at its articulation with the quadrate (Figs. 1D, 2A, B, D); on the bucco-caudal face of a maxilla (Figs. 1D, 2E, F); and in the alveolar processes of a dentary between teeth (Figs. 1D, 2G, H). The surangular and the maxilla each display a nodule of secondary cartilage (Figs. 2A, E) while the dentary displays smaller cartilaginous islets (Fig. 2G). These cartilages are composed of ovoid lacunae (Figs. 2B, D, F, H), interpreted as remnants of hypertrophied cartilage cells separated by a bright mineralized extracellular matrix, the latter appearing darker in the section (Fig. 2D). These two nodules and little islets are undergoing endochondral ossification. This is most visible on the surangular where chondroclastic resorption is evidenced by large erosion bays (Figs. 2B, D). Subsequent bone apposition is evidenced by bone struts on the walls of some of these erosion bays (Fig. 2D). These bone struts are also seen in the nodule of the maxilla (indicated by the white arrows in Fig. 2F) and in the islet (indicated by the black arrows in Fig. 2H). Secondary cartilages of birds also undergo resorption and endochondral ossification (Fig. 2C). We identify unambiguously these cartilages on *Hypacrosaurus* as secondary and not primary for two reasons: (1) they display the typical cellular organization of avian secondary cartilages as described below, an arrangement completely different from the one displayed by primary cartilage; (2) they are located on pre-existing membrane bones and therefore can only have arisen secondarily, after bone formation.

As expected, the endochondral bones that were sectioned displayed remnants of primary cartilage on their edges: the basisphenoid, the basioccipital, the supraoccipital and the exoccipital (i.e., chondrocranial bones forming the basicranium), the quadrate, the prootic and the sclerotic (i.e., endochondral bones forming the sensory capsules). No cartilage was found on the laterosphenoid, the orbitosphenoid or the presphenoid; we suggest that this is more a preservation bias rather than an unambiguous absence. The primary cartilage of these specimens is organized into long straight tubes of hypertrophic chondrocytes, separated by bone trabeculae (Fig. 3). This tubular arrangement, oriented in one direction is observed in growth cartilages that provide linear growth (i.e., epiphyseal plates of long bones and synchondroses of the cranial base). These cartilaginous tubes give an undulating shape to the junction between bone and calcified cartilage (i.e., the chondro-osseous junction, otherwise straight in mammals and non-avian sauropsids, Barreto et al., 1993; Horner et al., 2001), which is characteristic of the epiphyseal growth plates of birds and non-avian dinosaurs (Barreto et al., 1993; Horner et al., 2001; see also the tibial epiphyseal growth plate of *Hypacrosaurus* in Fig. 3C). Secondary cartilage distinctively lacks this linear organization and therefore, primary and secondary cartilage cannot be misidentified. One could argue that the islets in the dentary are nothing more than remnants of Meckel's cartilage, a primary cartilage rod that progressively becomes enveloped by the dentary during development (Hall, 2005). However, these islets have no topographical relationship with Meckel's cartilage, they are located in the dorsal region of the dentary, while Meckel's cartilage would be located ventrally, as it is in extant amniotes, and they differ histologically from the primary

cartilage of Meckel's, that usually stays hyaline in most taxa (i.e., uncalcified, with few and small chondrocytes encased in a very large amount of extracellular matrix, Hall 2005). Moreover, no remnants of Meckel's cartilage were found in the dentary of MOR 548. We suggest that the hyaline nature of Meckel's cartilage did not allow its fossilization.

Discussion

From a histological perspective, little is known about dinosaur skull development, especially during early ontogenetic phases. Indeed, only four studies on juvenile and adult non-avian dinosaurs describe the histology of cranial bones: the frontoparietals of pachycephalosaurs (Goodwin and Horner, 2004; Horner and Horner, 2009); the parietals of *Centrosaurus* (Tumarkin-Deratzian, 2010) and *Triceratops* (Scannella and Horner, 2010; Horner and Lamm, 2011). There are, to our knowledge, no studies on the bone microstructure of earlier ontogenetic stages (e.g., embryos, post- and circum-hatching stages) of dinosaur skulls. This study investigating dinosaurian secondary chondrogenesis presents the youngest non-avian dinosaur skulls ever shown from a histological perspective.

In extant birds, secondary cartilages resist and absorb mechanical stress. Secondary cartilages arise in sites experiencing mechanical stresses (e. g. Figure 1C) such as sutures, articular surfaces (in order to prevent abrasion, Hall, 1967) and points of insertion of ligaments or masticatory muscles (Hall, 2000). Likewise, *Hypacrosaurus*

displays secondary cartilages at three sites where mechanical factors can be inferred (Figure 1D):

(1) On the surangular, the location of the nodule is consistent with preventing abrasion, because it is where it articulates with the quadrate. Moreover, since the section is located in the vicinity of the insertion sites of masticatory muscles (such as *M. depressor mandibulae* and *M. Pterygoideus ventralis*, Holliday, 2009), a muscular induction could also be considered. Similarly, the surangular of birds displays secondary cartilages (Figs. 1C, 2C; Hall, 1967, 1971b; Murray, 1963).

(2) The location of the nodule on the maxilla could be puzzling at first; it is not a suture, nor an articular surface, nor has it previously been described as being a muscle insertion site. However, as the nodule directly faces the coronoid process of the dentary we hypothesize that this nodule might have arisen in response to the “pressure” of the coronoid process; or in response to the mechanical stress of a ligamentous or muscular insertion, possibly linking the coronoid process and the maxilla. Further investigation of the histology of a coronoid process in this particular area could shed light on this possibility and be of interest for hadrosaur jaw mechanics.

(3) Finally, we hypothesize that the development of secondary cartilaginous islets in the dentary was induced by the mechanical stress of tooth formation and growth. The growth of the dentary is different in non-avian dinosaurs and extant birds because the latter do not possess teeth. Therefore, no direct comparison with extant birds is possible. However, similar secondary cartilage islands are found in mammalian alveolar processes, and their formation allows rapid growth in the mandible (Goret-Nicaise, 1986; Goret-

Nicaise et al., 1984). Therefore, we hypothesize that this represents a convergent evolution allowing fast growth in the mandible of *Hypacrosaurus*. This is also supported by the bone microstructure (not only of the dentary, but in the vast majority of the investigated areas, *data not shown*), showing a highly cellular and fibrous primary bone, with numerous and large vascular spaces (e.g., Figs. 2A, E); altogether suggesting an extremely high velocity of growth (if not an embryonic potential of growth).

In extant birds, secondary cartilages are found in the cranial vault, in the face, and mostly in the skeleton involved with the masticatory function (Fig. 1C and see review in Hall, 2000). The formation of this skeletal tissue is species-specific and is dictated by the mechanical forces of the mode of feeding (Hall, 1968a; 2000). Similarly, in *Hypacrosaurus* we identified secondary cartilage on bones directly involved with the chewing function, the dentary and the surangular in the lower jaw, and the maxilla in the upper jaw (Fig. 1D). It is highly probable that more sites were present but were impossible to identify because of poor preservation. It is also possible that additional sites of secondary chondrogenesis existed during the embryonic development of *Hypacrosaurus*, or that new and different nodules arise at later ontogenetic stages, as in birds (Hall, 1967, 1971b). Any confusion with primary cartilage of endochondral bones of the skull can be avoided, first because it is not found on the same types of bones, and second, because it is organized differently with straight tubes of cartilage, oriented in one direction and with an undulating chondro-osseous junction. This undulating chondro-osseous junction is also present in the epiphyseal growth cartilages of the long bones of non-avian dinosaurs and birds (Figure 3C; Barreto et al., 1993; Horner et al., 2001). This

is a shared derived anatomical character corroborating the inclusion of birds within the Dinosauria (Horner et al., 2001). We describe it here for the first time in the cartilaginous skull of a non-avian dinosaur. Most importantly, this study indicates that the craniofacial development of birds and at least one clade of dinosaurs, the Ornithischia, is adapted to resist mechanical stress through secondary chondrogenesis.

Induced fracture repair did not produce secondary cartilage in lissamphibians (Hall and Hanken, 1985) or in lepidosaurians (Irwin and Ferguson, 1986), and in crocodilians (the closest living relatives of birds), secondary chondrogenesis was not observed during the development of *Alligator mississippiensis* (Vickaryous and Hall, 2008), nor has it been reported during reptilian development (Irwin and Ferguson, 1986). Therefore, because this process fails to occur in any other extant lissamphibian and non-avian sauropsid, we hypothesize that avian and *Hypacrosaurus* secondary cartilages are homologous. If this is the case, and as a result of its inferred presence in their common ancestor secondary cartilage would be present in the other dinosaurian clade, the Saurischia. The alternate hypothesis, that this complex ability of the periosteum to switch from osteogenesis to chondrogenesis evolved independently, seems less plausible. The discovery of “avian” secondary cartilage in a non-avian dinosaur further solidifies the dinosaurian origin of birds and suggests the creation of the more appropriate term “dinosaurian” secondary cartilage. Further histological analyses are underway to study members of the Saurischia, such as non-avian theropod material.

Materials and Methods

The disarticulated MOR (Museum of the Rockies) 548 specimens were collected from an exceptional hadrosaur nesting ground that has yielded dozens of disarticulated embryos and post hatchlings from at least fifteen individuals of *Hypacrosaurus stebingeri* (Ornithischia, Lambeosaurinae), in the Upper Cretaceous (Campanian) Two Medicine Formation of Montana (Horner and Currie, 1994). The elements used in this study were selected carefully from the collections, in order to represent the approximate same growth stage (i.e., post-hatching, a few months old) with an estimated skull length of 20 cm. A composite skull of a similar size is still on display at the MOR. So far, no texture by which secondary cartilage could be recognized on gross examination was found. In order not to lose any data concerning the size and the morphology of the disarticulated bones, molds and casts were made prior to histological analysis. In total, twenty-five elements were sectioned (Table 1) according to standard fossil thin-sectioning techniques (Lamm, 2007). Specimens were embedded in polyester resin and sectioned with a diamond powder disk on a precision saw. Two to five thin-sections were made of each element, with various cut orientations (i.e., sagittal, parasagittal, transverse and coronal) in order to study multiple potential secondary chondrogenesis sites such as articulations and sutural areas involving membrane bones (Table 2). Sections were then mounted on glass slides, ground and polished. Completed thin section slides were observed with a Nikon Optiphot-Pol polarizing microscope. Photomicrographs were taken with a Nikon DS-Fi1 digital sight camera and the NIS-Elements BR 3.0 software.

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Table 4.1. List of the thin-sectioned bones. Bones showing cartilage (remnants of primary cartilage for endochondral bones, and secondary cartilage for membrane bones) are indicated by an asterisk (*).

Endochondral bones	Membrane bones
basioccipital*	dentary* prefrontal
basisphenoid*	frontal premaxilla
exoccipital*	jugal quadratojugal
laterosphenoid	lacrimal squamosal
orbitosphenoid	maxilla* surangular*
presphenoid	nasal
prootic*	palatine
quadrate*	parietal
sclerotic*	postorbital
supraoccipital*	prementary

Table 4.2. List of the articulations studied for the investigation of secondary chondrogenesis. The elements studied were all disarticulated, but the numerous sections allowed an examination of several sutural edges of a bone (or the inferred areas of contact with other bones). The first-named component indicates the bone that was sectioned. The asterisk (*) indicates where secondary cartilage was found.

Articulations	
dentary-prementary	nasal-frontal
dentary-surangular	nasal-prefrontal
frontal-frontal	palatine-maxilla
frontal-nasal	palatine-pterygoid
frontal-parietal	parietal-frontal
frontal-postorbital	parietal-squamosal
jugal-lacrimal	postorbital-frontal
jugal-maxilla	postorbital-parietal
jugal-quadratojugal	prefrontal-nasal
lacrimal-premaxilla	squamosal-parietal
maxilla-jugal	squamosal-postorbital
maxilla-premaxilla	squamosal-quadrate
maxilla-pterygoid	surangular-quadrate*

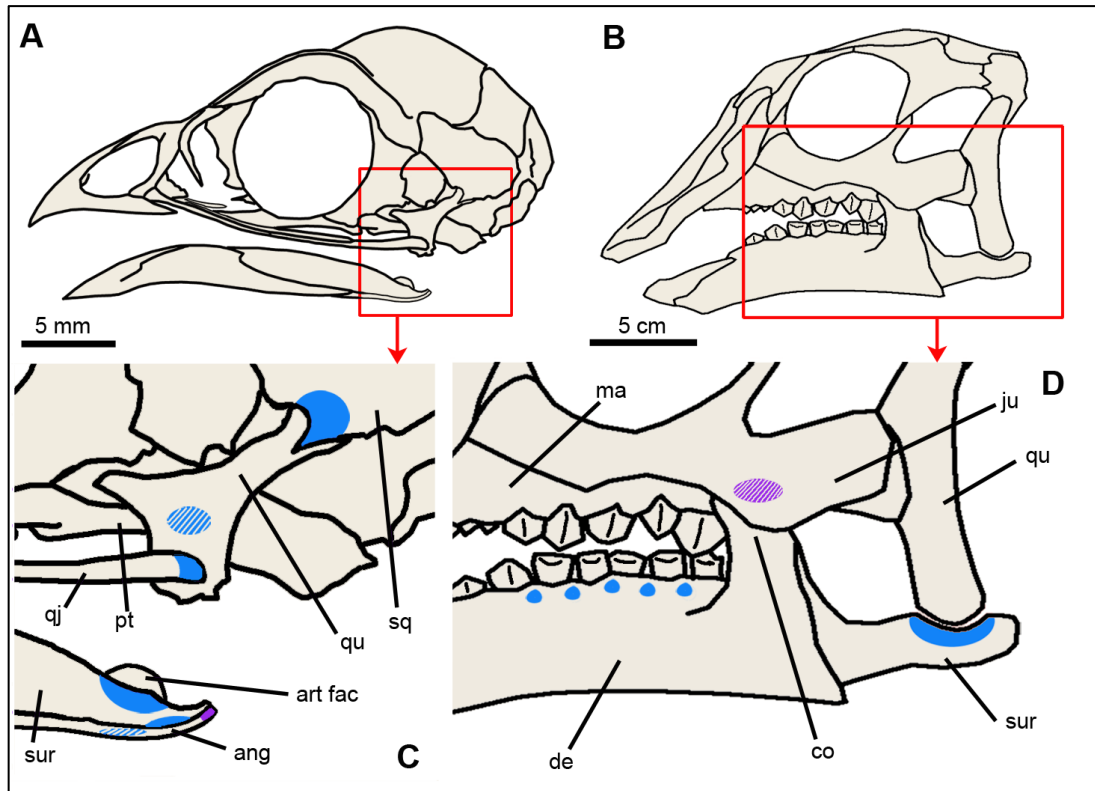


Figure 4.1. Head skeleton and distribution of secondary cartilage in a newly-hatched chick and a post-hatching *Hypacrosaurus*. (A) Skull diagram of a 2 day-old chick *Gallus*. (B) Skull diagram of a post-hatching *Hypacrosaurus*. (C, D) Detail in the red box in (A) and (B) respectively. Locations of secondary cartilage are indicated in blue (at articulations) and purple (at muscle or ligament insertions). Diagonal lines indicate that secondary cartilage is not located in the first plane of the figure, but more internally (on the lingual faces). In (C), secondary cartilage is found at the following articulations: pterygoid-quadratojugal, quadratojugal-quadratojugal, squamosal-quadratojugal, surangular-articular facet, surangular-Meckel's cartilage, and angular-Meckel's cartilage (based on Hall, 1968a). It is also found on the distal tip of the angular at the insertion site of *M. depressor mandibulae* (Hall, 1968a). Note that these sites change during ontogeny, i.e., more and different sites are present in the embryonic chick (Murray, 1963). In (D), secondary cartilage is found at the surangular-quadratojugal articulation; on the bucco-caudal face of the maxilla (in contact with the coronoid process of the dentary), and in the alveolar processes of the dentary between teeth. ang, angular; art fac, articular facet of Meckel's cartilage; co, coronoid process; de, dentary; ju, jugal; ma, maxilla; pt, pterygoid; qj, quadratojugal; qu, quadratojugal; sq, squamosal; sur, surangular.

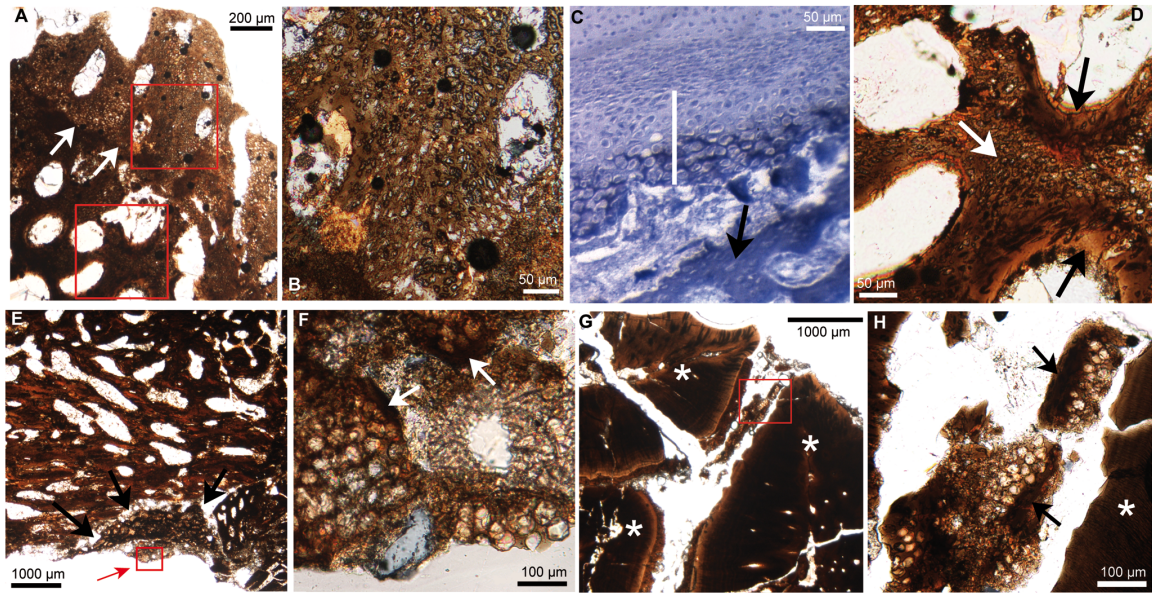


Figure 4.2. Thin-sections showing secondary cartilage. (A) Cross section of the surangular (at the quadrate articulation) of *Hypacrosaurus*. White arrows indicate the limit between bone and secondary cartilage. (B) Detail in upper red box in (A). The ovoid lacunae are remnants of hypertrophied chondrocytes. Resorption is evidenced by erosion bays. (C) Cross section in a 16 day-old-chick embryo showing Meckel's primary cartilage (uncalcified) above, the perichondrium below it and secondary cartilage (white bar) on eroded surangular bone struts (black arrow). Sudan black B shows that the most mature secondary cartilage is calcified (in dark blue). Adapted from a figure in Hall, 1968b. (D) Detail in lower red box in (A). Secondary cartilage (white arrow) is undergoing resorption and endochondral ossification (black arrows). (E) Coronal section in a maxilla. The nodule of secondary cartilage (black arrows) has globular hypertrophied chondrocytes. The area in the small red box (indicated by the red arrow) is detailed in figure (F). (F) Detail of red box in (E). The globular and hypertrophied chondrocyte lacunae are encased in a small amount of extracellular matrix. The white arrows indicate bone struts. (G) Cross section in the caudal part of a dentary showing teeth (white asterisk). (H) Detail of red box in (G). An islet of secondary cartilage is located between a tooth (indicated by the asterisk on the right) and alveolar bone (left). The black arrows show bone struts. Photographs taken under natural light.

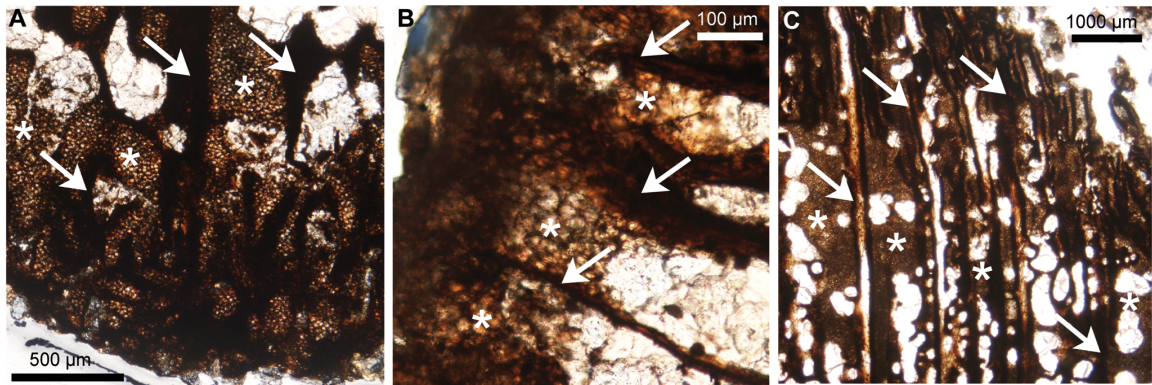


Figure 4.3. Thin-sections showing remnants of primary cartilage in *Hypacrosaurus*. Longitudinal sections a quadrate (distal end) (A), a basisphenoid (B) and a tibia (proximal end) (C). Primary cartilage is organized into long straight tubes (asterisks), oriented toward the direction of growth, and separated by bone trabeculae (arrows). The junction between these cartilaginous tubes and the bone trabeculae, i.e., the chondro-osseous junction, is undulating (as opposed to straight). Photographs taken under natural light.

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CHAPTER FIVE

SECONDARY CARTILAGE REVEALED IN A NON-AVIAN DINOSAUR EMBRYO

Contribution of Authors and Co-Authors

Manuscript in Chapter 5

Author: Alida M. Bailleul

Contributions: Conceived the study, performed experiments, analyzed data, interpreted results and wrote the manuscript.

Co-Author: Brian K. Hall

Contributions: Conceived the study, analyzed data, interpreted results and commented on the manuscript.

Co-Author: John R. Horner

Contributions: Provided funding and access to specimens. Conceived the study, analyzed data, interpreted results and commented on the manuscript.

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Abstract

The skull and jaws of extant birds possess secondary cartilage, a tissue that arises after bone formation during embryonic development at articulations, ligamentous and muscular insertions. Using histological analysis, we discovered secondary cartilage in a non-avian dinosaur embryo, *Hypacrosaurus stebingeri* (Ornithischia, Lambeosaurinae). This finding extends our previous report of secondary cartilage in post-hatching specimens of the same dinosaur species. It provides the first information on the ontogeny of avian and dinosaurian secondary cartilages, and further stresses their developmental similarities. Secondary cartilage was found in an embryonic dentary within a tooth socket where it is hypothesized to have arisen due to mechanical stresses generated during tooth formation. Two patterns were discerned: secondary cartilage is more restricted in location in this *Hypacrosaurus* embryo, than it is in *Hypacrosaurus* post-hatchlings; secondary cartilage occurs at far more sites in bird embryos and nestlings than in *Hypacrosaurus*. This suggests an increase in the number of sites of secondary cartilage during the evolution of birds. We hypothesize that secondary cartilage provided advantages in the fine manipulation of food and was selected over other types of tissues/articulations during the evolution of the highly specialized avian beak from the jaws of their dinosaurian ancestors.

Introduction

The skulls and jaws of extant birds possess a specific type of cartilage known as *secondary cartilage* (SC), so named because it arises secondarily on pre-existing membrane bones (Beresford, 1981; Bock, 1960; Hall, 1967, 1968, 1971, 1986, 2000; Lengelé et al., 1996; Murray, 1963; Murray and Smiles, 1965) and not before bone (primarily), as does the primary cartilage model of endochondral bones (see Hall, 2005). SC arises during embryonic and early post-hatching development as an articular cartilage at joints or as nodules associated with ligamentous or muscular insertions, in all cases arising in response to intermittent pressure and shear (Hall, 1967; Murray, 1963; Murray and Smiles, 1965). SC forms because of the ability of the ‘osteogenic’ periosteal precursor cells to form chondroblasts in addition to osteoblasts (Hall, 2000). This chondrogenic potential of the periosteum — the presence of osteochondroprogenitor cells (Hall, 1967, 1968) — is only found in birds among living sauropsids and lissamphibians, which do not form SC but rather accommodate stress and strain with syndesmoses, i.e., the deposition of dense networks of collagen fibers at articulations (Hall and Hanken, 1985; Irwin and Fergusson, 1986; Payne et al., 2011; Vickaryous and Hall, 2008). Outside sauropsids and lissamphibians, a tissue known as SC is found in mammals and teleosts (Benjamin, 1989; Gillis et al., 2006; Pritchard et al., 1956; Vinkka-Puhakka and Thesleff, 1992). Due to their phylogenetic distribution, teleostean, mammalian and avian SCs are hypothesized to be homoplastic (Hall, 2000, see Bailleul et al., 2012 for further details). A notable difference is that mechanical stimulation is not required for the

initiation of mammalian SCs (Vinkka-Puhakka and Thesleff, 1992), while it is needed for the formation of avian SCs (Hall, 2000).

In a previous study (Bailleul et al., 2012), we reported for the first time the presence of SC in a non-avian dinosaur, *Hypacrosaurus stebingeri* (Ornithischia, Lambeosaurinae), and hypothesized that it was homologous to “avian” SC; see Bailleul et al., 2012 for the criteria used to establish homology of avian and dinosaurian SC. That study (Bailleul et al., 2012) focused on post-hatching specimens. To investigate secondary chondrogenesis through ontogeny and to compare the avian and ornithischian patterns it was necessary to investigate non-avian dinosaur embryos. In the present study using histological analyses of isolated skull elements of embryonic *H. stebingeri* (Fig. 1A), *Maiasaura peeblesorum* (Hadrosaurinae) and a Hadrosauridae indet., we report for the first time the presence of SC in a non-avian dinosaur embryo. From our analyses we propose that different patterns of the onset of secondary chondrogenesis exist in ornithischian and saurischian (avian) embryos, that these different patterns reflect different biomechanical conditions within avian and dinosaurian embryos that may be explained by the evolution of the highly specialized avian beak from the jaws of their dinosaurian ancestors.

Material and Methods

Ethics

All necessary permits were obtained for the described field studies. Permission for the collection of MOR 1038 was granted by the Montana Department of Natural

Resources (Helena, MT). The other MOR specimens were collected with land owner permission from private land (and therefore do not require any specific permit).

The six embryonic skull bones examined in this study (Table 1) were all disarticulated and collected from nesting horizons in the Two Medicine (TM) and Judith River (JR) Formations (Upper Cretaceous) in Montana. They belong to three closely related species: *Hypacrosaurus stebingeri*, *Maiasaura peeblesorum* (Hadrosaurinae) and a Hadrosauridae indet. The *Hypacrosaurus* specimens MOR 559 come from a nesting ground located on Blacktail Creek, Glacier County (Locality TM-066; see Horner and Currie, 1994) and these embryonic bones were weathering out of the eggs of a clutch. This is the very same locality that yielded the numerous post-hatching specimens MOR 548 used in our previous study (Bailleul et al., 2012). The embryonic *Maiasaura* MOR-YPM.430.Sa was collected in the Willow Creek Anticline, in a nesting ground near Choteau, Teton County (Locality TM-160; see Horner, 1994, 1999). This bone was associated with its egg. Finally, the Hadrosauridae indet., was also collected from a nesting ground located North of Kremlin, Hill County (Locality JR-144Q). Table 2 shows all anatomical locations where secondary chondrogenesis was sought (in priority, the three previously known cartilaginous sites (Bailleul et al., 2012), but also any other sites potentially subject to pressure and shear). Archival molds and casts were made prior to sectioning. The disarticulated bones were embedded in epoxy resin and cut with a diamond powder disk on a precision saw. All elements were serially thin-sectioned (except for the parietal and frontal), mounted on plastic slides and then ground and

polished by hand on a Buehler Ecomet grinder. Finished thin-sections were studied by light microscopy under normal and polarized light.

Results

Since SC arises during embryonic development (Murray, 1963) and persists after hatching in extant birds (Hall, 1967, 1968) it was necessary to investigate in priority the same anatomical locations that showed SC in the *H. stebingeri* post-hatchlings (Bailleul et al., 2012). These are (1) the dorso-caudal face of the surangular (at its articulation with the quadrate; Figs. 1A, B, C); (2) the bucco-caudal face of the maxilla (directly facing the coronoid process of the dentary; Figs. 1A, D, E); and (3) alveolar spaces between dentary teeth (Figs. 1A, F-H).

As a result of this survey, a single SC islet was found in location 3 within a tooth socket in the embryonic dentary of *H. stebingeri* (Figs. 1A, F-H). This islet is unique in our sample, as no SC was present on localities 1 or 2 (Figs. 1A, C, D), nor on any of the other sites investigated (Table 2).

This islet is composed of large round cells, typical of hypertrophied chondrocytes (embedded in a sparse extracellular matrix) and differs from the surrounding flattened osteocytes in the alveolar bone (Fig. 1H). A small layer of dentine is present in the immediate vicinity of the cartilaginous islet (Fig. 1G). As interpreted for the SC islets found between the dentary teeth of the *H. stebingeri* post-hatchlings (Bailleul et al., 2012), this islet is hypothesized to have arisen due to the stress generated by odontogenesis (Goret-Nicaise, 1986; Goret-Nicaise et al., 1984). Such SCs had already

been found in the alveolar processes of the human dentary and maxilla by Masquelin (1878) and Schaffer (see review in Beresford, 1981) over 130 years ago. The possible confusion of this cartilage with Meckel's cartilage (MC) was discussed extensively in a study on birds (Murray, 1963). MC is unlikely to be found in a tooth socket; it is localized more ventrally within the dentary in vertebrates. Moreover, in all the serial thin-sections, no remnant of MC was found, the Meckelian groove being entirely filled with calcite. This suggests that MC was made of unmineralized hyaline cartilage at that stage (it remains as a permanent cartilaginous rod in most extant taxa, Hall, 2005), and that the alveolar cartilage is indeed SC.

Discussion

In this study, we report for the first time the presence of SC in a non-avian dinosaur embryo, the ornithischian *H. stebingeri* (Fig. 1H). This supports the conclusions of our previous study in which SC was found in post-hatching skulls of the same species (Bailleul et al., 2012). The combined results of our two studies show the distribution and persistence of secondary chondrogenesis through two ontogenetic stages of one non-avian dinosaur species. This distribution can now be compared to the patterns observed in extant birds (Hall, 1967, 1968; Murray, 1963).

First, that SC was found in an embryo, and second, that this site persists after hatching (Bailleul et al., 2012) (as it does in extant birds, Hall, 1967, 1968; Murray, 1963) provides further support for the similarity of avian and ornithischian SC. As we previously hypothesized (Bailleul et al., 2012), this strongly suggests that secondary

chondrogenesis (or the chondrogenic potential of the periosteum) is a synapomorphy for the clade containing the last common ancestor of *H. stebingeri* and extant birds, with all its descendants, i.e., the Dinosauria (Gauthier, 1986 and Fig. 2). Indeed, even though the condition in non-avian saurischians is still unknown, it is more plausible to assume that this complex ability of the periosteum (to switch from osteogenesis to chondrogenesis) evolved once in all the Dinosauria, rather than twice and independently in the Ornithischia and the Aves (Bailleul et al., 2012).

Before we give a paleobiological interpretation to our findings, it is important to take taphonomy into account. It may be that additional mineralized SC nodules existed at this embryonic stage but were removed by some post-mortem process without leaving a trace of their former presence (e.g. see Behrensmeyer et al., 2000). However, given the excellent microstructural preservation, and the absence of abrasion on the edges of the bones (where SC would be found if it were present), our biological interpretation is that the SCs of locations 1 and 2 had not yet arisen (Figs. 1C, E), or had arisen but had not yet calcified.

In chick embryos, SCs arise halfway through development (on the 11th day out of 21 days of incubation) and is calcified in part much later (by the 15th day, Hall, 1971). The size of *Hypacrosaurus* eggs, with diameters between 18 and 22 cm (Horner, 1999), suggests a much longer incubation time for *Hypacrosaurus* than for domestic chickens. Among birds, larger eggs correlate with longer incubation periods (Ricklefs and Starck, 1998), which would give ample time for new SC sites to arise and calcify.

Based on the volume of an average *Hypacrosaurus* egg (estimated at 3900 ml; Horner and Currie, 1994), we calculated a weight of 4251g (see Williams et al., 1984 for the formula) and estimated the incubation time as 74 days (based on the relationship determined by Rahn and Ar (1974) linking incubation time and egg weight). Moreover, the size (see rostro-caudal length in Table 1) of the embryonic bones of these specimens and a comparison with the skeletal reconstructions in Horner and Currie (1994) suggests that the embryos were between 2/3 and 3/4 of the way through embryonic development, leaving approximately 20 to 25 days left until hatching, which is much more time than that available to chicken embryos to calcify their nine SC centers (i.e. 6 days). Nevertheless, we found that SC was extremely rare in our sample of ornithischian embryos, with only one SC islet present in a dentary. Later in ontogeny, the number of SC nodules increases (with three sites after hatching, Bailleul et al., 2012), but the number of sites is still lower than that observed in bird hatchlings (seven sites for nestling chicks, Hall, 1968). Although, the limited sample size of the embryonic material in this study does not allow any definitive conclusion or generalization, these preliminary results do suggest that the relative abundance (and contribution) of SC differs during the normal development of the skull of extant birds and non-avian dinosaurs.

These different patterns of secondary chondrogenesis through ontogeny could be explained by either of two hypotheses, one developmental, one evolutionary. First, a difference in the embryonic motility of the avian and ornithischian embryos could be considered, with a lower motility and/or a higher threshold for the initiation of secondary chondrogenesis in ornithischian embryos. Indeed, movement during the embryogenesis

of the chick is necessary for the normal development of SC (Hall, 1968). If chick embryos are paralyzed, SC does not form in the skull, but does on the clavicle (the only post-cranial membrane bone) because of the passive movements of the amnion (i.e., clavicle SC requires less movement to be initiated than do skull SCs, Hall, 1968).

Second, the increase in the number of sites of SC in the bird lineage could be linked to the evolution of the highly specialized avian beak. A factor responsible for this increase could be mainly mechanical, i.e., intermittent pressure and shear within the beak, increasing during evolution (and possibly different from the forces acting on the dinosaurian jaws). For example it is known that the black skimmer, *Rynchops niger*, that flies with its lower jaw in the water (with tremendous pressure acting on it), possesses much larger amount of SC than birds with other lifestyles (Bock, 1960). A study focusing on SC in relation to lifestyle in birds of various sizes and feeding adaptations should shed light on the functional significance of secondary chondrogenesis. Moreover, the increase in the number of sites of SC in the bird lineage must have been under selection as joints, articulations, muscular and ligamentous insertions originated. Indeed, during the evolution of the bird beak, with all its numerous and diverse adaptations (e.g., Zusi, 1993), SC could have provided advantages in the fine manipulation of food (e.g., Hunt, 1996), and been selected over other types of tissues/articulations. Again, these functional hypotheses are preliminary considering the small sample size of our study (and the unknown condition in non-avian theropods). Nevertheless, this study does suggest that a change in the pattern of secondary chondrogenesis was an important step during the evolution of the avian beak from dinosaurian jaws.

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Table 5.1. List of the hadrosaurid membrane bones thin-sectioned and examined in this study. The asterisks designate bones that had remnants of SC.

Taxon	Specimen no.	Ontogenetic stage	Element	Length (rosto-caudal) (cm)	Formation and locality
Hadrosauridae indet.	MOR 1038	embryonic	Surangular	2.1	Judith River: JR-144Q
<i>Hypacrosaurus stebingeri</i>	MOR 559	embryonic	Dentary*	5.1	Two Medicine: TM-066
<i>Hypacrosaurus stebingeri</i>	MOR 559	embryonic	Frontal	3.6	Two Medicine: TM-066
<i>Hypacrosaurus stebingeri</i>	MOR 559	embryonic	Maxilla	5.0	Two Medicine: TM-066
<i>Hypacrosaurus stebingeri</i>	MOR 559	embryonic	Parietal	3.2	Two Medicine: TM-066
<i>Maiasaura peeblesorum</i>	MOR-YMP430.Sa	embryonic	Surangular	1.7	Two Medicine: TM-160
<i>Hypacrosaurus stebingeri</i>	MOR 548	post-hatching (in Bailleul et al., 2012)	Dentary*	9.0	Two Medicine: TM-066
<i>Hypacrosaurus stebingeri</i>	MOR 548	post-hatching (in Bailleul et al., 2012)	Maxilla*	8.8	Two Medicine: TM-066
<i>Hypacrosaurus stebingeri</i>	MOR 548	post-hatching (in Bailleul et al., 2012)	Surangular*	4.1	Two Medicine: TM-066

Table 5.2. List of the sites analysed for secondary chondrogenesis. The asterisk indicates where SC was found.

Articulations or contact zones
dentary-predentary
dentary-surangular
mandibular symphysis
maxilla-coronoid process of dentary (Location 2)
maxilla-jugal
maxilla-premaxilla
maxilla-pterygoid
surangular-angular
surangular-quadrates (Location 1)
Alveolar processes
dentary* (Location 3)
maxilla
Muscle insertion sites (see Holliday, 2009)
m. pterygoideus ventralis (surangular)
m. pseudotemporalis profundus (surangular/mandibular fossa)
m. pseudotemporalis superficialis (coronoid process/mandibular fossa)
m. adductor mandibulae externus profundus (coronoid process)
m. adductor mandibulae externus medialis (coronoid process/surangular)
m. adductor mandibulae externus superficialis (surangular)
m. adductor mandibulae posterior (mandibular fossa)
m. depressor mandibulae (surangular)
Sutural areas
frontal-frontal
frontal-nasal
frontal-parietal
parietal-frontal
parietal-squamosal

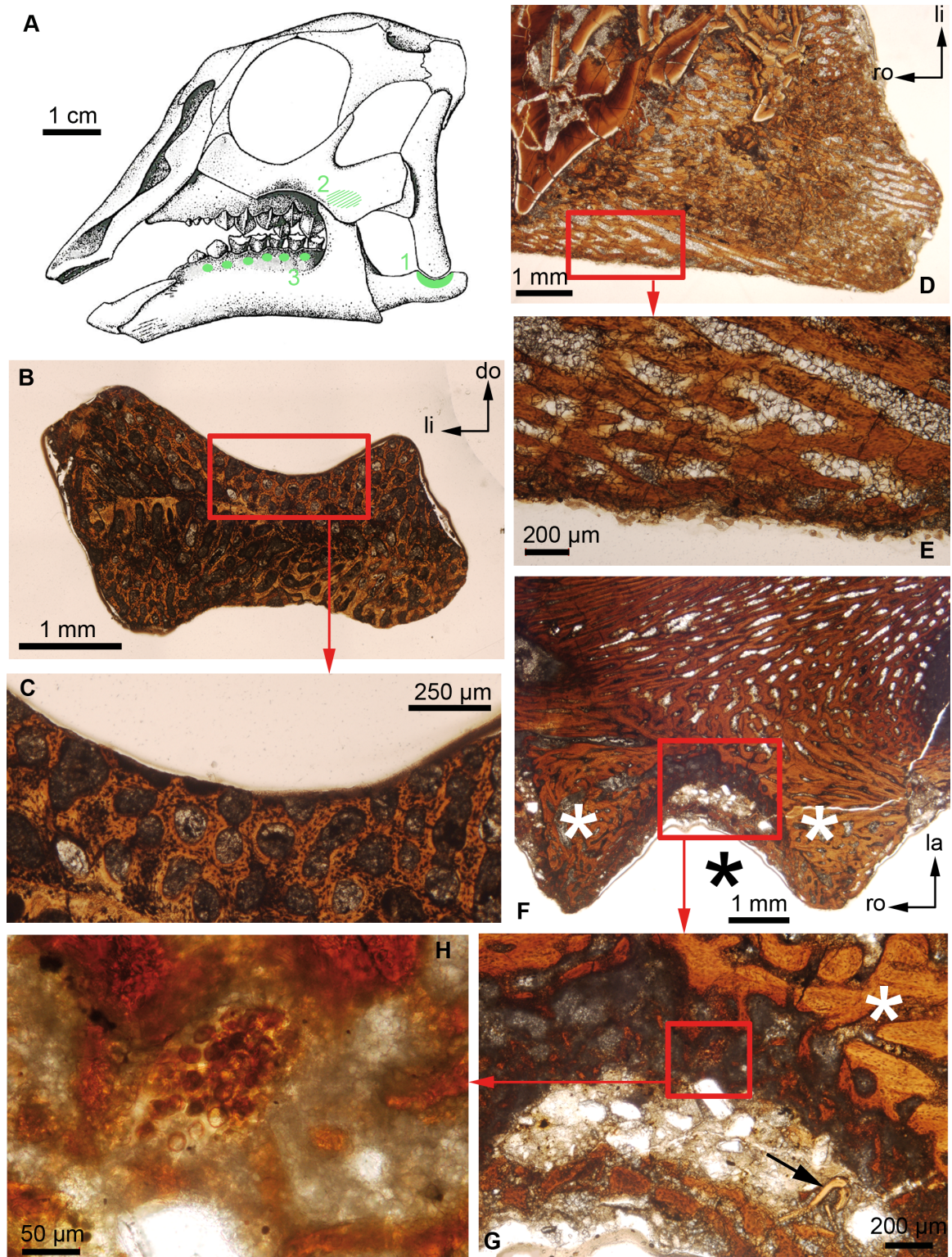


Figure 5.1. Secondary chondrogenesis investigated in hadrosaurid embryos. (A) Reconstruction of the embryonic skull of *Hypacrosaurus stebingeri*, reproduced with permission (Horner and Currie, 1994) with anatomical locations 1, 2 and 3 in green. (B)

Transverse section of the surangular of a Hadrosauridae indet. (MOR 1038). (C) Close-up of the red box in (B). The dorso-caudal face (Location 1) does not show any remnant of SC. (D) Coronal section of the maxilla of *Hypacrosaurus stebingeri* (MOR 559). (E) Close-up of the red box in (D). The bucco-caudal face of the maxilla (Location 2) does not show any remnants of SC. (F) Coronal section of the dentary of *Hypacrosaurus stebingeri* (MOR 559). (G) Close-up of the red box in (F). The arrow indicates a remnant of dentine. (H) Close-up of the red box in (G). (F) and (G) show alveolar bone (white asterisks) and incomplete alveoli with missing teeth (black asterisk; Location 3). (G) and (H) show a SC islet. All sections are shown under natural light. do, dorsal; la, labial; li, lingual; ro, rostral.

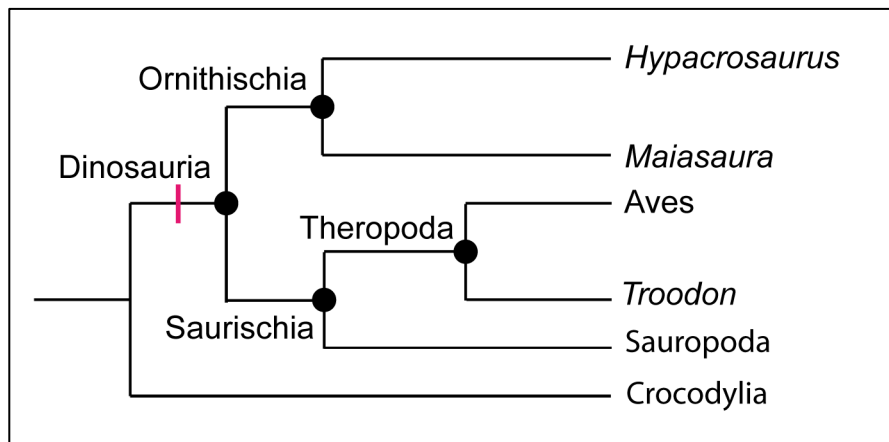


Figure 5.2. Phylogenetic relationships of some dinosaurian species and clades. Cladistic analysis (e.g., Weishampel et al., 2004) divides the Dinosauria into the Ornithischia (including *Hypacrosaurus* and *Maiasaura* from this study) and the Saurischia (with the Sauropoda and the Theropoda, the latter including birds). Based on the distribution of secondary cartilages discussed in this paper, secondary chondrogenesis is hypothesized to be a synapomorphy of the Dinosauria (red dash).

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CHAPTER SIX

CHONDROID BONE IN DINOSAUR EMBRYOS AND NESTLINGS
(ORNITHISCHIA: HADROSAURIDAE): INSIGHTS ON THE GROWTH OF THE
SKULL AND THE EVOLUTION OF SKELETAL TISSUES

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Abstract

In histology textbooks, the vertebrate skeleton is represented as almost entirely made of bone and cartilage. This is a false dichotomy and in fact, a continuum of intermediate tissues between bone and cartilage exists. Chondroid bone (CB; or chondroid tissue), one of the most well-known intermediate tissues, has been reported in mammals, birds and crocodilians. It accommodates 1) rapid growth of the skull and 2) the development of craniofacial sutures. Since CB is present in the extant phylogenetic bracket of the Dinosauria, we hypothesized that it was also present in non-avian dinosaurs. By means of paleohistological examination and microradiography, we report for the first time the presence of CB in non-avian dinosaur embryos and nestlings (Ornithischia: Hadrosauridae). It was found in five locations: 1) scattered within the bone trabeculae of an embryonic surangular; 2) and 3) in the coronoid process and in the alveolar processes of an embryonic dentary; 4) in the mandibular symphyses of an embryonic and a post-hatching dentary; 5) at the fronto-postorbital suture of an embryo. In these areas, CB was present in large amounts, suggesting that it played an important role in the rapid growth of the hadrosaurian skull during embryonic development. Moreover, the CB present in the sutural borders of a *Hypacrosaurus* frontal suggests that it was also involved in sutural growth, as it has been reported to be in mammalian and avian sutures. This is the first step taken to document and understand dinosaurian sutures from a histological perspective and it sheds light on an old problem by reporting the presence of CB in an additional clade within the Vertebrata. It is parsimonious to propose that CB in the chick embryo, *Gallus gallus*, the American alligator, *Alligator*

mississippiensis and the hadrosaurs of the present study are homologous and that CB arose once and was inherited from their common ancestor.

Key words: Archosauria, Dinosauria, Hadrosauridae, Chondroid bone/tissue, Skull Growth, Sutural Growth, Evolution of Skeletal Tissues.

Introduction

The term ‘paleohistology’ refers to the study of the bone and tooth microstructure of fossil vertebrates. The vast majority of studies involve analysis of tetrapod limb bone and the identification of types of bony tissues that reflect different growth rates, ontogenetic stages, phylogenetic positions or biomechanical factors (Padian, 2013). It is reasonable to focus exclusively on bone, because it composes the overwhelming majority of the fossilized remnants of tetrapods. Cartilage is the second most abundant supporting connective tissue in tetrapods (extant or fossil) and very few investigators have focused on fossilized cartilage (e.g., Barretto et al., 1993; Horner et al., 2001). In histology textbooks (especially those with medical applications), the skeleton is presented as made entirely of these two tissues: bone and cartilage; but this is a false dichotomy. Indeed, it is known that there is actually a continuum of tissues between bone and cartilage, known as ‘intermediate tissues’ (Hall, 2005; Smith and Hall, 1990; Witten et al., 2010), because they share features of both of these tissues. Studies on intermediate tissues in fossils are almost nonexistent (except perhaps in early vertebrates e.g., Ørvig, 1951) because 1) they are hard to identify, even in extant species, 2) they are much more rare than bone or cartilage, and 3) because the terminology employed is obscure and inconsistent. The most

well-known intermediate tissues are secondary cartilage and chondroid bone (Beresford, 1981; and see Hall, 2005, Chapter 5). Secondary cartilage was recently reported in hadrosaur embryos and nestlings (Bailleul et al., 2012, 2013; and see discussion), therefore, here, we will focus on chondroid bone in the same taxa.

Generalities about Chondroid Bone

As mentioned earlier, the terminology of chondroid bone is very inconsistent and it has been designated by various names since the early 1900s (see review by Beresford, 1981). Therefore, it is important to note that it might be difficult to accurately review where chondroid bone has been found in some instances (but see next paragraph, and Chapter 5 in Hall, 2005, 2014 *in press*). The term “chondroid tissue” has been proposed to replace the older term “chondroid bone” by Goret-Nicaise and Dhem (1982). We think that both terms are legitimate and we will consider them synonymous in this study.

Chondroid bone (CB) is intermediate between bone and cartilage because it has cartilage-like rounded cells that are closely packed together and that are embedded in a bone-like matrix (Beresford, 1981; Gillis et al., 2006; Hall, 1971, 1972; Lengelé et al., 1990; Murray, 1963). This extracellular matrix (ECM) possesses collagen type I which is typical of bone, and collagen type II which is typical of cartilage (Goret-Nicaise, 1984). It has been found in all the craniofacial bones and the mandible (in both endochondral and membrane bones) of human fetuses and infants (Goret-Nicaise, 1984, 1986; Goret-Nicaise and Dhem, 1982, 1985; 1988; Manzanares et al., 1988; and see Fig. 1), cat fetuses (Goret-Nicaise et al., 1984) and chick embryos (Lengelé, 1997; Lengelé et al., 1990, 1996a, 1996b). It plays two major roles: it facilitates 1) rapid growth (Gillis et al.,

2006; Goret-Nicaise, 1986; Huyseune and Verraes, 1986; Taylor et al., 1994); and 2) sutural growth (and later sutural fusion). Indeed, its growth rate was estimated by fluorescence labeling in the cat mandible at 44 to 67 microns/day, while the rate of lamellar bone formation was only 5.3 to 8.9 microns/day (Goret-Nicaise, 1986). It was reported in **all** the cranial sutural edges in humans (from 20 weeks-old fetuses until at least 9 months old babies; Goret-Nicaise et al., 1988), and in all the cranio-facial sutural edges in chick embryos (at the 9th, 12th and 14th day of incubation; Lengelé et al., 1990, 1996a, 1996b). More recently, Rafferty and Herring (1999) found CB in the nasofrontal suture of 4 to 6 month-old miniature pigs. These studies have shown that during early ontogenesis, it is CB that forms the sutural borders, not bone (*contra* Kokich, 1976; Pritchard et al., 1956).

CB is thought to arise directly from mesenchymal cells, i.e., these latter can give rise to chondroblasts, osteoblasts or chondroid bone cells depending on the stimuli (Lengelé, 1997; Lengelé et al., 1996b; but see Discussion for alternative hypotheses). The stimulus of CB is thought to be tension (and not compression like primary or secondary cartilage; Hall, 1967; Lengelé, 1997). While the extent of calcification of CB and woven bone are similar, CB is more mineralized than lamellar bone but less mineralized than calcified cartilage (Goret-Nicaise and Dhem, 1985). Curiously, it has a unique mode of calcification, different from that of bone and cartilage: it calcifies centripetally around the cell lacunae and irregularly (Goret-Nicaise and Dhem, 1982). Note that this unique calcification has been reported in humans and chick embryos, but it might not be true for all vertebrates.

Ontogenetically, this tissue is resorbed quickly in embryonic bones but it may persist at later stages of development as residual blocks scattered in bone trabeculae, and in articular and sutural areas (Lengelé et al., 1996a, 1996b). It can also arise after hatching (or after birth) in zones that are still under tensional stress. CB has not been investigated in many taxa, but beside from mammals and birds (the preferred laboratory animals in skeletal biology and histology), it has been found in agnathans (Ørvig, 1951), teleosteans (e.g., Gillis et al., 2006, Huysseune and Verraes, 1986, Taylor et al., 1994), and more recently in the skull of *Alligator mississippiensis* embryos (Vickaryous and Hall, 2008; see their figures 6G and 6H p 411). Therefore, from a phylogenetic perspective, CB seems to be widely distributed among Vertebrata.

Chondroid Bone and Dinosaurs

One important fact to highlight from our summary above is that two species that belong to the extant phylogenetic bracket of the Dinosauria, the chick, *Gallus gallus*, and the American alligator, *Alligator mississippiensis*, possess CB (Hall, 1971, 1972; Lengelé et al., 1990; Vickaryous and Hall, 2008). This strongly suggests that CB could have been involved in the craniofacial development of non-avian dinosaurs (Witmer, 1995). Note that there is always a possibility that CB arose in parallel in crocodilians and birds. Whether avian and crocodilian CB is homologous or analogous, it is important to investigate whether or not non-avian dinosaurs had the ability to form CB and how ancient this tissue was within the Archosauria. Therefore, the skull of some Lambeosaurine and Hadrosaurine dinosaur embryos and juveniles were analyzed by means of paleohistological techniques and microradiography (Table 1). Paleontological

crews of the Museum of the Rockies (MOR, Bozeman, Montana) have unearthed hundreds of remains of young hadrosaurs from nesting grounds (Horner, 1982; Horner and Currie, 1994; Horner and Makela, 1979; Horner et al., 2000), and this abundant material was therefore selected for this investigation. Reporting CB in hadrosaurs would bring two important insights on the growth of their skull: 1) First, even though it is commonly accepted that most hadrosaurs grew fast, fast growth has never been reported in the cranium, and most studies concern the post-cranium (e.g., Cooper et al., 2008; Horner et al., 2000; Padian et al., 2001). Moreover, the presence, absence or relative abundance of this tissue in hadrosaurs would give qualitative insights on the growth rate of their skull. 2) Second, this study could shed light on the mode of sutural growth of hadrosaur skulls, i.e., indicate whether or not they used CB as a vector of growth in the sutures. Indeed, sutures are often mentioned in paleontological studies because they are used for maturity assessment (e.g., Bakker and Williams, 1988; Sereno et al., 2009; Longrich and Field, 2012). However very little is known about the sutures of dinosaurs (or even extant archosaurs) from a histological perspective and only a few living mammalian species have been sectioned (i.e., some humans, Kokich, 1976; Koskinen et al., 1976; Latham, 1971; Miroué and Rosenberg, 1975; Opperman, 2000; Persson and Thilander, 1977; Sitsen, 1933; and some rats and rabbits, Moss 1958; Persson, 1973; Persson et al., 1978; Persson and Roy, 1979; Pritchard et al., 1956). For future paleontological studies, it is important to document the osteohistology of sutures in order to understand their morphology and their (potential) relationship to ontogeny.

Finally a third point to note is the possible importance of the study of CB to understand the evolution of skeletal tissues within the Archosauria or the evolution of skeletal tissues in general. Indeed, as mentioned earlier, CB has been observed in birds (e.g., Lengelé et al., 1990), reptiles (Vickaryous and Hall, 2008) and mammals (e.g., Goret-Nicaise, 1984) but neither in anuran nor in urodele amphibians (e.g., Beresford, 1981; Hall, 2003). This study could give more insights on the phylogenetic distribution of this intermediate tissue.

Material and Methods

Paleohistology

In extant species, CB can be identified histologically with the use of stains on decalcified sections (e.g., with Mallory's trichrome, Vickaryous and Hall, 2008; or Methylene blue, Lengelé et al., 1990). Although it is possible to stain decalcified archaeological bone to observe histological structures (e.g., with Toluidine blue, Garland 1989), stains used on dinosaur bones cannot show histological structures or different types of tissues *per se*, as they are usually histochemical (e.g., Sudan black can show lipids, Pawlicki, 1977; the Hoescht 33258 dye reveals DNA; Schweitzer et al., 1997). Therefore, we could not use a specific stain that would allow the identification of CB in these hadrosaurs. Instead, CB was identified qualitatively on undecalcified and unstained sections (i.e., standard paleohistological sections). As mentioned earlier, CB cells are round (cartilage-like) and embedded in a bone-like matrix. It is possible to differentiate

CB from bone or cartilage under natural light (see Results section for complete explanation).

For this study, we re-analyzed the palaeohistological thin-sections that were made for two previous studies on secondary cartilage (Bailleul et al., 2012, 2013) because secondary cartilage and CB are often found in the same areas. About a hundred sections were analyzed, from a total of 21 separate skull bones (see Table 1). All elements were found disarticulated and were collected from nesting horizons or isolated nests in the Two Medicine and Judith River formations (Upper Cretaceous), Montana, USA (see Bailleul et al., 2012, 2013 and Horner and Currie, 1994 for more information about the localities).

Paleohistological methods were employed according to the procedure for small specimens presented in Lamm (2013). The embryonic bones were embedded in epoxy resin and cut with a Norton 5" diamond blade on an Isomet precision saw. Thick sections (between 1.0 and 1.3 mm) were mounted on plastic (Plexiglas) slides with cyanoacrylate glue, then ground and polished by hand on a Buehler Ecomet grinder with silicon carbide paper of decreasing grit sizes. Finished thin-sections (with a thickness surrounding 100 microns) were studied by light microscopy under normal and polarized light with a Nikon Optiphot-Pol polarizing microscope. Photographs were taken with a Nikon DS-Fi1 digital sight camera and the NIS Elements BR 4.13 software.

Microradiography

In extant species, another efficient method to identify CB is microradiography, an X-ray technique that shows the mineral distribution in calcified tissues at the microscopic

scale (e.g., Goret-Nicaise and Dhem, 1982). Under microradiography, CB appears as radioopaque mineralized struts or islets containing irregular patches of confluent cellular lacunae that are radiotransparent (i.e., they appear dark). These struts and islets can be adjacent to woven bone or surrounded by lamellar bone, which have a distinct radiopacity and cell organization (Fig. 1A-D). Microradiography is also used on archaeological bone (e.g., Garland, 1989) and to our knowledge, only one other study performed microradiography on Mesozoic fossils (on reptilian and amphibian teeth from the Triassic, Wyckoff et al., 1963). Since paleohistological and histological methods are not standardized, we encountered problems while performing microradiography analysis, and we were only able to microradiograph five slides successfully (Table 2). Even though this is a small sample size, this study introduces a powerful tool that can be used by palaeohistologists in the future.

All microradiography experiments were performed at the *Université Catholique de Louvain* (UCL, Brussels, Belgium). The pre-made paleohistological thin-sections (70 to 90 microns thick, except for slide DE1-12 that was as thick as 175 microns in some places) were microradiographed in contact with a fine grain emulsion (VRP-M, Slavich-Geola, Lithuania), exposed to long-wavelength X radiations produced by a Machlett tube (Baltograph BF- 50/20, Balteau, Liege, Belgium) at 14 kV and 15 mA. In standardized sections (i.e., on extant material), the exposure usually lasts around 50 min, but because the X-rays did not go through the Plexiglas used at the MOR (i.e., it was too radio-opaque), the exposure lasted 4 hours for a film-focus distance of 106mm. At UCL, the sections were previously flipped and the Plexiglas (not the bone) was ground down to

about 100 microns in order to make it less radio-opaque. The microradiographs were observed and photographed with a Nikon Digital Sight DS-5MC camera (NIS Elements BR 3 software) attached to a microscope Leitz DMRB (Leica).

Results

In the present study, we report for the first time the presence of CB in some non-avian dinosaurs. CB was found in five different locations: 1) scattered within the bone trabeculae of an embryonic surangular (Fig. 2); 2) in the coronoid process of an embryonic dentary (Fig. 3); 3) in the alveolar processes of an embryonic dentary (Fig. 3); 4) in the mandibular symphyses of an embryonic and a post-hatching dentary (Figs 4 and 5); and 5) in the sutural borders of an embryonic frontal (at the fronto-postorbital suture, Fig. 6). Note that locations 2), 3) and 4) are all from the same bone (a dentary of a *Hypacrosaurus* embryo, MOR 559; Figs 3 and 4). Even though CB was most evident in these five locations, it was also present deep within the bone trabeculae and at the periosteal borders of the other embryonic bones (Table 1), but it was much more scattered and scarce in the post-hatching bones of this sample (*data not shown*).

As mentioned earlier, under natural light, this tissue possesses round cell lacunae that are closely packed in clusters, and are embedded in a bone-like matrix. Figure 2 shows transverse sections of an embryonic hadrosaurid surangular (MOR 1038) with a central string of tissue that runs along the whole length of the bone (Figs 2A-D). Indeed, this bone was serially sectioned (giving a total of 10 thin sections) and this central tissue can be observed on each slide (*data not shown*, Fig. 2 only presents images from slide SU1-

4). At higher magnification, numerous round cell lacunae can be observed (white arrows, Fig. 2E) and such roundness cannot be attributed to standard bone cell lacunae. Adjacent osteocyte lacunae can be seen (e.g., green arrow, Fig 2E) and they are much more elongated than the CB cell lacunae in the center. The CB matrix is brighter and more translucent (light brown) than the bone matrix (darker brown, Figs 2B, 2D). This difference in light transmission can be seen under polarized light as well (Fig. 2C). Finally, figures 1F and 1G show this same tissue in an adjacent section. Alas, we were not able to obtain a microradiograph of this embryonic surangular because the center of the slide peeled off during experimentation.

Figures 3 through 6 show microradiographs (**in black and white**) and their corresponding natural light photographs (**in color**). Note that the corresponding natural light pictures represent the exact same locations, with the same scale, as the microradiographs. However, since it was not possible to re-take pictures after microradiography had been performed, the corresponding natural light pictures are sometimes taken from an adjacent section (about 1.0 to 1.3 mm away). It is the case for figures 3G, 3J, 4F-G, 4I, 6C and 6E. Figure 3 represents longitudinal sections of an embryonic dentary (MOR 559, previously published in Bailleul et al., 2013). As mentioned earlier, CB can be identified under microradiography because it appears as patches of radiopaque matrix (appearing light) that contain radiotransparent (i.e., that appear dark), irregular and confluent cellular lacunae (indicated by white arrows) surrounded by bone layers with a more homogenous radiopacity and containing fewer cell lacunae (indicated by black arrows). Such clusters can be observed in the coronoid

process (Figs 3D, 3F) and in the alveolar processes (Figs. 3H-I). It is absolutely comparable to the CB observed in the microradiographs of extant human fetuses (Fig. 1) and extant bird embryos (see figures of Lengelé et al., 1990 and Lengelé et al., 1996a). In the coronoid process, CB is present deep within the bone trabeculae and is organized as little islets surrounded by lamellar bone (Figs 3D and 3F) but in the alveolar processes, it is organized into struts that radiate out in the alveoli (Figs 3H-I). The white arrows in these microradiographs show clear-cut, typical characteristics of CB and not those of other tissues (such as woven bone, or osteoid that is radio-transparent in microradiographs). The corresponding natural light pictures show two types of morphologies: round cell lacunae closely packed together in clusters (with a very high cellular density, Figs 3C, 3E); and a more ‘normal’ appearance, where the cells are not organized in clusters but are distributed evenly throughout the alveoli (Fig. 3J). Had we not performed microradiography, the latter tissue would have probably been identified as ‘normal’ woven bone rather than CB (Fig. 3J). These CB struts in the alveoli ensure rapid growth and they are most likely more recent than the deeper islets of CB surrounded by bone in the coronoid process.

Figure 4 shows cross-sections through the mandibular symphysis (at the most rostral tip of the dentary) of the same *Hypacrosaurus* embryo (MOR 559). All the microradiographs also show islets of CB on the periosteal borders (white arrows, Figs 4D-E, 4H). On the lingual face of this mandibular symphysis, natural light pictures show a light tissue with a high cellular density surrounded by struts of darker lamellar bone (Figs 4B-C). Cementing lines are present between the internal CB and the more external

lamellar bone, attesting the resorption of CB followed by lamellar bone apposition (Figs 4B-C). Note that this difference in color (light vs. brown) was also observed in the surangular (Fig. 2), but not in the coronoid process and the alveoli (Fig. 3; see discussion for further elaboration). At the very dorsal tip of the mandibular symphysis, the microradiograph indicates the presence of CB (Fig. 4H), but under natural light, it almost looks like a diagenetically altered bone (Figs 4F-G, 4I). Indeed, the tissue appears very thin and the shape of the cell lacunae are irregular (Fig. 4I) instead of round like those presented in Fig. 2E. This is curious and it would appear that CB cell lacunae can have multiple morphologies (see discussion). We wish to emphasize here that even though this tissue (at the most rostral tip of the mandibular symphysis) has the appearance of a ‘diagenetically altered’ bone in natural light, it is unlikely that this observed morphology is the product of diagenesis for two reasons: 1) the corresponding microradiograph (Fig. 4H) shows a pristine preservation of the mineral distribution, comparable to that of the CB of extant species (see Figs 1A,C); 2) beside from the rostral tip of this bone, all periosteal borders present histological structures that are well preserved (e.g., Fig. 4B). Only a very localized diagenetic alteration at the mandibular symphysis (and not on the rest of the bone) could explain these differences, but this does not seem like a plausible explanation.

This ‘diagenetically altered’ appearance is in fact present in the mandibular symphysis of a *Hypacrosaurus* post-hatching specimen as well (MOR 548), at the exact same location (Fig. 5). Even though we could not perform microradiography on this slide (because it is mounted on glass and X-rays cannot go through glass), the fact that 1) this

tissue presents a similar appearance as to that of MOR 559 (Fig. 4) and 2) that it is present at the exact same location as that of MOR 559, suggest this tissue is CB (Figs 5B-D). There is a clear limit between the light CB located superficially and the darker bone located more internally (Fig. 5C, see blue line). Just like the embryo MOR 559, the periosteal borders of MOR 548 show good histological preservation (*data not shown*) suggesting that this change in color in the mandibular symphysis is not due to diagenetic alteration, but instead could be a characteristic of CB in paleohistological ground sections. These results (Figs. 4 and 5) suggest that CB was present during embryogenesis and persisted at least a few months after hatching in the mandibular symphysis of *Hypacrosaurus*. Note that CB has previously been found in the mandibular symphyses of cat embryos and newborns (Goret-Nicaise et al., 1984).

Figure 6 shows transverse sections in a frontal of a *Hypacrosaurus* embryo (MOR 559). The microradiographs show once again struts and islets of CB (Figs 6B and 6D). These struts are located on the periosteal surface (rather than deeper within the bone) of the fronto-postorbital sutural border. Indeed, at this suture, almost all the sutural “bony” projections are made of CB and not of bone. This is very similar to what has been observed in the metopic suture of human fetuses (Manzanares et al., 1988) and other bird sutures (Lengelé et al., 1990, 1996a). The corresponding natural light pictures (Figs 6C and 6E) look like “normal” bone, although cellular density can be very high in some areas (black arrows, Fig. 6C).

Lastly, we analyzed a fossilized endochondral bone (a laterosphenoid, Fig. 7) that showed remnants of calcified cartilage, in order to document how CB and calcified

cartilage differ under microradiography. Indeed, one could argue that we are misidentifying CB cells for cartilage cells (or *vice-versa*) since they can share a similar round shape. Figure 7 shows sections through the laterosphenoid of a post-hatching Lambeosaurinae (MOR 1015). Remnants of the early embryonic cartilage model can be seen within the bone trabeculae (Figs 7B-C). Articular cartilage can also be seen on the surface that articulates with the postorbital (Fig. 7E). The corresponding microradiographs show cartilage cell lacunae that are very round (instead of irregular), individualized (instead of confluent) and embedded in an extremely radiopaque matrix (instead of a moderately radiopaque matrix, Figs 7D and 7F, blue arrows). This appearance is similar to that of the primary and the secondary cartilage of extant species seen under microradiography (e.g., see Figure 3C in Goret-Nicaise and Dhem, 1985; and Figure 5 p 33 in Lengelé, 1997). Moreover, the density of cartilage cells is much lower than that found in CB clusters. This shows that in these hadrosaurs, calcified cartilage and CB have very different characteristics on microradiographs and that is it difficult to misidentify them for one another.

Discussion

As mentioned earlier, CB (or chondroid tissue) is widely distributed among vertebrates. It has been reported in fossil agnathans and placoderms (Ørvig, 1951), in teleosts (e.g., in the kype of the Atlantic salmon, *Salmo salar*, Gillis et al 2006; in the pharyngeal jaws of some African cichlids, Huysseune, 1985; in bony cysts of the yellow perch, *Perca flavescens*, Taylor et al., 1994), in mammals (in deer antlers, Wislocki et al.,

1947; in the growing skull of human fetuses and infants, e.g., Goret-Nicaise, 1986; at muscle, tendon and ligament attachments of rabbit long bones, Hurov, 1986; in the cat skull, Goret-Nicaise et al., 1984; in miniature pig sutures, Rafferty and Herring, 1999), in crocodiles (in the embryonic skull of *Alligator mississippiensis*, Vickaryous and Hall, 2008) and in birds (in the skull of chick embryos, Hall, 1971, 1972; Lengelé, 1997; Lengelé et al., 1990, 1996a, 1996b; Murray, 1963). It is also found as a transitional tissue in bone sarcomas and tumors of human patients (see Beresford, 1981 and Hall, 2005, 2014 *in press* for full reviews). It appears that the terminology used for CB and other intermediate tissues is still inconsistent. For example, ‘chondroid tissue’ was recently described during the formation of the notochord of some geckos (Jonasson et al., 2012). However, its mode of formation (differentiating from the **chordoid** tissue of the notochord) is very different from that of ‘chondroid tissue’ *sensu* Goret-Nicaise and Dhem (1982) and these studies designate two different tissues. This demonstrates one of the difficulties when studying CB. Nevertheless, here, we report for the first time the presence of CB (synonymous with the “chondroid tissue” *sensu* Goret-Nicaise and Dhem, 1982; and Lengelé et al., 1990, 1996) in some non-avian dinosaurs (Ornithischia, Hadrosauridae). The identification of this tissue was made possible by means of paleohistological examination under natural light (Fig. 2) and microradiography (Figs 3-6). As mentioned previously, it was found in five locations: scattered within the bone trabeculae of an embryonic surangular (Fig. 2); in the coronoid process of an embryonic dentary (Fig. 3); in the alveolar processes of an embryonic dentary (Fig. 3); in the

mandibular symphyses of an embryonic and a post-hatching dentary (Figs 4 and 5); and in the sutural borders of an embryonic frontal (at the fronto-postorbital suture, Fig. 6).

Before discussing the physiological and the phylogenetic implications of these findings, we explore morphological criteria identified as characteristic of CB under natural light in standard paleohistological thin-sections. This will be useful for future paleohistologists interested in this tissue.

Identification of Chondroid
Bone with Microradiography
versus Natural Light Microscopy

The microradiographs obtained from these hadrosaurs look exactly like those of humans (Fig. 1) and chicks (see Lengelé et al., 1990, Lengelé et al., 1996a) that possess CB (compare Fig. 1 with Figs. 3-6). Both the extant species and the hadrosaurs show islets of mineralized matrix with clusters of confluent cellular lacunae, typical of CB. This suggests that our identification in the fossil bone is correct. When comparing microradiographs with their corresponding natural light pictures, we found two noteworthy results. **Firstly**, while CB always has the same appearance under microradiography, different corresponding morphologies can be observed under natural light: the ECM of CB can be much brighter than the ECM of the surrounding bone (Figs 2B, 2D, 2F-G and 4B-C), it can have the appearance of normal woven bone (Figs 3J, 6C, 6E), or of a diagenetically altered bone (Figs. 4F-G, 4I). Moreover, the cell lacunae can appear round, i.e., almost like hypertrophied cartilage cells (Fig. 2E), or more irregular (Figs 4G, 4I). Note that CB cells are sometimes known to present features of hypertrophied chondrocytes (e.g., Tuominen et al., 1996). In all the cases, the cellular

density was always higher than that of the surrounding bone, or that of calcified cartilage (Fig. 7). **Second**, some areas that look like CB under natural light (with clusters of round cells closely packed together) look similar to bone under microradiography (see Figs 3C-D, blue arrows). These conflicting results did not enable us to find a clear-cut relationship between microradiography and natural light pictures of CB. Nevertheless, we propose four characteristics that CB presents under natural light in paleohistological ground sections. Note, however, that these characteristics are not necessarily accurate for CB in ground- or decalcified sections of extant animals. Also note that those are preliminary results since further examination and discrimination of this tissue need to be made with paleohistological sections. The characteristics of CB are as follows:

- 1) It presents large ‘cartilage-like’ cell lacunae (similar in morphology to those of hypertrophic cells of extant vertebrates), or irregular cell lacunae.
- 2) Its cell density is always higher than that of the surrounding woven or lamellar bone.
- 3) Its ECM is more translucent than that of bone (for a section thickness of approximately 80 to 120 μm).
- 4) It may have the appearance of a diagenetically altered bone.

This diversity of morphologies could directly reflect skeletal diversity (i.e., there would be multiple types of CB). Indeed, Beresford (1981) classified two types of CB (type I and type II, see Chapter 1) and noted that it is difficult to draw limits between intermediate tissues, as they can form a “jungle of overlapping categories”. Another example of reported skeletal diversity is that in Benjamin, 1990, where six different types of cranial

cartilage where reported in the black molly, *Poecilia sphenops* (also see Witten et al., 2010). To demonstrate that hadrosaurs possess different types of CB is beyond the scope of this paper, but we would like to note that this is a plausible hypothesis.

It cannot be ruled out that this morphological diversity does not reflect reality but instead is due to diagenesis (e.g., the latter could have altered the overall appearance of the tissues but not the orientation of their crystals). However, as mentioned in the results sections, both the microradiographs and the natural light images of the periosteal borders of the sampled elements showed good preservation (e.g, compare Fig. 4H with Figs 1A,C). According to our results, diagenetic alterations seems to have been minor. Perhaps a bigger sample size, or a comparison between microradiographs and serial sections of some extant species stained with Masson's trichrome and/or Mallory's trichrome (i.e., what was used in Gillis et al., 2006 and Vickaryous and Hall, 2008) could help answer our questions about this morphological diversity.

Chondroid Bone in Hadrosaurs: Implications for Skull Growth

In analogy to the five locations presented in this study, CB has been found 1) on the surangular of chick embryos (Lengel  et al., 1996a), 2) in the coronoid process of human fetuses and infants (Goret-Nicaise, 1981), 3) in the alveolar processes during the growth of the tooth buds in human fetuses and infants (Goret-Nicaise et al., 1984), 4) in the mandibular symphysis of the cat and the human fetus (Goret-Nicaise, 1986; Goret-Nicaise et al., 1984); and 5) at all the sutural borders of the skull of human fetuses and infants (Goret-Nicaise et al., 1988, Manzanares et al., 1988) and chick embryos (Lengel 

et al., 1990, 1996a). Finding this tissue in these duck-billed dinosaurs has two important implications for their skull growth:

1) As mentioned earlier, CB is an adaptation to rapid growth (Gillis et al., 2006; Goret-Nicaise, 1986; Huysseune and Verraes, 1986; Taylor et al., 1994). The fact that CB was found in large amounts in these dinosaur embryos suggests a rapid embryonic skull growth. Alas for now, we can only make qualitative statements since a comparison between microradiographs of slow and fast-growing animals (with quantitative information) has never been undertaken. This rapid development is not surprising because it is already known that the post-cranium of hadrosaurs grew fast and that they were more endotherms than ectotherms (Cooper et al., 2008; Horner et al., 2000; Padian et al., 2001)

2) We also report the presence of CB at the fronto-postorbital suture of *Hypacrosaurus*. The vast majority of the “bony” struts at these sutural borders are made of CB (Figs 6B, 6D), in a similar manner as to what has been observed in mammalian and avian sutures (Manzanares et al., 1988; Lengelé et al., 1990, 1996a). Goret-Nicaise et al., (1988) found that CB was the major driving force of “bone” lengthening at the sutural borders, and that it was not comparable to periosteal growth. Manzanares et al., (1988) concluded that CB also had a role in sutural fusion (in the metopic suture of 6-months-old infants).

Our results suggest that CB might also have been involved in the sutural growth of hadrosaurs. Many dinosaur studies use the degree of closure of sutures to assess the maturity of their specimens and in turn, if they are identified as ‘adults’, this sometimes is

considered grounds for naming a new species (e.g., Bakker and Williams, 1988; Sereno et al., 2009). However, such conclusions should be reconsidered since there is a lack of understanding of sutures in non-mammalian species (including the phylogenetic bracket of the Dinosauria) in terms of 1) pattern (i.e., the order in which sutures fuse through ontogeny), 2) morphology and 3) histology (see Herring, 2000 for an excellent review). Therefore, it is important to start documenting and understanding the osteohistology of sutures in extant and extinct archosaurs. Our results suggest that sutural growth was very similar in birds, mammals and hadrosaurs (at least during embryonic development), but other preliminary results suggest that this similarity fades through ontogeny and leads to more unique modes of fusion (Bailleul and Horner, 2013). Even though CB was only found in one suture of *Hypacrosaurus* (i.e., the only suture that was microradiographed), this is the first step in understanding more about sutural growth in non-avian dinosaurs, a process that we need to comprehend to accurately assess their maturity.

Chondroid Bone: Phylogenetic Implications

CB has not been studied in many clades of vertebrates, therefore hasty and preliminary conclusions should not be made. However, it is not surprising to find CB in non-avian dinosaurs, because members of their extant phylogenetic bracket also form this tissue (Hall, 1971, 1972; Lengel   et al., 1990; Vickaryous and Hall, 2008). It is parsimonious to say that the CB in *Gallus gallus*, *Alligator mississippiensis*, and hadrosaurs are homologous and that it was present in their most recent common ancestor. If our hypothesis of homology holds true, CB would be present in the other clades of

dinosaurs as well, but only more paleohistological and microradiography examinations could verify this.

It is not clear exactly when CB arose within the Archosauria, and if it is homologous to the CB found in less derived vertebrates (e.g., in Gillis et al., 2006; Ørvig, 1951). Nevertheless, this study sheds some new light on an old problem, reporting the presence of CB in an additional clade within the Vertebrata. This may be of importance for those who study skeletal diversity and the evolution of skeletal tissues.

One last issue that we would like to discuss is the relationship between CB and secondary cartilage, an intermediate tissue that arises secondarily on membrane bones and that has previously been reported in some hadrosaur skulls (Bailleul et al., 2012, 2013).

Dinosaurian Chondroid Bone and Secondary Cartilage

Secondary cartilage and chondroid bone are often found together throughout the skull of mammals and birds (Goret-Nicaise and Dhem, 1982; Hall, 1971, 1972; Lengelé, 1996b). In our small sample size, we found CB (present study) where dinosaurian secondary cartilage had previously been reported (Bailleul et al., 2013, in the alveoli of MOR 559). We also discovered a new secondary cartilage location while we were looking for CB, on the coronoid process of a post-hatching *Hypacrosaurus* (slides DE10 and DE13 from project 1988-13, MOR 548, *data not shown*).

The possibility of the existence of multiple types of CB, as presented in the first part of the discussion, was partly suggested by the fact that CB cell lacunae present two different morphologies: they can have the appearance of hypertrophied cartilage (Fig. 2E) or can

appear irregular (e.g., Figs 4G, 4I). Even though it has been reported that CB cells sometimes have the appearance of hypertrophied chondrocytes (Mizoguchi et al., 1997; Tuominen et al., 1996), since we could not perform microradiography on the element present in Figure 2 (MOR 1038), the possibility that those cells are actually secondary cartilage cells, and not CB cells, cannot be ruled out. Secondary cartilage is usually organized as nodules on the periosteal surface of membrane bones (Hall, 1967; Hall, 1968), while here, it is present as a long central strut. The absence of resorption suggests that it is not an external secondary cartilage nodule that has been relocated from the periosteal surface to the interior of the bone. Rather, these CB cells could be transforming into cartilage cells via metaplasia (Beresford, 1981). Lengelé et al. (1996b) showed that CB and secondary cartilage were two autonomous tissues, arising independently from the cephalic mesenchymal cells under different biomechanical inductions. However, if indeed there is not one, but many types of CB, perhaps metaplasia between CB and secondary cartilage cells is possible in some cases. Metaplasia, the permanent transformation of cell identity from one cell type to another (Beresford, 1981; Hall, 2005), is a common mechanism in vertebrate lineages in skeletal and non-skeletal tissues: for e.g., hypertrophied chondrocytes can transform directly into osteoblasts in turtle and lizard long bones (Haines, 1969); fibroblasts can transform into fibrocartilage (e.g., at mammalian tendon or ligament insertions, Gao et al., 1996; Hurov, 1986; McLean and Bloom 1940; during antlerogenesis; and at the anterior tip of the rat penile bone and during the fracture repair of bones in some frogs and lizards; see Beresford, 1981 and Hall, 2005, 2014 *in press* for full review). Metaplasia has also been reported in the extant

phylogenetic bracket of non-avian dinosaurs: the osteoderms of *Alligator mississippiensis* arise by *in situ* transformation of dense irregular connective tissue (perhaps a population of fibroblasts; M. Vickaryous, *personal communication*) into bone (Vickaryous and Hall, 2008). Tenocytes of avian tendons can transform into osteoblasts (Adams and Organ, 2005; Agabalyan et al., 2013) and most importantly, secondary cartilage can transform into CB in paralyzed embryonic chicks (Hall, 1972). Moreover, even though it is not possible to know the exact mode of formation of fossilized tissues, due to the unusual histology of some dinosaur mineralized tissues, metaplastic transformations have been hypothesized to play a role in the formation of dinosaur skulls (Goodwin and Horner, 2004; Horner and Lamm 2011; Horner et al., in prep; Hieronymus and Witmer, 2008) and osteoderms (Main et al., 2005, Scheyer and Sander, 2004). It appears also that this unusual histology was much more abundant in the skulls of dinosaur than in those of extant mammals and birds (J. Horner *personal observations*). Therefore, these numerous examples of metaplasia suggest that it is reasonable to hypothesize the transformation of CB cells into secondary cartilage cells in our sample. Of course, this is beyond the scope of this paper, and we are simply considering additional possibilities. More neontological studies on extant archosaurs are necessary for the current and futures advances in the growing field of dinosaur paleohistology.

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Table 6.1. List of bones sectioned and analyzed in the present study.

Taxon	Specimen	Ontogenetic stage	Element	Formation and locality
<i>Hypacrosaurus stebingeri</i>	MOR 559	Embryo	Dentary	Two Medicine: TM-066
			Frontal	
			Maxilla	
			Parietal	
	MOR 548	Post-hatching (nestling?)	Dentary	Two Medicine: TM-066
			Frontal	
			Jugal	
			Lacrima	
			Maxilla	
			Nasal	
			Palatine	
			Parietal	
			Postorbital	
			Predentary	
			Prefrontal	
			Premaxilla	
			Quadratojugal	
			Squamosal	
			Surangular	
Lambeosaurinae indet.	MOR 1015	Post-hatching (nestling?)	Laterosphenoid	Judith River: JR-144Q
Hadrosauridae indet.	MOR 1038	Embryo	Surangular	Judith River: JR-144Q

Table 6.2. List of thin-sections analyzed with microradiography.

Species	Specimen	Ontogenetic stage	Element	Thin-section
<i>Hypacrosaurus stebingeri</i>	MOR 559	Embryo	Dentary	DE1-2
			Dentary	DE1-14
			Frontal	FR1-3
Lambeosaurinae	MOR 1015	Post-hatching (nestling?)	Laterosphenoid	LS1-5
Hadrosauridae	MOR 1038	Embryo	Surangular	SU1-4

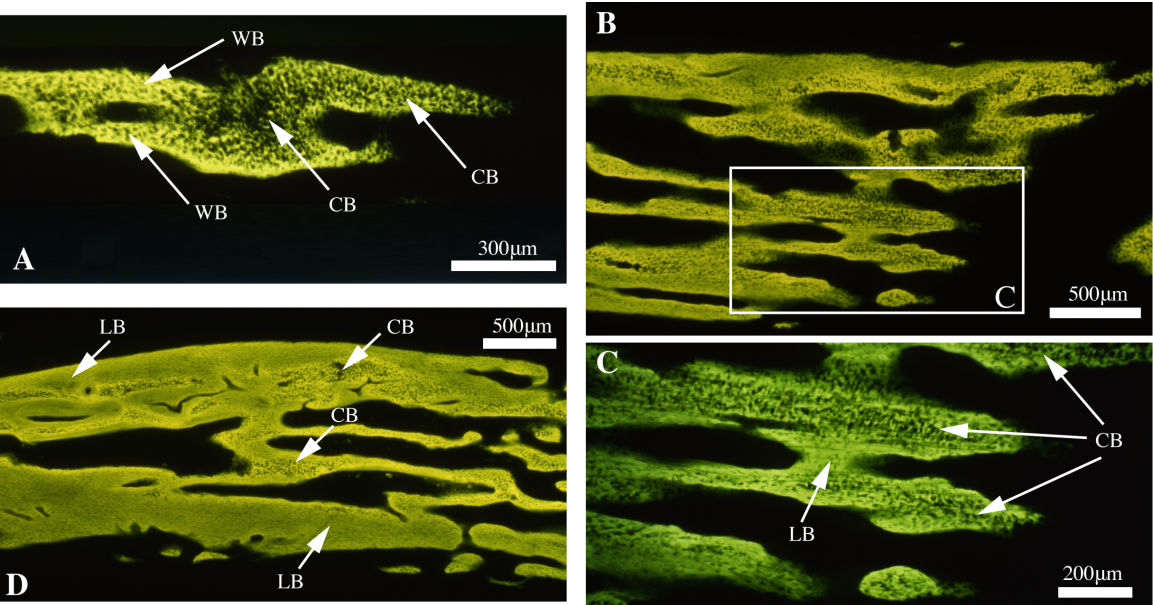


Figure 6.1. Microradiographs of the cranial vault in human fetuses and infants. A) Microradiograph of the cranial vault of a human fetus. Chondroid bone (CB) is indicated by areas where the cells are very numerous and irregular, with confluent cellular lacunae. Woven bone appears highly radiopaque, with fewer and more individualized cellular lacunae. B) Growing sagittal suture of a full term fetus. C) Detail of the white box in B). The sutural edges are almost entirely made of CB, with some areas composed of lamellar bone. D) Cranial vault at a more mature stage in an infant. CB is still present deep within the lamellar bone trabeculae. Abbreviations: CB, Chondroid bone; LB, Lamellar bone; WB, Woven bone. Courtesy of B. Lengelé, UCL.

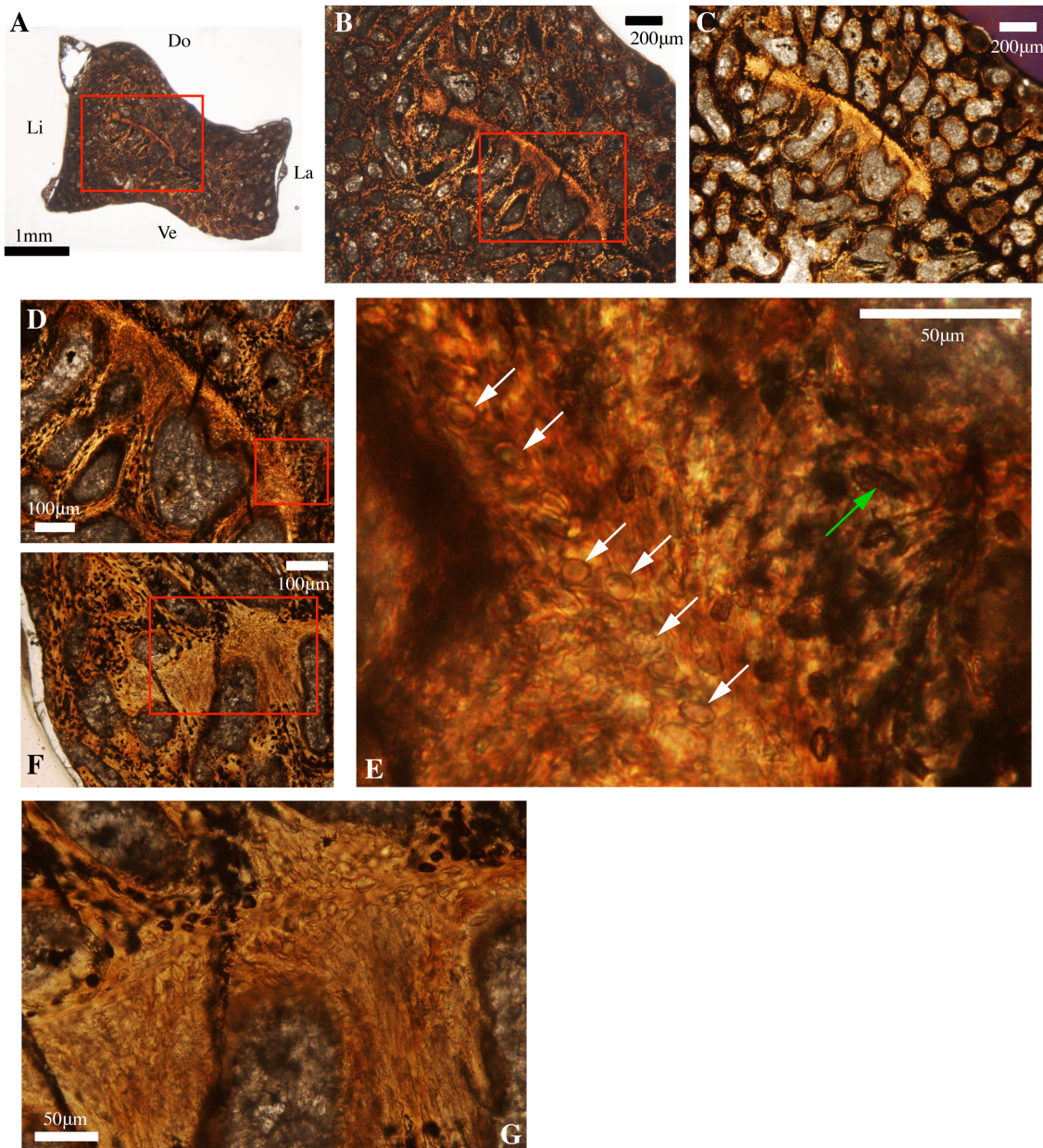


Figure 6.2. Cross sections in the surangular of a hadrosaur embryo (MOR 1038, Hadrosauridae indet.) under natural and polarized light. A) Cross section under natural light. B) Detail of the box in A) showing a long strut of CB under natural light. C) Same detail of the box in A) under polarized light. D) Detail of the box in B showing CB at a higher magnification. This tissue appears brighter than the surrounding bone under natural light. E) Detail of the box in D), showing CB cells that are very round and cartilage-like (white arrows). Compare with the elongated morphology of an osteocyte (green arrow). F) Adjacent section of this same bone showing the central strut of CB. G) Detail of the box in F. Abbreviations: Do, Dorsal; La, Labial; Li, Lingual; Ve, Ventral.

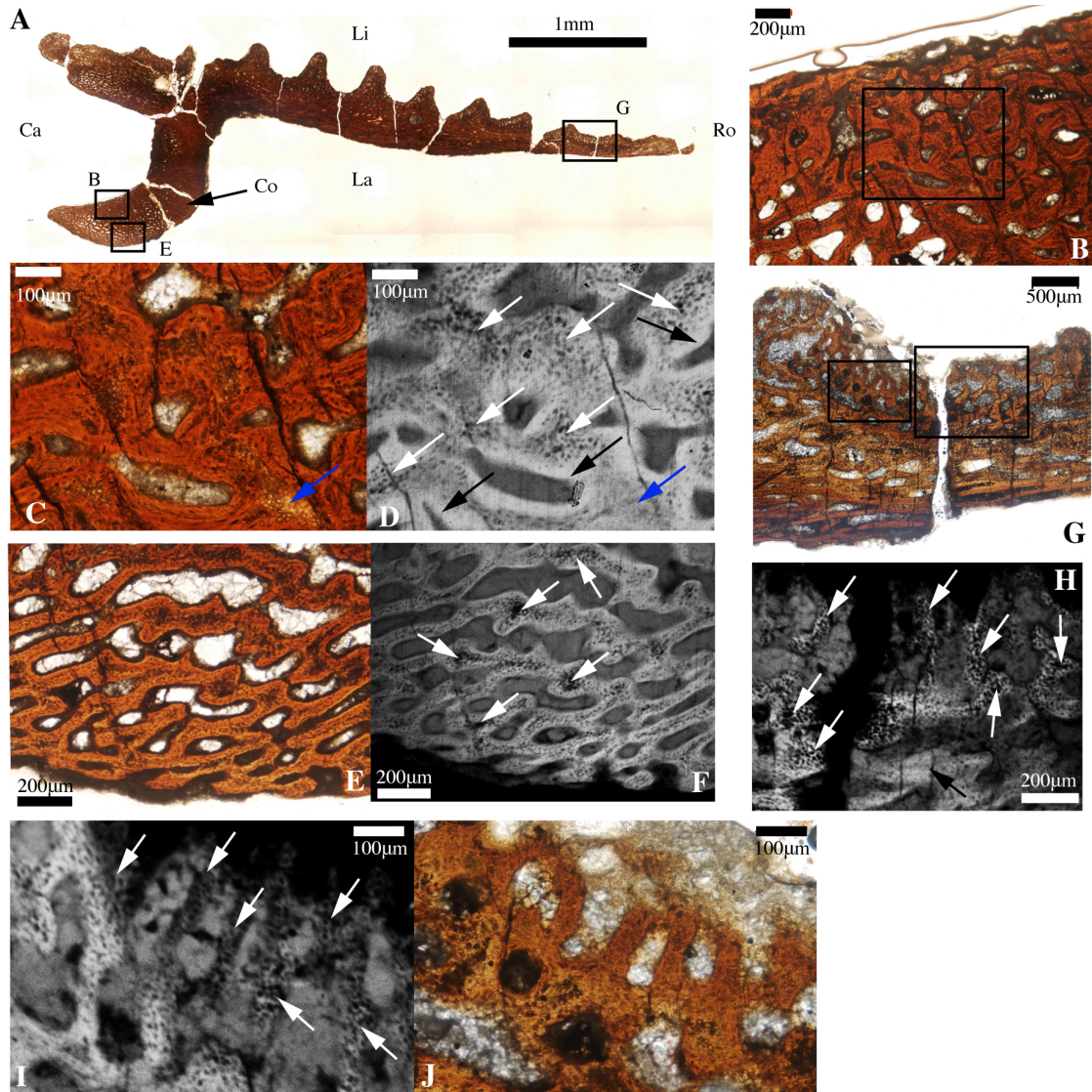


Figure 6.3. Longitudinal sections in the dentary of a *Hypacrosaurus* embryo (MOR 559) under natural light with corresponding microradiographic aspect of framed areas. A) Longitudinal section under natural light. B) Detail of the upper box on the coronoid process in A). C) Detail of the box in B) under natural light. Clusters of numerous cells can be observed in the center of the bone trabeculae. D) Corresponding microradiograph of C) showing CB (white arrows) and bone (black arrows). The blue arrow designates an area where microradiography and natural light pictures have contradicting results (see discussion). E) Detail of the lower box on the coronoid process in A). F) Corresponding microradiograph of E). CB is indicated by white arrows. G) Detail of the right box in A). H) Microradiograph of the right box in G), showing many struts of CB radiating into the alveoli (white arrows), and bone deeper within the element (black arrow). I) Detail of the left box in G). CB is indicated by white arrows. J) Corresponding natural light photograph of I). CB has the appearance of “normal” woven bone under natural light. Abbreviations: Ca, Caudal; Co, Coronoid process; La, Labial; Li, Lingual; Ro, Rostral.

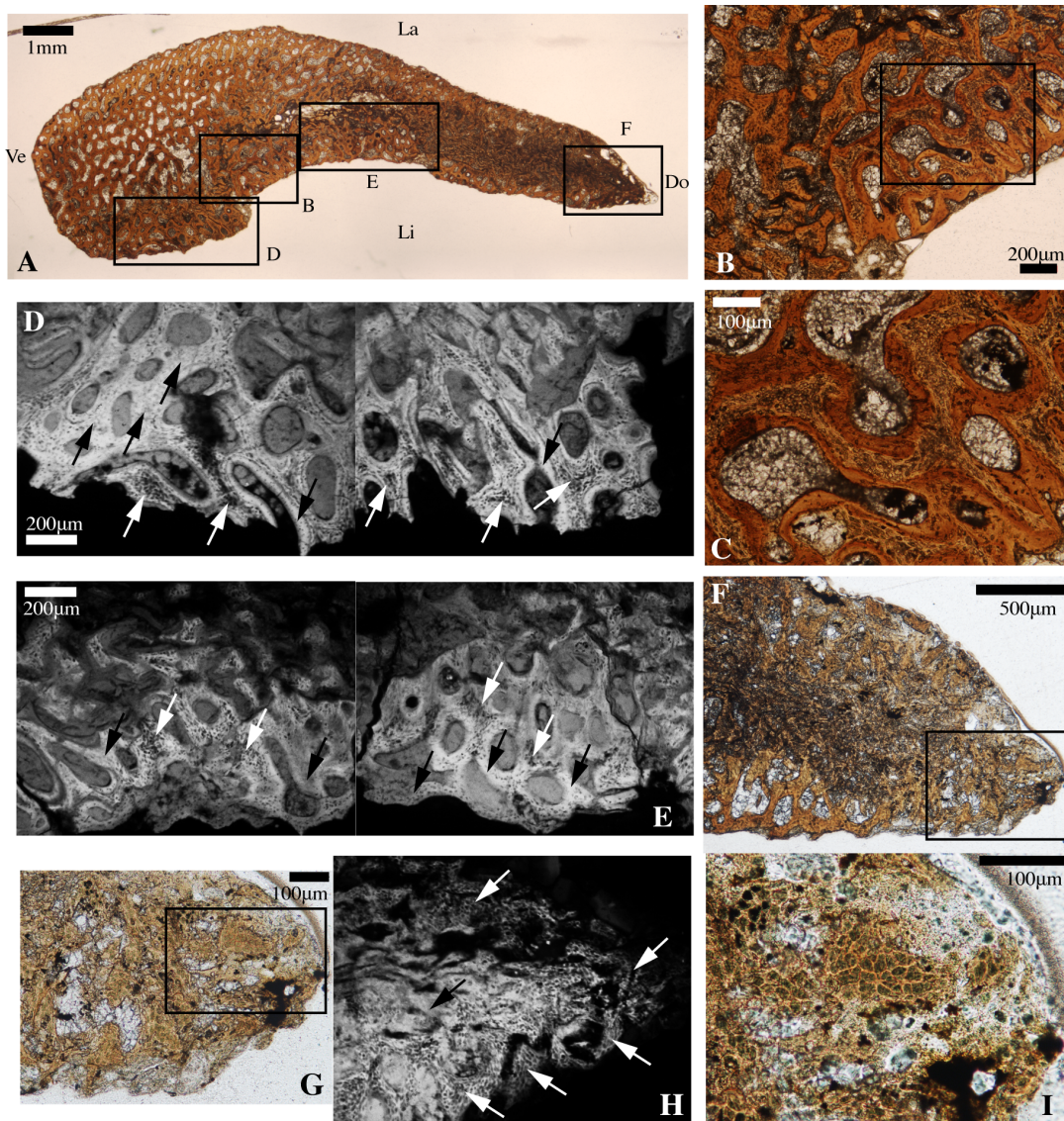


Figure 6.4. Cross sections in the mandibular symphysis of the dentary of a *Hypacrosaurus* embryo (MOR 559) under natural light with corresponding microradiographs. A) Cross section under natural light. B) Detail of the box B in A). C) Detail of the box in B). Struts of CB appear lighter and with a higher cellular density than that of the surrounding bone under natural light. D) Microradiograph of the Box D in A). The periosteal borders show that CB is very well distributed (white arrows), surrounded by bone struts (black arrows). Bone is also presents deeper within the element. E) Microradiograph of the Box E in A). The same observations as those of figure E) can be seen. F) Detail of the box F in A) under natural light. G) Detail of the box in F). CB has a “diagenetically altered” appearance under natural light. H) Corresponding microradiograph of G). CB is extremely abundant at the dorsal tip of this mandibular symphysis. I) Detail of the box in G), showing irregular CB cell lacunae and their ‘diagenetically altered’ appearance. Abbreviations: Do, Dorsal; La, Labial; Li, Lingual; Ve, Ventral.

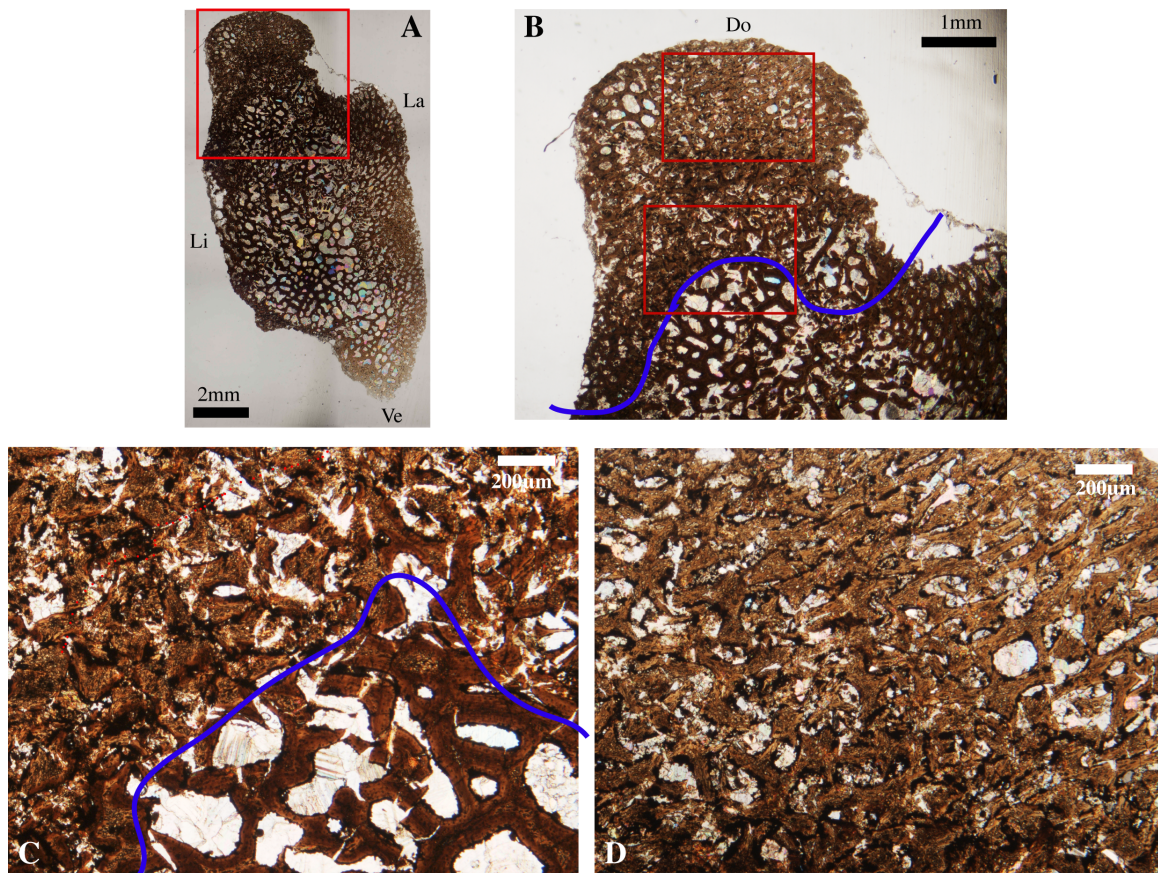


Figure 6.4. Cross sections in the mandibular symphysis of the dentary of a post-hatching *Hypacrosaurus* (MOR 548) under natural light. A) Cross section under natural light. B) Detail of the box in A). C) Detail of the lower box in B). There is a clear limit (blue line) between bone on the interior side and CB on the peripheral side. D) Detail of the upper box in B) showing the “diagenetically altered” appearance of CB, its bright extracellular matrix and its high cell density. Abbreviations: Do, Dorsal; La, Labial; Li, Lingual; Ve, Ventral.

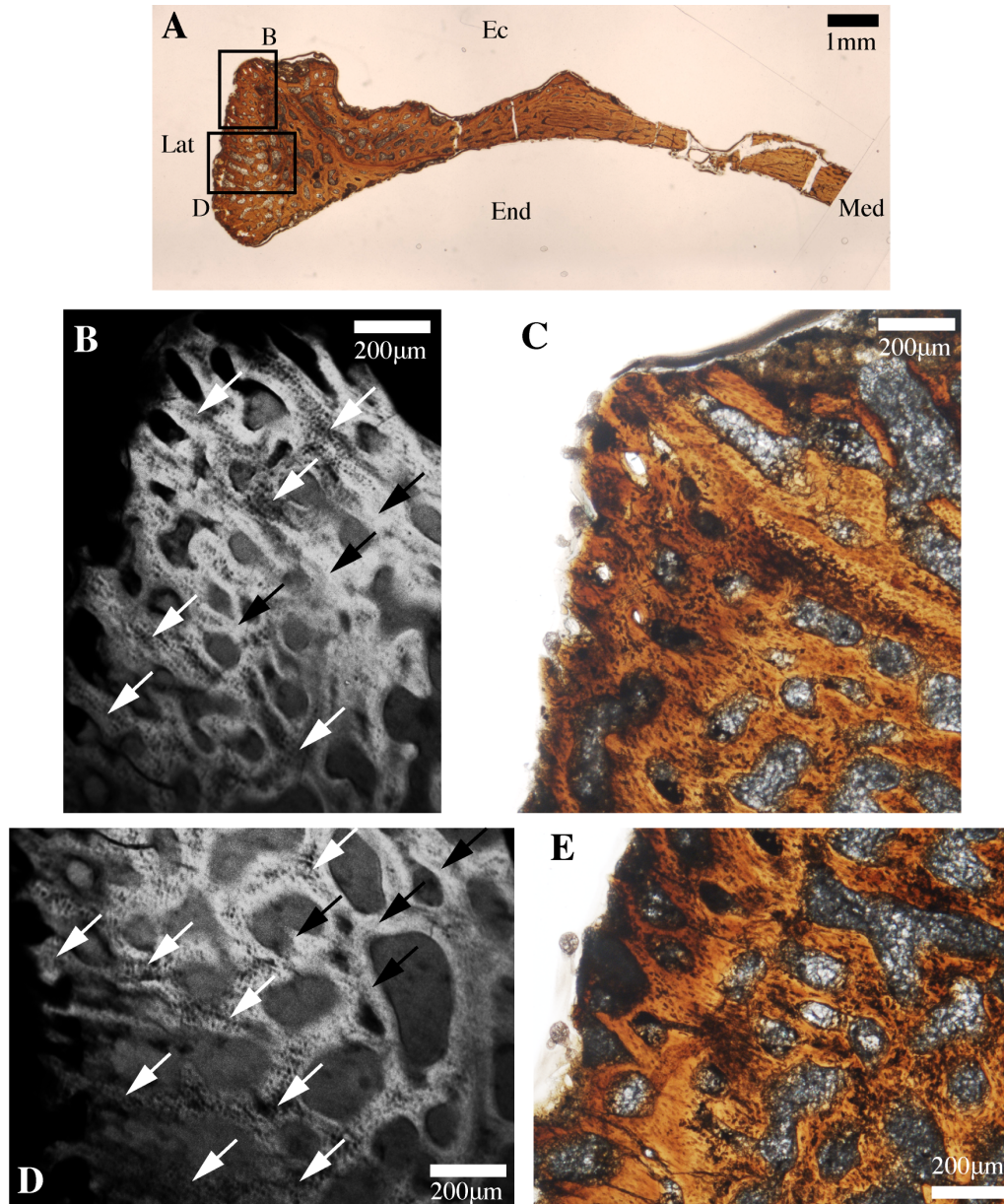


Figure 6.6. Cross sections in the frontal of an embryonic *Hypacrosaurus* (MOR 559) under natural light with corresponding microradiographs. A) Cross section under natural light. B) Detail of the upper box in A) under microradiography. Many sutural struts are composed of CB (white arrows). Bone (black arrows) is located deeper within the element. C) Corresponding natural light picture of B). D) Detail of the lower box in A) under microradiography. The same observations as those of figure B) can be seen. E) Corresponding natural light picture of D). Under natural light (C and E), CB looks like 'normal' woven bone. Abbreviations: Ec, Ectocranial side; End, Endocranial side; Lat, Lateral; Med, Medial.

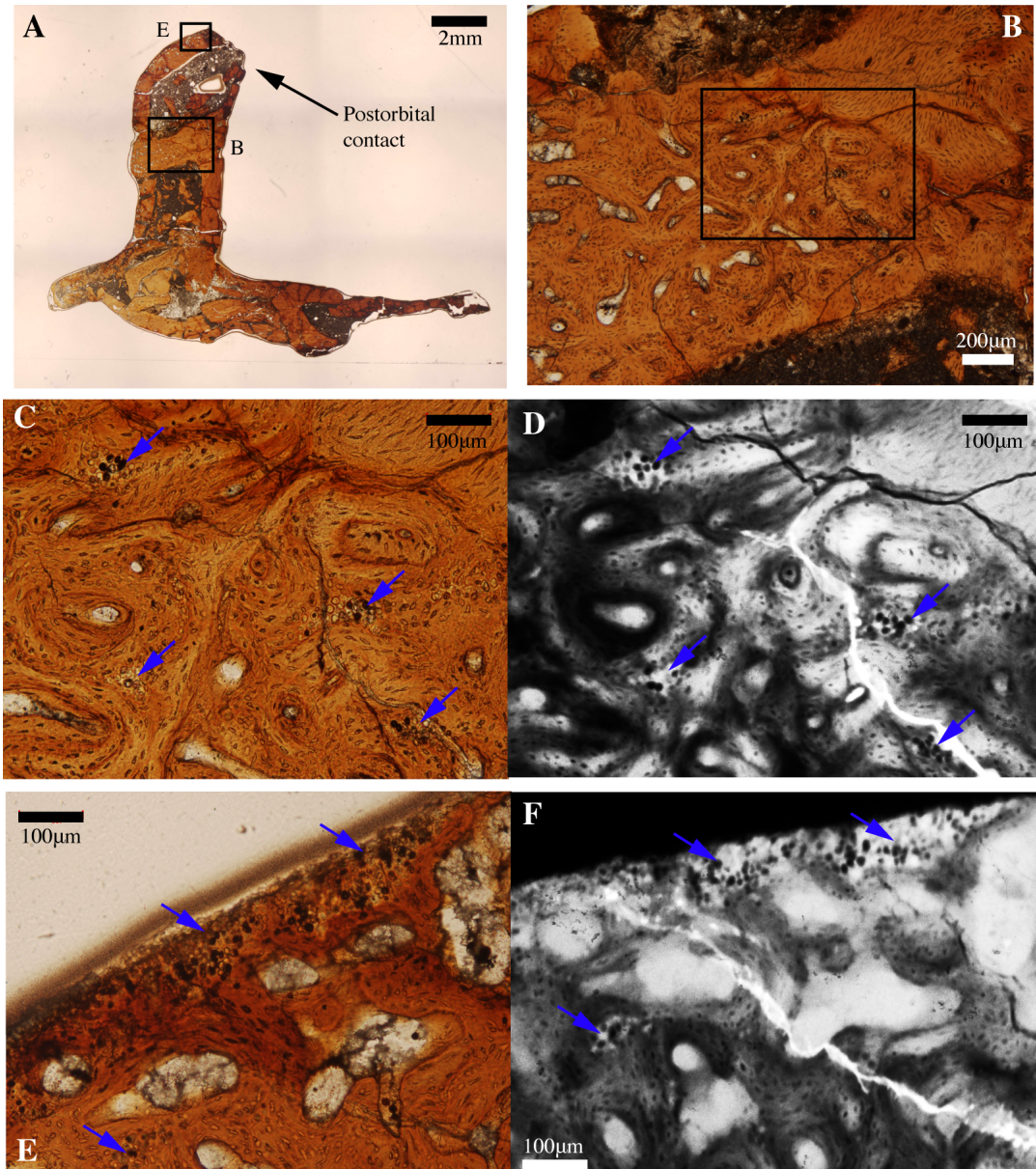


Figure 6.7. Cross section in the laterosphenoid of a *Lambeosaurinae* indet (MOR 1015) under natural light with corresponding microradiographs to show the appearance of calcified cartilage. A) Cross section under natural light. B) Detail of the lower box in A) under natural light. C) Detail of the box in B). Islets of calcified cartilage (remnants of the primary cartilage model, blue arrows) are isolated within bone struts. D) Corresponding microradiograph of C). The cartilage islands have cartilage cells that are really round and individualized (as opposed to CB when observed under microradiography) and embedded in a really bright extracellular matrix (blue arrows). E) Detail of the upper box in A) showing articular cartilage (where it articulates with the postorbital, blue arrows). F) Corresponding microradiograph of E). The same observations as those of figure D) can be seen (blue arrows).

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CHAPTER SEVEN

CONCLUSIONS

The morphological degree of sutural closure has been used to assess the maturity of non-avian dinosaur specimens for decades even though 1) this method was developed mostly for use within the clade Mammalia and 2) it has never been tested in the extant phylogenetic bracket of the Dinosauria. To help remedy the problem, this dissertation investigated the sutures in the skulls of the closest extant relatives of non-avian dinosaurs at both the morphological and the microscopic level.

Regarding the order in which sutures close in the skulls of emus and American alligators, the results demonstrated that sutures progressively close in the former species, but progressively ‘re-open’ during ontogeny in the latter. Some sutural obliterations coincided with the onset of maturity in emus, but in alligators, only two sutures obliterate completely during development. This showed that, contrary to common beliefs, open sutures do not necessarily indicate juvenescence and in a similar fashion fused sutures do not always imply adulthood in extant archosaurs. The enigmatic pattern of *Alligator mississippiensis* was explained by changes in its diet during ontogeny and it was concluded that the degree of sutural closure is not a good proxy for maturity in this species. Extrapolated to non-avian dinosaurs, these results suggest that all previous assessment of maturity solely relying on this method should be carefully reconsidered.

The sutural histology through ontogeny was also investigated in some extant archosaurs and non-avian dinosaurs. The results showed that both avian and mammalian

sutures possess a periosteum and consequently have very similar mineralized tissues at their sutural borders. However, the sutures of American alligators lacked a periosteum and form their sutural mineralized tissues via metaplasia. Metaplastic tissues were also found in the sutural areas of many dinosaurs, which suggested that, like crocodilian sutures, dinosaurian sutures lacked a periosteum as well. This demonstrated that the sutures of mammals and non-avian dinosaurs form and grow with considerably different mechanisms.

The findings of this dissertation emphasize the dangers associated with applying conclusions based on mammalian observations to non-avian dinosaurs. Mammals are not the correct extant analogues for the Dinosauria and results show that their sutures are different both at the morphological level (in terms of sequence and timing of fusion) and at the histological level. In the near future, sequences of sutural closure have to be investigated in the skulls and in the vertebral columns of many other archosaurian species. Echoing this dissertation, the potential ‘non-ontogenetic’ signals reflected by these sequences have to be taken into account. As of today, sutural fusion is not a robust method to assess ontogenetic stages and should not be considered grounds to name new species of non-avian dinosaurs. The most accurate method for this purpose remains limb-bone paleohistology (e.g., Horner et al., 2000; Padian et al., 2001).

Moreover, this dissertation improves the understanding of the initial phases of sutural development in non-avian dinosaurs (hadrosaurs) by focusing mainly on two tissues found in the skulls of extant birds and mammals: chondroid bone and secondary cartilage. Results show that chondroid bone is found early during ontogeny at sutural

borders (during embryonic and early post-hatching development) where it most likely allowed extremely rapid sutural growth. The investigation of chondroid bone in the sutures of other vertebrates might reveal that this tissue is more widely distributed in the Vertebrata than previously thought. Secondary cartilage was not found within sutures, but rather was only present at jaw articulations in embryonic and nestling hadrosaurs. This may have served to act as a shock absorber while these dinosaurs were chewing. Even though it is already well established that birds are the descendants of dinosaurs (e.g., Xu et al., 2014), this mineralized tissue represents yet another dinosaurian characteristic (at the microscopic scale) that further cements the dinosaurian origin of birds, hence the creation of the term “dinosaurian secondary cartilage”. In order to further understand the functional significance of secondary cartilage in the skulls of non-avian dinosaurs, it will be necessary to investigate this tissue in extant birds that present different jaw adaptations.

To summarize, this dissertation assessed that sutural fusion is not a robust indicator of ontogeny in non-avian dinosaurs. It also shed light on the early stages of sutural development in non-avian dinosaurs and gave insights into the evolution of skeletal tissues within the Archosauria. Hopefully, it will encourage paleontologists to perform more studies in the extant phylogenetic bracket of the Dinosauria.

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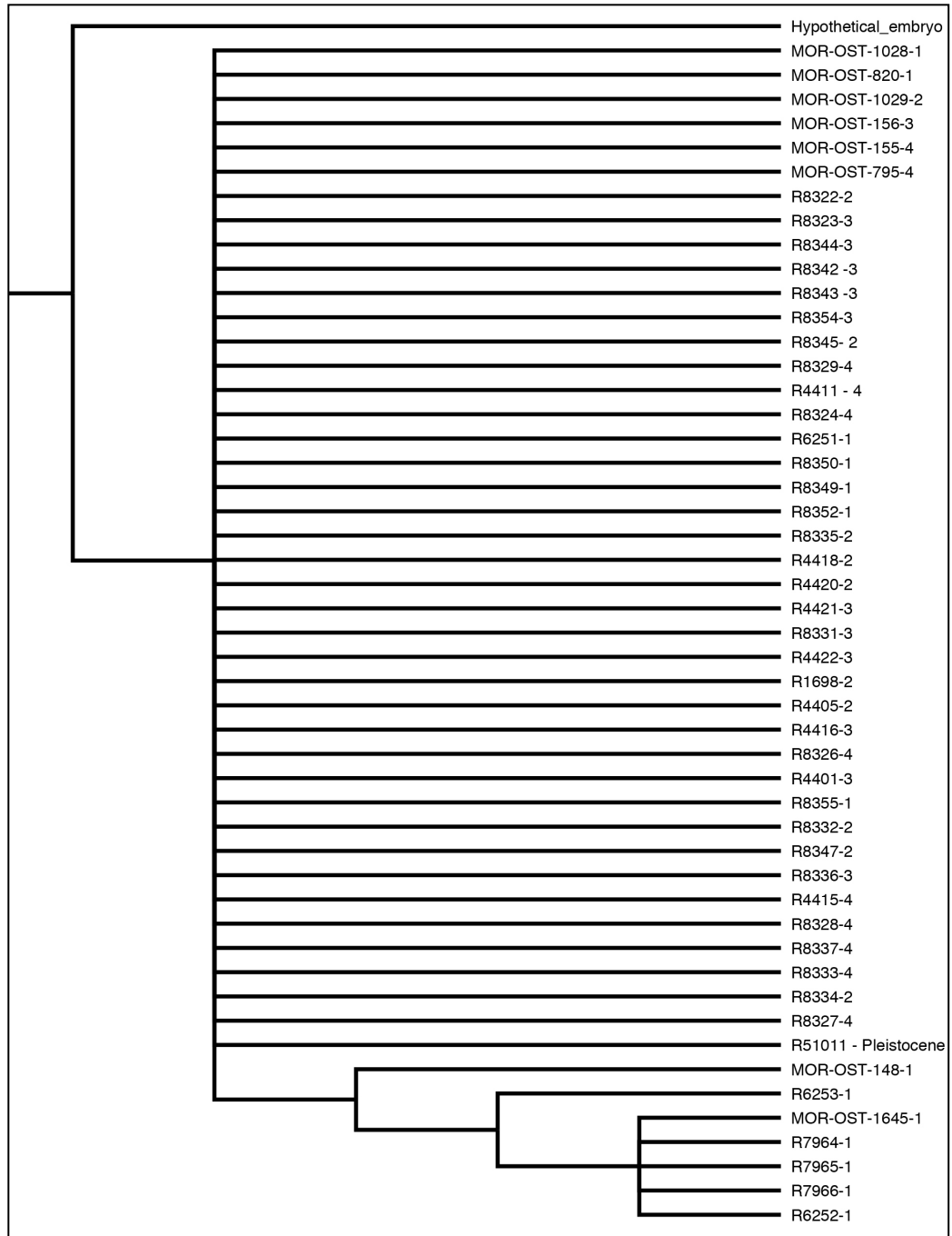
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APPENDICES

APPENDIX A

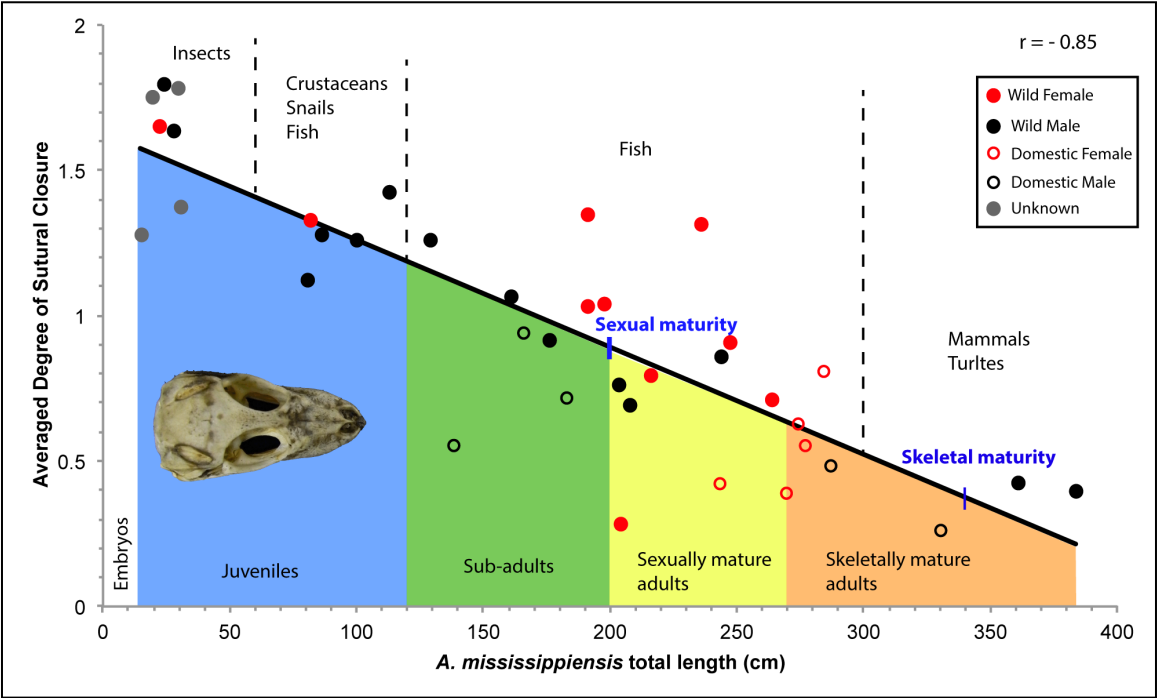
STRICT CONSENSUS PHYLOGENETIC TREE FOR *A. MISSISSIPPIENSIS*



The numbers (1 through 4) following each museum specimen number indicate ontogenetic categories: 1 for juveniles, 2 for sub-adults, 3 for sexually mature adults and 4 for skeletally mature adults.

APPENDIX B

RELATIONSHIP BETWEEN TOTAL LENGTH AND AVERAGED DEGREE OF
SUTURAL CLOSURE IN *A. MISSISSIPPIENSIS* (n=38)



APPENDIX C

SUTURAL CLOSURE SCORES (AND AVERAGES) FOR *D. NOVAEHOLLANDIAE*

	Character/suture number	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	Averaged degree of closure	Average per category
Juveniles	MOR-OST-1799	0	2	2	0.5	1	0	0.5	0.5	0	1	2	0	2	2	2	0	2	1	0	0	0	0.88	0.91
	MOR-OST-1807	2	2	2	0.5	0.5	0.5	1.5	0	1	1	1	2	1.5	1	1	1	2	2	0.5	0	0	1.10	
	MOR-OST-1806	1	2	2	0.5	0.5	0.5	0.5	0	0	1	2	2	1	1	1	1	1		0.5	1	0	0.93	
	MOR-OST-1805	1	2	1.5	0.5	0.5	0.5	0	0	0	1	1	1	1		1	0.5	1	1	1.5	2	1.5	0.93	
	ROM R7945	1	1	0	1	1	1	1	1	1	2	1	1		1	1	1	1	1	2	1		1.05	
	ROM R7644				1	1	1	1	0	1	1				1	1	1						0.90	
	ROM R7630					1		0.5	0	0	1			0	0	0	0	0	1				0.32	
Sub-adults	MOR-OST-1802	1	1.5	2	1.5	1.5	2	2	2	1	1	2	2	2	1	1				1.5	1	1	1.47	1.45
	MOR-OST-1800	2	2	2	1.5	1	1	2	1	1	1	1.5	2	2	2	2	0.5	1		2	2	2	1.58	
	MOR-OST-1809	2	1	2	1	1	1	1	1	1.5	2	1.5	1	2.5	3	2.5	2	0	0.5	2	2	2	1.55	
	MOR-OST-1808	2	2	2	1.5	2	2	2	1	1	1	2	2	1.5	0	1	2	2		2	2	2	1.65	
	MOR-OST-1298				0.5	0.5	1	0.5	0	0	1			1	1	1	0.5						0.64	
	MOR-OST-232	2	3	3	2	3	3	2.5	3	3	3	1	2	3	3	3	2	2	1	2	2	2.5	2.43	
	MOR-OST-1803	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	2	1	2	2	2	2.67	
Sexually mature adults	ROM R6843	2	3	2	3	3	3	3	3	3	3	1.5	2		3	3	3	2	1	2	2	2	2.48	2.52
	MOR-OST-1815	2	1	2	1	2	3	3	3	3	3	1.5	1	3	3	3	3	1	1	2	2	2	2.17	
	MOR-OST-1814	1.5	1	2	1	2	3	3	3	3	3	1.5	1	3	3	3	3	1	1	1.5	1	2	2.07	
	MOR-OST-1813	1	1.5	2.5	1.5	3	3	2.5	3	3	3	1.5	1	3	3	3	3	1.5	0.5	1	1	1	2.07	
	MOR-OST-1812	1	1.5	2.5	1	2.5	3	3	3	3	3	0	1	3	3	3	3	1.5	0	1	1	1	1.95	
	MOR-OST-1811	2	2	2	2	3	3	3	3	3	3	1	1	3	3	3	3	1.5	0	2.5	2	1	2.24	
	MOR-OST-1810	0	1	1	1	1	1	2	3	3	3	1	1	2.5	3	3	3	0.5	0	1	0	1	1.52	
Skeletally mature adults	ROM R7654	1	1	2	1	2	2	2	3	3	3	1	1		3	3	3	1	0.5	2	1	2	1.88	2.73
	MOR-OST-186	1.5	3	3	2	3	3	2.5	3	3			2	3	3	3							2.73	
	MOR-OST-1297	1.5	2	3	2.5	3	3	2.5	3	3	3		1	2	3	3	3						2.57	

APPENDIX D

SUTURAL CLOSURE SCORES (AND AVERAGES) FOR *A. MISSISSIPPIENSIS*

[illegible]

31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61	63	65	67	69
2	1	2	2	1	2	2	2	2	1	0									1
1	1		2	1	1	2	1	1	0.5	1									0
1		2	2	2	2	2	2	1	1	0									1
2			2		2	2	2	2		1									2
			1	1	1	2	2	1	2	1									2
2	2	1	2	1	2	1	1	2	1	2									1
2			2	2	1	1	2	2	2	1									2
2	2	1	2	2	1	1	2	2	2	1									2
1	2	0	2	1	1	2	1	2	2	1									2
1	2	0	0	1	0	1	2	2	2	1									0
		1	1	1	1	1		1		2	2	1							1
	1	1	1	1	1	1	2	1.5	2	1	2	2	2	1	1		1	1	1
1	2	1	1	1	1	1	1	1	1	1		1							1
1	1	1	1	1	1	1	1	1		1									2
	2	2	1	1	1	1		2		0									1
1	0	0	0	1	1	1	1	2	1	2									1
2	2	1	0	0	1	1		2		1			2	2	1	0	1	1	1
1	1	1	1	1	1	2	0	2	1	1									1
		1	1	1	1	1	0			1			1	1	2	0	1	1	1
1	1	1	1	1	1	1	1	2		1									1
1	1	1	1	1	1	1	1	1.5	1	1			2	1	1	0	1	1	1
1	1	2	0	0	1	0	2	1	1	1									1
0			0	0	0	1	0	1	2	1	1	1	1		1	0	1	1	1
2	1	1	1	0	1	1	1	2	1	1						0			2
			0	0	1	0		1		0				0	0	0	0	1	1
1	2	1	0	0	1	0.5	1	1	2	1			0	1	2	0	2	2	1
0	0	0	0	0	0	0	1	0	0	0									0
	0	0		0	0	0				1			1	0	0	0	1	0	0
3	1	1	1	0	1	0		1	1	0									1
3	0	1	0	0	1	1	1	1	1										1
	1	1	0	0	0	0	2	1	1	0	1	1	1	0	1	0	0	0	2
1	1	0	0	0	0	1	0	1	0	0	2	1	0	1	2	0	1	1	1
1	1	0	0	0	1	1		1	2	1			2	2	1	0	1	1	1
1		1	1	1	1	1	1	2		1	1	1	2	1	1	0	1	1	1
0	1	0	0	0	0	0		1		0			1	0	1	0	0	1	1
1	1	0	0	0	0	0	1	2	0.5	0	0	0	0	0	1	0	0	0	0
1	1	0	0	0	1	1	1	1	1	2			1	2	1	0	1	2	1
0	0	0	0	0	0	0		1	1	3									0
1	1	0	0	0	0	0	1	2	2	2									1
2	2	1	0	0	0	0	1	1	0	1									1
0	0	0			0		1	0	0	0									0
1	1	0	0	0	2	0	0	0	1	0									1
0	0	0	0	0	1	1		2	2	1			2	1	2	1	2	2	1
0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
0	0	0					0	0	1	1			1	0	1	0.5	1	0	0
0	0	0	0	0	0	0	0	0	1	1			1	1	1	0	1	1	1

71	73	75	77	79	Degree of closure (averaged)	Average per category
2	2	2	2	2	1.78	1.43
0	1	0	1	1	1.28	
1	2	2	1	1.5	1.66	
2	1	2	0	2	1.80	
1	2	2	1	2	1.64	
2	1	1	2	2	1.38	
2	2	1	2	2	1.75	
2	2	2	0	2	1.67	
1	2	2	2	2	1.41	
1	0	1	2	1.5	0.98	
2	1	1	2	1	1.28	
1	1	1	2	1	1.33	
1	1	1	2	1	1.12	
2	2	1	2	1	1.26	
1	1	1	2	1	1.42	
1	1	1	0	1	1.02	1.02
1	2	1	2	0	0.91	
1	2	1	2	1	1.34	
2	1	1	0	1	1.03	
2	2	1	2	1	1.26	
1	1	2	1	1	1.07	
1	2	1	1	1	0.94	
	2	1	1	1	0.72	
1	1	1	1	2	1.30	
1	1	1	1	1	0.55	
1	1	1	2	0	1.04	
0	1	0	0	0	0.33	0.69
1	0	0	0	0	0.29	
1	1	1	0	0	0.76	
2	1	1	0	0	0.90	
1	2	1	0	0	0.71	
	2	0	0	1	0.69	
2	2	1	0	1	0.79	
2	2	1	1	1	1.32	
1	1	1	0	0	0.42	
1	1	0	0	0	0.39	
1	2	1	2	1	0.86	
2	2	1	0	0	0.55	0.52
1	1	1	0	0	0.63	
2	2	0	0	0	0.77	
1	1.5	0	0	0	0.38	
1	1	0	0	0	0.48	
1	2	0	0	0	0.81	
0	1	0	0	0	0.26	
1	1	1	0	0	0.43	

1	0	0	0	0	0.39	
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APPENDIX E

SUTURAL INTERDIGITATION SCORES (AND AVERAGES) FOR *D.*
NOVAEHOLLANDIAE

	Character/suture number	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	Average per category
Juveniles	MOR-OST-1799	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05
	MOR-OST-1807	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	MOR-OST-1806	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	MOR-OST-1805	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	ROM R7945	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	ROM R7644				0	1	1	0	0	0	0				0	0	0						
Sub-adults	ROM R7630					1		0	0	0	0			0	0	0	0	0	0				0.20
	MOR-OST-1802	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0			1	0	0	
	MOR-OST-1800	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0		1	0	0	
	MOR-OST-1809	0	0	0	0	0	1	0	0.5	0	0	0	0	0		0	0	0	0	1	0	0	
	MOR-OST-1808	0	0	1	1	1	1	0	0.5	0	0	0	0	0	0	1	0	0		0	0	1	
	MOR-OST-1298				1	1	1	0	0	0	0			0	0	0	0						
Skeletally mature adults	MOR-OST-1815	1	0	0	0	1						0	0					0	0	1	0	0	0.17
	MOR-OST-1814	0	0	0	0	1						0	0					0	0	1	0	0	
	MOR-OST-1813	0	0	0	0			0				0	0					0	0	1	0	0	
	MOR-OST-1812	0	0	0	0	0	0					0	0					0	0	1	0	0	
	MOR-OST-1811	0	0	0	1							0	0					0	0	1	0	0	
	MOR-OST-1810	0	0	0	1	1	1	0				0	0	0				0	0	1	0	0	
	ROM R7654	0	0	0	1	1	1	0	0	0	0	0	0		0	0	0	0	0	0	0	2	
	MOR-OST-186	0			0			0				0	0										
	MOR-OST-1297	0	0		0			0				0	0										
	MOR-OST-1803											0						1	1	1	1	0	
Sexually mature adults	ROM R6843	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0.13
	MOR-OST-232	0			0			0				0	0	0			0	0	0	1	0	0	

APPENDIX F

SUTURAL INTERDIGITATION SCORES (AND AVERAGES) FOR A.
MISSISSIPPIENSIS

	Character/suture number	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34
Juveniles	R6252	0	0	0	0	0	0	0	0	0	0	0	0		0		0	0
	MOR OST 1645		0	0	0	0	0	0	0	0	0	0	0		0		0	1
	R7964		0	0	0	0	0	0	0	0	0	0	0		0		0	
	R7965	0	0	0	0	0	0	0	0	0	0	0	0				0	
	R7966	0	0	0	0	0	0			0	0							
	R6251	0	1	0	0	0	1	0	0	0	0	1	0		1		0	1
	R6253	0	0							0	0						0	
	MOR-OST-148	0	1	0	0	0	0	0	0	0	0	0	0		0		0	0
	MOR-OST-1028	0	1	0	0	0	0	0	0	0	0	1	1		1		1	0
	MOR-OST-820	0	1	0	0	0	0	0	1	0	1	1	1		1		0	0
	R8350	0	1	1	0	0	1	0	2	0	1	0	1		1			
	R8349	0	1	1	0	0	0	0	1	0	1	0	1		1			1
	R8352	0	1	1	0	0	0	0	1	0	1	0	1		1		1	1
	R8354	0	1	0	0	1	1	1	1	0	1	1	1		1		1	1
	R8355	1	1	0	0	1	0	0	1	0	1	0	0					1
Sub-adults	MOR-OST-1029	0	1	0.5	0	1	1	0	1	1	1	1	1		1		1	1
	R8332	0	1	1	0	0	0	0	1	1	1	1	1		1		1	1
	R8347	1	1	1	0	1	0	0	0	1	1	1	1		1		1	1
	R8322	0	1	1	0	1	0	0	1	0	0	0	1				1	1
	R8345	0	1	0	0	0	1	1	1	0	1	1	1		1		1	1
	R8335	0	1	1	0	1	0	0	1	1	1	0	1		1		1	1
	R4418	0	2	1	1	1	0	0	1	1	1	0	1		1		1	1
	R4420	0	1	1	1	1	1	1	1	1	1	0	1		1		1	
	R1698	0	1	0	0	0	0	1	1	1	0	0	1		1		1	1
	R4405	0	1	1	0	0	0	0	1	0	1	0	1					
	R8334	0	2	1	1	1	1	1	2	1	1	0	0		1		1	1
Sexually mature adults	MOR-OST-156	0	2	2	0	1	1	1	2	1	1	1	1		1		1	2
	R8323			1	0	0	0	0	1	0	1	1	1				1	1
	R8344	0	1	1	0	0	0	0	1	0	1	1	1		1			1
	R8342	1	1	1	1	1	1	0	2	0	1	1	1		1		1	1
	R8343		2	2	1	1	1	1	2	1	1	1	1		1			1
	R4421	0	1	1	1	1	1	1	1	1	1	0	1		1		1	1
	R4422	2	1	1		1	1	1	1	0	1	1	1		1		1	
	R4401	1	2	1	1	1	1	1	2	0	1	1	1		1		1	1
	R4416	1	1	1	1	1	1	1	1	1	1	1	1		1		1	1
	R8331	0	1	1	0	1	1	1	1	1	1	1	1		1		1	1
	R8336	1	1	1	0	1	1	0	1	1	1	0	1		1		1	2
Skeletally mature adults	R8329	0	2	2	1	1	1	0	1	1	2	1	1		1		1	1
	R4411	0	2	1	1	1	1	1	1	1	1	1	1		1		2	2
	R8324	2	2	1	2	1	1	1	2	1	1	1	2		1		1	2
	R8326	1	2	1	1	1	1	1	1	1	1	1	1		1		1	1
	MOR-OST-155	0	2	2	1	1	1	0	1	1	1	1	1		1		1	2
	MOR-OST-795		2	2	1	1	2	1	2	1	1	2	2		2		2	2

R4415	1	1	2	1	2	1	1	1	1	2	1	1		1		2	1
R8328														1			
R8337														1		1	1
R8333																1	1
R8327		1.5	1	1	1	1	1	1	1	1	1	1	1	1		1	1

36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	74
0	0	0	0	0	0	0	0	0									0	0	0
	0	0	0	0	0	1	0	0									0	0	0
0	0	0	0	0	0	0	0	0									0	0	0
	0		0	0	0	0		0									0	1	0
	0	0	0	0	0	0	0	0									0	0	0
1	1	0	1	0	0	0	0	0									1	0	0
	0	0	0	0	0	0	0	0									0	0	0
1	0	0	1	0	0	0	0	0									0	0	1
1	1	0	0	0	1	1	0	0									1	0	1
1	1	0	1	0	0	1	1	0.5									1	1	1
1	1	0	1	0		0		1	0	1							1	0	1
1	1	0	0	0	0	0	0	1	0	0	0	0	0		1	1	0	1	1
1	1	0	0	0	0	0	0	1		1							1	0	1
1	1	0	1	0	0	1	0	1									1	0	0
1	1	1	1	0		1		0									1	1	1
1	1	1	1	0	1	1	1	0									1	1	1
1	1	1	1	1		1		0			0	0	0	0	0	0	1	1	0
1	1	1	1	0	1	1	1	0									1	1	0
	1	1	1	0	0			1			0	0	1	0	1	1	1	1	1
1	1	0	1	0	1	1		1									1	0	0
1	1	1	1	0	0	0	1	0			0	0	0	0	1	1	1	1	1
1	1	1	1	0	1	0	1	1										1	1
	1	1	1	0	1	1	1	0	1	1	1		1	0	0	0	1		1
1	1	1	1	1	1	1	1	0									1	1	1
	1	1	1	0		0		0				0	0	0	1	0	1	1	1
1	1	1	1	0	1	1	1	0			1	0	1	0	0	0	1	1	1
2	2	1	1	0	1	1	1	0									1	1	1
1	1	2	1	0				1			1	1	1	0	1	1	1	1	1
1	1	1	1	0		1	1	1									1	1	1
1	1	1	1	0	0	1	1										1	1	1
1	2	2	2	1	0	1	1	1	1	2	1	1	1	0	1	1	1	1	1
1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	0	0	1		1
1	1	1	1	1	1	1		1	1	2	1	0	0	0	0	0	1	1	1
1	2	2	1	0	1	1	1	1	1	2	0	1	1	0	1	1	1	1	1
1	1	1	1	0		1		0			1	0	1	0	1	0	1	1	1
1	2	1	1	0		1	1	1			1	1	1	0	1	0	1	1	1
1	1	1	1	1	1	1	1	0			1	0	1	0	1	0	1	1	0

1	2	2	1	1		1	1	1			0	1	1	0	1	1	1	0	0
2	2	2	2	0	1	2	1	1	1	2	1	1	1	0	1	1	1	1	0
2	2	2	2	1	1	2	1	2			1	1	1	0	0	1	2	1	1
1	2	2	1	1	1	1	1	0									1	1	2
2	2	2	1	0	1	1	1	0									1	1	1
2	2		1		1	1	1	1									2	1	1
1	2	2	1	1	1	1	1	0									1	1	1
						1	1	1									1	1	1
2					1	1	1	0.5			1	1	1	0	1	1	1	2	1
1						1	1	0									1	1	
2	2	2	2	1	?	1	1										2	1	1

76	78	80	Average per category
0	0	0	0.30
0	0	0	
0	0	0	
0	0	0	
0	0	0	
0	1	0	
0	0	0	
1	0	0	
1	0	0	
1	2	0	
1	0	0	
1	0	0	
1	0	0	
1	2	0	
1	2	0	0.71
1	2	0	
1	2	0	
1	2	1	
1	0	0	
2	2	0	
1		0	
2	2	0	
1	2	0	
1	2	0	
2	2	0	0.94
2	2	1	
2	2	0	

2	2	1	
2	2	1	
2	2	0	
2	2	0	
2	2	0	
2	2	0	
1	2	0	
2	2	0	
2	2	0	1.18
2	2	0	
2	2	0	
2	2	1	
2	2	0	
2	2	0	
	2	0	
2	2	1	
1	2		
1	2	0	
	2	0	
2	2	0	

APPENDIX G

CHARACTER LIST FOR THE SUTURES AND SYNCHONDROSES OF *D.*
NOVAEHOLLANDIAE

Character 1: nasal-mesethmoid: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 2: nasal-mesethmoid: (0) straight; (1) interdigitated; (2) very interdigitated

Character 3: nasal-prefrontal: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 4: nasal-prefrontal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 5: nasal-frontal: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 6: nasal-frontal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 7: interfrontal: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 8: interfrontal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 9: frontal-parietal: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 10: frontal-parietal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 11: interparietal: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 12: interparietal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 13: supraoccipital-parietal: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 14: supraoccipital-parietal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 15: exoccipital-squamosal (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 16: exoccipital-squamosal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 17: exoccipital-supraoccipital: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 18: exoccipital-supraoccipital: (0) straight; (1) interdigitated; (2) very interdigitated

Character 19: exoccipital basioccipital: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 20: exoccipital-basioccipital: (0) straight; (1) interdigitated; (2) very interdigitated
Character 21: premaxillo-nasal patency: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 22: premaxillo-nasal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 23: frontal-mesethmoid: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 24: frontal-mesethmoid: (0) straight; (1) interdigitated; (2) very interdigitated

Character 25: parietal-squamosal patency: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 26: parietal-squamosal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 27: laterosphenoid-parietal patency: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 28: Laterosphenoid-parietal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 29: Laterosphenoid-squamosal: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 30: Laterosphenoid-squamosal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 31: (Palatal view) Basiparasphenoid-basioccipital: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 32: (Palatal view) Basiparasphenoid-basioccipital: (0) straight; (1) interdigitated; (2) very interdigitated

Character 33: (Palatal view) pterygoid-vomer: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 34: (Palatal view) pterygoid- vomer: (0) straight; (1) interdigitated; (2) very interdigitated

Character 35: (Palatal view) vomer-palatine patency: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 36: (Palatal view) vomer-palatine: (0) straight; (1) interdigitated; (2) very interdigitated

Character 37: (Palatal view) maxilla-premaxilla patency: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 38: (Palatal view) maxilla-premaxilla: (0) straight; (1) interdigitated; (2) very interdigitated

Character 39: (Palatal view) premaxilla-vomer: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 40: (Palatal view) premaxilla-vomer: (0) straight; (1) interdigitated; (2) very interdigitated

Character 41: (Dorsal view) Maxilla-premaxilla patency: (0) open; (1) partially closed; (2) closed (but still visible), (3) completely obliterated

Character 42: (Dorsal view) Maxilla-premaxilla: (0) straight; (1) interdigitated; (2) very interdigitated

APPENDIX H

CHARACTER LIST FOR THE SUTURES AND SYNCHONDROSES OF A.
MISSISSIPPIENSIS

Character 1: interpremaxillary: (0) open; (1) partially closed; (2) closed (but still visible);
(3) completely obliterated

Character 2: interpremaxillary: (0) straight; (1) interdigitated; (2) very interdigitated

Character 3: premax-maxilla: (0) open; (1) partially closed; (2) closed (but still visible);
(3) completely obliterated

Character 4: premaxilla-maxilla: (0) straight; (1) interdigitated; (2) very interdigitated

Character 5: premaxilla-nasal: (0) open; (1) partially closed; (2) closed (but still visible);
(3) completely obliterated

Character 6: premaxilla-nasal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 7: internasal: (0) open; (1) partially closed; (2) closed (but still visible); (3)
completely obliterated

Character 8: internasal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 9: nasal-maxilla: (0) open; (1) partially closed; (2) closed (but still visible); (3)
completely obliterated

Character 10: nasal-maxilla: (0) straight; (1) interdigitated; (2) very interdigitated

Character 11: nasal-prefrontal: (0) open; (1) partially closed; (2) closed (but still visible);
(3) completely obliterated

Character 12: nasal-prefrontal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 13: prefrontal-lachrymal: (0) open; (1) partially closed; (2) closed (but still
visible); (3) completely obliterated

Character 14: prefrontal-lachrymal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 15: maxilla-lachrymal: (0) open; (1) partially closed; (2) closed (but still

visible); (3) completely obliterated

Character 16: maxilla-lachrymal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 17: lachrymal-jugal: (0) open; (1) partially closed; (2) closed (but still visible);
(3) completely obliterated

Character 18: lachrymal-jugal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 19: maxilla-jugal: ((0) open; (1) partially closed; (2) closed (but still visible);
(3) completely obliterated

Character 20: maxilla-jugal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 21: frontal-prefrontal: ((0) open; (1) partially closed; (2) closed (but still
visible); (3) completely obliterated

Character 22: frontal-prefrontal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 23: nasal-frontal: (0) open; (1) partially closed; (2) closed (but still visible); (3)
completely obliterated

Character 24: nasal-frontal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 25: interfrontal: (0) open; (1) partially closed; (2) closed (but still visible); (3)
completely obliterated

Character 26: interfrontal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 27: frontal-parietal: (0) open; (1) partially closed; (2) closed (but still visible);
(3) completely obliterated

Character 28: frontal-parietal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 29: interparietal: (0) open; (1) partially closed; (2) closed (but still visible); (3)
completely obliterated

Character 30: interparietal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 31: parietal-squamosal: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 32: parietal-squamosal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 33: frontal-postorbital: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 34: frontal-postorbital: (0) straight; (1) interdigitated; (2) very interdigitated

Character 35: postorbital-squamosal: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 36: postorbital-squamosal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 37: (palatal view) premaxilla-maxilla: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 38: (palatal view) premaxilla-maxilla: (0) straight; (1) interdigitated; (2) very interdigitated

Character 39: (palatal view) intermaxillary: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 40: (palatal view) intermaxillary (0) straight; (1) interdigitated; (2) very interdigitated

Character 41: (palatal view) maxilla-palatine: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 42: (palatal view) maxilla-palatine: (0) straight; (1) interdigitated; (2) very

interdigitated

Character 43: (palatal view) interpalatine: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 44: (palatal view) interpalatine: (0) straight; (1) interdigitated; (2) very interdigitated

Character 45: supraoccipital-parietal: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 46: supraoccipital-parietal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 47: exoccipital-squamosal (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 48: exoccipital-squamosal: (0) straight; (1) interdigitated; (2) very interdigitated

Character 49: exoccipital-supraoccipital: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 50: exoccipital-supraoccipital: (0) straight; (1) interdigitated; (2) very interdigitated

Character 51: exoccipital-basioccipital: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 52: exoccipital-basioccipital: (0) straight; (1) interdigitated; (2) very interdigitated

Character 53: (endocranial view) parietal-supraoccipital : (0) open; (1) partially closed;

(2) closed (but still visible); (3) completely obliterated

Character 54: (endocranial view) parietal-supraoccipital : (0) straight, (1) interdig, (2) very interdigitated

Character 55: (endocranial view) parietal-frontal: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 56: (endocranial view) parietal-frontal: (0) straight, (1) interdig, (2) very interdigitated

Character 57: (medial view) laterosphenoid-prootic: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 58: (medial view) laterosphenoid-prootic: (0) straight; (1) interdigitated; (2) very interdigitated

Character 59: (medial view) laterosphenoid-basisphenoid: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 60: (medial view) laterosphenoid-basisphenoid: (0) straight; (1) interdigitated; (2) very interdigitated

Character 61: (medial view) basisphenoid-prootic: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 62: (medial view) basisphenoid-prootic: (0) straight; (1) interdigitated; (2) very interdigitated

Character 63: (medial view) exoccipital-prootic: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 64: (medial view) exoccipital-prootic: (0) straight; (1) interdigitated; (2) very

interdigitated

Character 65: (medial view) exoccipital-basioccipital: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 66: (medial view) exoccipital-basioccipital: (0) straight; (1) interdigitated; (2) very interdigitated

Character 67: (medial view) basioccipital-basisphenoid: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 68: (medial view) basioccipital-basisphenoid: (0) straight; (1) interdigitated; (2) very interdigitated

Character 69: (lateral view) laterosphenoid-pterygoid: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 70: (lateral view) laterosphenoid-pterygoid: (0) straight; (1) interdigitated; (2) very interdigitated

Character 71: L(lateral view) laterosphenoid-quadrato: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 72: (lateral view) laterosphenoid-quadrato: (0) straight; (1) interdigitated; (2) very interdigitated

Character 73: (lateral view) quadrato-pterygoid: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 74: (lateral view) quadrato-pterygoid: (0) straight; (1) interdigitated; (2) very interdigitated

Character 75: (ventro-caudal view) basisphenoid-pterygoid: (0) open; (1) partially closed;

(2) closed (but still visible); (3) completely obliterated

Character 76: (ventro-caudal view) basisphenoid-pterygoid: (0) straight; (1) interdigitated; (2) very interdigitated

Character 77: (ventro-caudal view) basisphenoid-basioccipital: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 78: (ventro-caudal view) basisphenoid-basioccipital: (0) straight; (1) interdigitated; (2) very interdigitated

Character 79: (ventro-caudal view) exoccipital-quadrato: (0) open; (1) partially closed; (2) closed (but still visible); (3) completely obliterated

Character 80: (ventro-caudal view) exoccipital-quadrato: (0) straight; (1) interdigitated; (2) very interdigitated

APPENDIX I

CHARACTER MATRIX FOR *D. NOVAEHOLLANDIAE*

Hypothetical embryo

0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0

MOR-OST-1298

?	?	?	?	?	?	0&1	1	0&1	1	1	1	0&1	0
0	0	0	0	1	0	?	?	?	?	1	0	1	0
1	0	0&1	0	?	?	?	?	?	?	?	?	?	?

MOR-OST-1297

1&2	0	2	0	3	?	2&3	0	3	?	3	?	2&3	0
3	?	3	?	3	?	?	?	1	0	2	0	3	?
3	?	3	?	?	?	?	?	?	?	?	?	?	?

MOR-OST-186

1&2	0	3	?	3	?	2	0	3	?	3	?	2&3	0
3	?	3	?	3	?	?	?	2	0	3	0	3	?
3	?	3	?	?	?	?	?	?	?	?	?	?	?

MOR-OST-232

2	0	3	?	3	?	2	0	3	?	3	?	2&3	0
3	?	3	?	3	?	1	0	2	0	3	0	3	?
3	?	2	0	2	0	1	0	2	1	2	0	2&3	0

ROM R7630

?	?	?	?	?	?	?	?	1	1	?	?	0&1	0
0	0	0	0	1	0	?	?	?	?	0	0	0	0
0	0	0	0	0	0	1	0	?	?	?	?	?	?

ROM R7644

?	?	?	?	?	?	1	0	1	1	1	1	1	0
0	0	1	0	1	0	?	?	?	?	?	?	1	0

1 0 1 0 ? ? ? ? ? ? ? ? ?

ROM R7945

1 0 1 0 0 0 1 0 1 0 1 1 1 0

1 0 1 0 2 0 1 0 1 0 ? ? 1 0

1 0 1 0 1 0 1 0 2 0 1 0 ? ?

ROM R7654

1 0 1 0 2 0 1 1 2 1 2 1 2 0

3 0 3 0 3 0 1 0 1 0 ? ? 3 0

3 0 3 0 1 0 0&1 0 2 0 1 0 2 2

ROM R6843

2 0 3 0 2 0 3 0 3 0 3 0 3 0

3 0 3 0 3 0 1&2 0 2 0 ? ? 3 0

3 0 3 0 2 0 1 0 2 0 2 0 2 0

MOR-OST-1803

3 ? 3 ? 3 ? 3 ? 3 ? 3 ? 3 ?

3 ? 3 ? 3 ? 2 0 3 ? 3 ? 3 ?

3 ? 3 ? 2 1 1 1 2 1 2 1 2 0

MOR-OST-1805

$$0 \& 1 \& 2 \quad 0 \quad 2 \quad 0 \quad 1 \& 2 \quad 0 \quad 0 \& 1 \quad 0 \quad 0 \& 1 \quad 0 \quad 0 \& 1 \quad 0 \quad 0$$

0 0 0 0 0 1 0 1 0 0&1&2 0 1 0

?	?	1	0	0&1	0	1	0	1	0	1&2	0	2	0
---	---	---	---	-----	---	---	---	---	---	-----	---	---	---

1&2 0

MOR-OST-1806

0&1&2	0	2	0	2	0	0&1	1	0&1	0	0&1	0	0&1
-------	---	---	---	---	---	-----	---	-----	---	-----	---	-----

0 0 0 0 0 1 0 2 0 2 0 1 0 1

0 1 0 1 0 1 0 ? ? 0&1 0 1 0 0

0

MOR-OST-1807

2	0	2	0	2	0	0&1	1	0&1	0	0&1	0	1&2	0
0	0	1	0	1	0	1	0	2	0	1&2	0	1	0
1	0	1	0	2	0	2	0	0&1	0	0	0	0	0

MOR-OST-1808

2	0	2	0	2	1	1&2	1	2	1	2	1	2	0
1	0&1	1	0	1	0	2	0	2	0	1&2	0	0	0
1	1	1&2&3	0	2	0	?	?	2	0	2	0	2	
1													

MOR-OST-1809

2	0	1	0	2	0	1	0	1	0	1	1	1	0
1	0&1	1&2	0	1&2&3	0	1&2	0	1	0	2&3	0	3	
?	2&3	0	1&2&3	0	0	0	0&1	0	2	1	2	0	
2	0												

MOR-OST-1810

0	0	1	0	1	0	1	1	1	1	1	1	2	0
3	?	3	?	3	?	1	0	1	0	2&3	0	3	?
3	?	3	?	0&1	0	0	0	1	1	0	0	1	0

MOR-OST-1811

2	0	2	0	2	0	2	1	3	?	3	?	3	?
3	?	3	?	3	?	1	0	1	0	3	?	3	?
3	?	3	?	1&2	0	0	0	2&3	1	2	0	1	0

MOR-OST-1812

1	0	1&2	0	2&3	0	1	0	2&3	0	3	?	3	?
3	?	3	?	3	?	0	0	1	0	3	?	3	?
3	?	3	?	1&2	0	0	0	1	1	1	0	1	0

MOR-OST-1813

1	0	1&2	0	2&3	0	1&2	0	3	?	3	?	2&3	0
3	?	3	?	3	?	1&2	0	1	0	3	?	3	?
3	?	3	?	1&2	0	0&1	0	1	1	1	0	1	0

MOR-OST-1814

1&2	0	1	0	2	0	1	0	1&2&3	1	3	?	3	
?	3	?	3	?	3	?	1&2	0	1	0	3	?	3
?	3	?	3	?	1	0	1	0	1&2	1	1	0	2
0													

MOR-OST-1815

[illegible]

MOR-OST-1800

[illegible]

MOR-OST-1802

1	0	1&2	0	2	0	1&2	0	1&2	1	2	1	2	0
2	0	1	0	1	0	2	0	2	0	2	0	1	0
1	0	1	0	?	?	?	?	1&2	1	1	0	1	0

MOR-OST-1799

[illegible]

APPENDIX J

CHARACTER MATRIX FOR *A. MISSISSIPPIENSIS*

Hypothetical embryo

[illegible]

MOR-OST-1645

?	?	1	0	2	0	2	0	2	0	2	0	2	0
2	0	2	0	1	0	?	0	1	0	3	?	1	0
1&2&3		?	1	0	1	1	?	?	2	0	1	0	1
0	2	0	1	0	1	1	0&1	0	1	0	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	?	0
0	0	0	1	0	0	0	1	0	1	0			

MOR-OST-148

2	0	1	1	2	0	2	0	1	0	2	0	2	0
1	0	1	0	1&2	0	2	0	1	0	3	?	1	0
3	?	2	0	2	0	1	1	2	0	2	0	1	1
1	0	2	0	2	0	2	0	1	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	2	0
2	0	2	1	2	1	0	0	2	0				

MOR-OST-1028

[illegible]

1 0 2 1 2 1 2 0 2 0

MOR-OST-820

1 0 0 1 1 0 1 0 1 0 1 0 1 0
 1 1 0 0 0 1 1 1 0 1 3 ? 0 1
 3 ? 1 0 2 0 0 1 0 1 1 0 0 1
 1 0 2 0 2 1 2 1 1 0&1 ? ? ? ?
 ? ? ? ? ? ? ? ? ? ? ? ? 0 1
 1 1 0 1 1 1 2 2 1&2 0

MOR-OST-1029

0 0 0&1 1 0 0&1 2 0 2 1 1 1 1 0
 1 1 1 1 1 1 1 1 0 1 3 ? 1 1
 3 ? 1 1 0 1 0 1 0 1 1 1 1 1
 1 0 1 1 2 1 1 1 2 0 ? ? ? ?
 ? ? ? ? ? ? ? ? ? ? ? ? 1 1
 1 1 1 1 1 1 0 2 1 0

MOR-OST-156

1 0 0 2 0 2 0&1&2 0 0&1 1 0 1 0
 1 0 2 0 1 0 1 0 1 0 1 3 ? 0
 1 3 ? 0 1 0 2 0 2 0 2 0 1 0
 1 0 0 1 1 0 1 0 1 0 0 ? ? ?
 ? ? ? ? ? ? ? ? ? ? ? ? 0
 1 0 1 0&1&2 1 0 2 0 2 0 0

MOR-OST-155

0 0 0 2 0 2 0 1 1 1 0 1 1&2 0
 0 1 1 1 1 1 0 1 0 1 3 ? 1 1
 3 ? 2 1 2 2 1 2 0 2 0 2 0 1
 0 0 1 1 1 1 0 1 1 0 ? ? ? ?

?	?	?	?	?	?	?	?	?	?	?	?	1	1
2	1	2	1	0	2	0	2	0	0				

MOR-OST-795

?	?	?	2	?	2	?	1	0	1	0	2	0	1
0	2	0	1	0	1	0	2	0	2	3	?	0	2
3	?	0	2	0	2	0	2	?	2	?	?	0	1
?	?	1	1	0	1	0	1	0	1	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	0	2
1	1	1&2	1	0	?	0	2	0	0				

ROM R8322

1	0	1	1	1	1	1	0	1	1	1	0	1	0
1	1	1	0	1	0	1	0	1	1	3	?	?	?
?	?	?	1	?	1	1	?	1	1	1	1	1	1
1	0	0	0	?	?	?	?	1	1	?	?	?	?
1	0	1	0	2	1	0	0	1	1	1	1	1	1
2	1	1	1	1	1	0	2	1	1				

ROM R8323

?	?	?	?	?	1	1	0	0	0	0	0	0	0
0	1	0	0	0	1	0	1	0	1	3	?	?	?
?	?	?	1	0	1	0	1	?	1	0	2	0	1
0	0	?	?	?	?	?	?	1	1	?	?	?	?
1	1	0	1	0	1	0	0	1	1	0	1	0	1
1	1	0	1	0	2	0	2	0	1				

ROM R8344

0	0	0	1	0	1	1	0	1	0	1	0	1	0
1	1	1	0	0	1	1	1	1	1	?	?	1	1
?	?	3	?	1	1	1	1	1	1	0	1	1	1

0	0	?	?	1	1	1	1	0	1	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
1	1	1	1	1	2	0	2	0	0				

ROM R8342

0	1	0	1	1	1	1	1	0	1	1	1	1	0
0	2	1	0	1	1	0	1	1	1	3	?	1	1
3	?	3	1	0	1	1	1	0	1	0	1	1	1
1	0	1	0	1	1	1	1	?	?	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
2	1	1	1	1	2	0	2	0	1				

ROM R8343

?	?	0	2	0	2	0	1	1	1	0	1	0	1
0	2	1	1	1	1	1	1	0	1	3	?	1	1
3	?	?	?	1	1	1	1	0	2	0	2	0	2
0	1	2	0	1	1	1	1	0	1	1	1	1	2
1	1	0	1	1	1	0	0	0	1	0	1	2	1
1	1	2	1	1	2	0	2	0	1				

ROM R8354

1	0	1	1	1	0	1	0	1	1	1	1	1	1
1	1	1	0	1	1	1	1	1	1	3	?	1	1
3	?	1	1	1	1	1	1	1	1	1	0	1	1
1	0	1	0	1	1	?	0	1	1	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	2	1
2	0	2	0	1	1	2	0	1	0				

ROM R8345

1	0	1	1	1	0	1	0	1	0	1	1	1	1
1	1	1	0	1	1	1	1	1	1	3	?	1	1

3	?	1	1	1	1	1	1	1	1	1	0	1	1
1	0	1	1	2	1	?	?	1	1	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
2	0	2	0	1	1	2	0	1	0				

ROM R8329

0&1&2		0	0	2	0	2	1	1	1	1	0	1	0
0	0	1	0	1	0	2	1	1	0	1	3	?	1
1	?	?	0	1	0	1	0	1	0	2	0	2	1
1	1	1	?	?	2	1	2	1	1	1	?	?	?
?	2	0	1	1	2	1	1	0	2	1	2	1	1
1	1	0	2	0	0	2	0	2	0	0			

ROM R4411

0	0	0	2	0	1	0	1	0	1	0	1	0	1
0	1	0	1	0	1	0	1	0	1	2&3	?	0	1
3	?	0	2	0	2	0	2	0	2	0	2	0	2
0	0	0	1	0	2	1	1	1	1	1	1	0	2
1	1	0	1	0	1	0	0	0	1	0	1	0	1
0	1	1	0	0	2	0	2	0	0				

ROM R8324

0	2	0	2	0	1	0	2	0	1	0	1	0	1
0	2	0	1	0	1	0	1	0	2	3	?	0	1
3	?	0	1	0	2	0	2	0	2	0	2	0	2
0	1	0	1	0	2	1	1	1	2	?	?	?	?
1	1	1	1	1	1	0	0	1	0	1	1	1	2
1	1	0	1	0	2	0	2	0	1				

ROM R6251

1	0	1	1	2	0	1	0	1	0	0	1	1	0
---	---	---	---	---	---	---	---	---	---	---	---	---	---

0	0	0	0	1	0	1	1	2	0	3	?	1	1
3	?	2	0	2	1	1	1	2	1	1	0	2	1
1	0	1	0	2	0	1	0	2	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
2	0	1	0	1	0	2	1	2	0				

ROM R8350

1	0	1	1	1	1	1	0	1	0	1	1	2	0
0	2	1	0	1	1	2	0	0	1	3	?	?	1
3	?	?	?	?	?	1	1	1	1	1	0	1	1
1	0	?	?	1	0	?	?	2	1	2	0	1	1
?	?	?	?	?	?	?	?	?	?	?	?	1	1
2	0	1	1	1	1	2	0	1	0				

ROM R8349

1	0	1	1	1	1	1	0	1	0	1	0	1	0
1	1	1	0	2	1	2	0	1	1	3	?	1	1
3	?	?	?	1	1	1	1	1	1	1	0	1	0
1	0	2	0	1&2	0	2	0	1	1	2	0	2	0
2	0	1	0	1	0	?	?	1	1	1	1	1	0
1	1	1	1	1	1	2	0	1	0				

ROM R8352

1	0	1	1	1	1	1	0	1	0	1	0	1	0
0	1	1	0	1	1	1	0	1	1	3	?	0	1
3	?	1	1	2	1	1	1	1	1	1	0	1	0
1	0	1	0	1	0	1	0	1	1	?	?	1	1
?	?	?	?	?	?	?	?	?	?	?	?	1	1
1	0	1	1	1	1	2	0	1	0				

ROM R8335

1	0	0	1	1	1	1	0	1	1	1	0	2	0
0	1	1	1	1	1	1	0	1	1	3	?	1	1
?	?	1	1	1	1	1	1	1	1	1	1	1	1
1	0	1	0	1&2	0	1	1	1	0	?	?	?	?
2	0	1	0	1	0	0	0	1	1	1	1	1	1
1	1	1	1	2	2	1	2	1	0				

ROM R4418

0	0	0	2	0	1	0	1	0	1	1	0	1	0
1	1	0	1	0	1	2	0	1	1	3	?	1	1
3	?	1	1	1	1	2	1	0	1	0	1	1	1
0	0	2	1	1	0	1	1	1	1	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	?
1	1	2	1	1	1	1	?	1	0				

ROM R4420

0	0	0	1	0	1	1	1	0	1	0	1	0	1
0	1	1	1	0	1	1	0	0	1	3	?	0	1
3	?	0	1	?	?	?	?	0	1	0	1	0	1
1	0	0	1	1	1	2	1	1	0	1	1	1	1
1	1	?	?	1	1	0	0	1	0	1	0	1	1
?	?	2	1	1	2	1	2	1	0				

ROM R4421

1	0	1	1	0	1	1	1	0	1	0	1	0	1
0	1	1	1	1	1	0	0	0	1	3	?	0	1
3	?	1	1	1	1	0	1	0	1	0	1	0	1
1	0	0	1	1	1	0	1	0	1	2	1	1	1
0	1	1	0	2	1	0	0	1	0	1	0	1	1
?	?	2	1	0	2	0	2	1	0				

ROM R8331

0	0	0	1	0	1	0	0	0	1	0	1	0	1
0	1	1	1	1&2	1	0	1	0	1	3	?	1	1
?	?	1	1	1	1	0	1	0	2	0	1	1	1
1	0	?	?	1	1	2	1	1	1	?	?	?	?
2	1	2	1	1	1	0	0	1	1	1	0	1	1
2	1	2	1	1	1	0	2	1	0				

ROM R4422

1	2	1	1	1	1	3	?	1	1	1	1	1&2	1
1	1	1	0	2	1	2&3	1	1	1	3	?	1	1
3	?	1	1	?	?	1	1	1	1	1	1	1	1
1	1	1	1	2	1	?	?	1	1	1	1	1	2
2	1	1	0	1	0	0	0	1	0	1	0	1	1
2	1	2	1	1	2	1	2	1	0				

ROM R1698

1	0	1	1	1	0	2	0	1	0	1	0	2	1
1	1	1	1	1	0	2	0	1	1	3	?	2	1
3	?	2	1	1	1	1	1	1	1	0	1	1	1
1	1	1	1	2	1	1	1	1	0	?	?	?	?
?	?	?	?	?	?	0	0	?	?	?	?	2	1
1	1	1	1	1	1	1	2	2	0				

ROM R4405

0	0	1	1	1	1	1	0	1	0	1	0	0	0
1	1	0	0	0	1	1	0	0	1	?	?	?	?
?	?	?	?	?	?	?	?	0	1	0	1	1	1
0	0	?	?	1	0	?	?	0	0	?	?	?	?
?	?	0	0	0	0	0	0	0	1	1	0	1	1

1 1 1 1 1 1 1 2 1 0

ROM R4416

0	1	0	1	0	1	0	1	0	1	0	1	0	1
0	1	0	1	0	1	0	1	0	1	3	?	0	1
3	?	0	1	1	1	0	1	0	1	0	1	0	1
0	0	?	?	1	1	?	?	0	0	?	?	?	?
1	1	0	0	1	1	0	0	0	1	1	0	1	1
1	1	1	1	1	2	0	2	0	0				

ROM R8326

0	1	0	2	0	1	0	1	0	1	0	1	0	1
0	1	0	1	0	1	0	1	0	1	3	?	1	1
3	?	1	1	1	1	0	1	0	2	0	2	0	1
0	1	1	1	2	1	2	1	2	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
1	1	1	2	1	2	0	2	0	0				

ROM R4401

0	1	0	2	0	1	0	1	0	1	0	1	0	1
0	2	0	0	0	1	0	1	0	1	3	?	1	1
3	?	1	1	1	1	0	1	0	2	0	2	0	1
0	0	1	1	2	1	0&1	1	0	1	0	1	0	2
0	0	0	1	1	1	0	0	0	1	0	1	0	1
1	1	1	1	0	2	0	2	0	0				

ROM R6253

1	0	2	0	?	?	?	?	?	?	?	?	?	?
?	?	1	0	2	0	?	?	?	?	?	?	?	?
3	?	2	0	?	?	?	?	2	0	2	0	1	0

1	0	2	0	2	0	2	0	1	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	2	0
2	0	2	0	1	0	2	0	2	0				

ROM R8355

1	1	0	1	1	0	2	0	2	1	2	0	2	0
1	1	?	0	2	1	2	0	2	0	3	?	?	?
?	?	?	?	2	1	2	1	1	1	1	1	1	1
1	0	?	?	2	1	?	?	0	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
1	1	1	1	1	1	2	2	1	0				

ROM R8332

0	0	0	1	0	1	0	0	1	0	1	0	1	0
0	1	1	1	0	1	1	1	0	1	?	?	2	1
?	?	2	1	2	1	1	1	0	1	0	1	1	1
1	1	?	?	2	1	?	?	1	0	?	?	?	?
2	0	2	0	1	0	0	0	1	0	1	0	1	1
1	1	2	0	1	1	2	2	0	0				

ROM R8347

1	1	1	1	1	1	2	0	1	1	2	0	2	0
1	0	1	1	1	1	1	1	2	1	3	?	1	1
3	?	1	1	1	1	1	1	1	1	1	1	1	1
2	0	0	1	2	1	1	1	1	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
1	1	2	0	1	1	2	2	1	0				

ROM R8336

0	1	0	1	0	1	0	0	0	1	0	1	0	0
0	1	1	1	1	1	0	0	0	1	2&3	?	1	1

3	?	1	1	1	2	0	1	0	1	0	1	1	1
1	1	1	1	1	1	1	1	2	0	?	?	?	?
1	1	2	0	1	1	0	0	1	1	2	0	1	1
1	1	2	0	1	2	2	2	1	0				

ROM R4415

0	1	0	1	0	2	0	1	0	2	0	1	0&1	1
0	1	0	1	0	2	0	1	0	1	3	?	1	1
3	?	1	2	1	1	0	1	0	2	0	2	2	1
0	1	0	1	0	1	1	1	0	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
1	1	1	1	0	2	0	2	0	1				

ROM R7964

?	?	1&2	0	2	0	2	0	2	0	2	0	2	0
2	0	2	0	1	0	1	0	2	0	?	?	2	0
3	?	1	0	?	?	2	0	2	0	2	0	2	0
2	0	2	0	1	0	1	0	0	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	0
1	0	2	0	2	0	1	0	1&2	0				

ROM R7965

1	0	2	0	2	0	2	0	2	0	2	0	2	0
2	0	2	0	2	0	2	0	2	0	?	?	?	?
?	?	2	0	?	?	?	?	2	0	?	?	2	0
2	0	2	0	2	0	?	?	1	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	2	0
2	1	1	0	2	0	0	0	2	0				

ROM R7966

1 0 2 0 2 0 2 0 2 0 2 0 ? ?

?	?	2	0	2	0	?	?	?	?	?	?	?	?
?	?	?	?	?	?	?	?	1	0	1	0	1	0
2	0	2	0	1	0	2	0	1	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	2	0
1	0	2	0	2	0	1	0	2	0				

ROM R6252

1	0	2	0	2	0	2	0	2	0	1	0	2	0
2	0	2	0	2	0	2	0	2	0	3	?	1	0
3	?	2	0	1	0	2	0	2	0	1	0	2	0
2	0	2	0	2	0	1	0	0	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	0
2	0	2	0	2	0	2	0	2	0				

ROM R8328

?	?	?	?	?	?	?	?	?	?	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	2	1
?	?	?	?	?	?	?	?	?	?	?	?	?	?
?	?	?	?	1	1	1	1	2	1	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	1	1
1	1	1	1	2	1	2	2	?	?				

ROM R8337

?	?	?	?	?	?	?	?	?	?	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	0	1
?	?	0	1	0	1	0	2	?	?	?	?	?	?
?	?	0	1	0	1	1	1	1	0&1	?	?	?	?
1	1	0	1	1	1	0&1	0	1	1	0	1	0	1
1	2	1	1	1	1	0	2	0	0				

ROM R8333

?	?	?	?	?	?	?	?	?	?	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	?	?
?	?	2	1	2	1	2	1	?	?	?	?	?	?
?	?	?	?	2	1	2	1	2	0	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	2	1
2	1	?	?	?	?	2	2	2	0				

ROM R8334

0	0	0	2	0	1	1	1	1	1	1	1	2	1
1	2	2	1	1	1	1	0	1	0	3	?	1	1
?	?	1	1	2	1	1	1	0	1	0	1	1	1
0&1	0	1	1	1	1	2	1	1	0	?	?	?	?
0	1	1	0	2	1	0	0	2	0	2	0	1	1
1	1	1	1	1	1	2	2	0	0				

ROM R8327

2&3	?	0	1&2	0	1	0&1&2	1	0	1	0	1	0	
1	0	1	0	1	0	1	0	1	0	1	3	?	0
1	?	?	0	1	0	1	0	2	0	2	0	2	0
2	0	1	?	?	1	1	1	1	3	?	?	?	?
?	?	?	?	?	?	?	?	?	?	?	?	?	0
2	2	1	2	1	1	2	0	2	0	0			

ROM R51011

0&1&2		1	0	1	?	?	0	1	0	1	0	1&2	1
1	?	?	?	?	?	?	?	?	?	?	3	?	0
1	3	?	0	2	0	2	0	2	?	1	?	?	?
?	?	?	?	?		0&1&2	1	0	1	0	0	?	?
?	?	?	?	?	?	?	?	?	?	?	?	?	?
?	?	2	0	?	?	0	0	2	2	0	0		

APPENDIX K

MAMMALIAN AND TELEOSTEAN SECONDARY CARTILAGES

Secondary cartilage has only been reported for two species of teleosts: *Poecilia sphenops*, on its dentary, maxilla and cleithrum (Benjamin, 1989), and *Salmo salar* on its dentary (Gillis et al., 2006; Witten and Hall, 2002). However, whether this is mechanically induced and/or maintained is unknown. In mammals, mechanical stimulation is not required for the initiation of secondary cartilages (it is required however for their maintenance). Mammalian secondary cartilages are mostly located in the mandible (in the symphysis, and on the alveolar, condylar, and coronoid processes of the dentary, Bareggi et al., 1994; Beresford, 1981; Goret-Nicaise, 1986; Goret-Nicaise et al., 1986; Richany et al., 1956; Vinkka-Puhakka and Thesleff, 1992) and on the clavicle (Tran and Hall, 1989). However, not all cartilages on the condylar process are secondary. In humans and in rats, the condylar cartilage is secondary, arising from periosteal cells of the condylar process. In mice however, the condylar cartilage is a sesamoid, arising, as does the patella (knee cap) in a separate aggregation of cells beside the condylar process, which subsequently fuses with the condylar process (Hall, 2005).

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