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Joint histology in *Alligator mississippiensis* challenges the identification of synovial joints in fossil archosaurs and inferences of cranial kinesis

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Archosaurs, like all vertebrates, have different types of joints that allow or restrict cranial kinesis, such as synovial joints and fibrous joints. In general, synovial joints are more kinetic than fibrous joints, because the former possess a fluid-filled cavity and articular cartilage that facilitate movement. Even though there is a considerable lack of data on the microstructure and the structure–function relationships in the joints of extant archosaurs, many functional inferences of cranial kinesis in fossil archosaurs have hinged on the assumption that elongated condylar joints are (i) synovial and/or (ii) kinetic. Cranial joint microstructure was investigated in an ontogenetic series of American alligators, *Alligator mississippiensis*. All the presumably synovial, condylar joints found within the head of the American alligator (the jaw joint, otic joint and laterosphenoid–postorbital (LS–PO) joint) were studied by means of paraffin histology and undecalcified histology paired with micro-computed tomography data to better visualize three-dimensional morphology. Results show that among the three condylar joints of *A. mississippiensis*, the jaw joint was synovial as expected, but the otherwise immobile otic and LS–PO joints lacked a synovial cavity. Therefore, condylar morphology does not always imply the presence of a synovial articulation nor mobility. These findings reveal an undocumented diversity in the joint structure of alligators and show that crocodylians and birds build novel, kinetic cranial joints differently. This complicates accurate identification of synovial joints and functional inferences of cranial kinesis in fossil archosaurs and tetrapods in general.

1. Introduction

Vertebrate cranial evolution is resplendent with modifications to bones, their articulations and ultimately adaptations for cranial development and function. Numerous changes occurred in the craniofacial and suspensorial skeletons of archosaur clades that impacted their aptitude for cranial kinesis or the movement of cranial joints other than the jaw joint [1,2]. Although both extant lineages of archosaurs, crocodylians and birds, independently diverged from a condition with limited kinesis (i.e. the partially kinetically competent condition in [3]) into akinetic and kinetic lineages, respectively, they each retain ill-understood vestiges of these cranial transformations. Among these cranial joints, the laterosphenoid–postorbital (LS–PO) and the otic (quadrate–squamosal) joints are key to understanding patterns of cranial development, function and phylogeny, yet little is known about their anatomy apart from basic osteology.

The diagnosis of cranial joint structure, whether a joint is synovial or fibrous for example, is fundamental to predicting the growth and functional characteristics of the joint and its surrounding tissues. Synovial joints possess articular cartilage, synovial fluid and capsular tissues that allow movement, while

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lubricating the joint and distributing forces [4]. In contrast, fibrous articulations are less mobile than synovial joints, although some examples of mobile, extensible syndesmoses are found in the mandibular symphyses and otic joints of snakes and lepidosaurs or within the cranial linkages of fishes for example [5–8].

The laterosphenoid is a characteristic of archosauriformes and results from the endochondral ossification of the pila antotica of the chondrocranium [9]. The element consistently articulates with the postorbital (which is a membrane bone) and occasionally the neighbouring quadrate and frontal elements in derived clades such as alligatorids [10]. The condylar LS–PO joint persists among virtually all pseudosuchian and dinosaurian clades, only to be finally obliterated through co-ossification within Euavialae, as *Archaeopteryx* retains the primitive archosauriform character state [11].

Archosauriforms also primitively possess a similarly condylar otic joint between the cartilaginous quadrate and the membranous squamosal bone [12]. The otic joint persists throughout Dinosauria and ultimately evolved into a highly mobile joint in birds. On the other hand, as crocodylomorphs sutured the quadrate to the braincase, the otic joint diminished in size and theoretically mobility, resulting in only a vestigial articulation that persists in Crocodylia [3,13,14].

Historically, the LS–PO and the otic joint have been described as synovial in structure, most likely as an assumption stemming from our knowledge of condylar, synovial and mobile joint anatomy that is ubiquitous among the appendicular skeleton. For example, the LS–PO joint of ornithopod dinosaurs and extant crocodylians, as well as the otic joint of extant crocodylians were described as synovial without any histological support [3,15]. Although the surrounding sutural contacts of the laterosphenoid and the quadrate and their sutured neighbours have always dissuaded researchers from inferring mobility at the two joints in extant crocodylians, the otic joints of non-crocodyliform archosauriforms are notoriously implicated in hypotheses of cranial kinesis, where the condylar shape of the joint is synonymized with the synovial nature and/or mobility of the joint. For example, otic joint kinesis (i.e. streptostyly, pleurokinesis) has been inferred in non-avian theropods and hadrosaurs, but not thyreophorans nor sauropods even though the otic joint morphologies are quite similar [3].

Thus, it is often challenging to accurately infer joint structure as well as joint function in fossil taxa such as extinct archosaurs, because (i) there are a considerable lack of data on the structure–function relationships in the cranial joints of extant archosaurs, and (ii) most osteological studies describing joint structure in extant species are not accompanied by histological examination. Because of this lack of clarity in joint anatomy, we still struggle to understand the origin of key features of the archosaur skull such as the laterosphenoid or the distribution of streptostyly in dinosaurs and pseudosuchians.

Here we test the common assumption that the condylar jaw, otic and LS–PO joints are synovial by means of osteology, micro-computed tomography (µCT) and histology in an ontogenetic series of American alligators, *Alligator mississippiensis*. This species was chosen for three reasons: (i) the microanatomy of cranial synovial joints is understudied in crocodylians (but see [16]) compared with those of birds [17,18–20]); (ii) these joints in American alligators have been directly or indirectly assumed to be synovial based on their condylar morphology [3,21,22]; and (iii) alligators are part of the extant phylogenetic bracket of dinosaurs (EPB; [23]) and their anatomy serves as a key test of homology, evolution and origins of the archosaur condition. In the rest of this paper, synovial joints will be identified by four histological criteria: (i) articular cartilage including its underlying calcified cartilage), (ii) a synovial cavity, (iii) a synovial membrane, and (iv) a fibrous capsule. At the osteological level (i.e. in defleshed specimens and in fossils), only calcified cartilage is left in most cases. This calcified articular cartilage, as well as the fibrous capsule, may also leave osteological correlates on bone surfaces of both extant specimens and fossils [24–28]. This study provides new findings on the histology, structure and diversity of cranial joints, which are key to understanding the evolution of archosaur and vertebrate cranial kinesis.

2. Material and methods

(a) Histology

The ontogenetic series consisted of three small individuals sampled via decalcified paraffin histology and one large individual sampled using un-decalcified bone histology (because only small samples can be cut on a microtome; see table 1 for specimen sizes). The three joints of interest (figure 1*b*) were extracted from frozen heads with either a Dremel equipped with a diamond blade or a hand saw.

(i) Paraffin histology

Extracted joints were fixed in 10% neutral-buffered formalin (NBF) for at least 48 h, decalcified in solutions of Cal-Ex (Fisher Scientific) for 24–48 h, transferred back to NBF and sent to an automated tissue processor overnight for dehydration, clearing and paraffin wax infiltration (Paraplast Plus, Fisher Scientific) before embedding. Sections were cut at 5 µm on a rotary microtome (Shandon Finesse Me+, ThermoFisher), placed in a warm water bath at 44°C with gelatin (Sta-on Surgipath, Leica) and mounted on charged slides (Superfrost Plus, Fisher Scientific). All three joints were cut axially (figures 2–4). Slides were stained using a modified Masson's trichrome [17,29] and coverslipped with Permount. Slides were scanned with an Aperio ScanScope CS and photographed using the software ImageScope v. 12.1.

(ii) Un-decalcified histology

The extracted joints were fixed in 10% NBF for two weeks, transferred to a solution of 70% ethanol (EtOH) for one week, then dehydrated in graded solutions of 80%, 95% and finally 100% EtOH (between 2 and 4 days for each solution, with one solution change). They were then cleared in xylene for 12 hours, air-dried and embedded in epoxy resin in a vacuum chamber for 5 min (Epothin 2TM, BuehlerTM). They were then cut on a tile saw and mounted on Plexiglass slides with cyanoacrylate glue. Thick sections were then ground by hand on a Buehler Metaserv grinder with silicon carbide paper of decreasing grit sizes (180, 320, 400, 600 and 800) and polished by hand with aluminium oxide powder (5 then 1 µm). Final slide thickness ranges between 100 and 150 µm. Slides were stained with a modified Masson's trichrome ([17]; save the deparaffinization steps). Slides were scanned on a V800 Epson scanner, observed with a microscope Olympus CX31 and photographed, using the software PixeLink µscope Essentials v. 2.3.

Table 1. List of specimens used in this study with their associated method for analysis.

specimen number	skull length (cm)/ontogenetic stage	method
MUVC-AL050	3.3 (hatchling)	paraffin histology
MUVC-AL052	3.3 (hatchling)	
MUVC-AL075	10 (juvenile)	
MUVC-AL039	30 (sexually mature)	un-decalcified histology
OUVC10606	2.9 (hatchling)	three-dimensional rendering/CT
MUVC-AL623	10.5 (juvenile)	
MUVC-AL721	26 (sexually mature)	
MUVC-AL805	approximately 20 (juvenile)	osteology
MUVC-AL806	approximately 20 (juvenile)	
MUVC-AL008	45.4 (skeletally mature)	

(b) Three-dimensional reconstructions and micro-computed tomography

Three-dimensional skeletal reconstructions were made using CT scans of three alligator heads that approximately matched the sizes of the heads samples via histology (table 1). Scans were conducted using a Siemens Inveon MicroCT (University of Missouri, 21 µm), a Siemens Somatom Definition scanner (University of Missouri, 600 µm) and a GE eXplore Locus *in vivo* Small Animal MicroCT scanner (Ohio University; 90 µm; data downloaded from [30]). Datasets were stacked and resliced using Avizo Lite to find slices that approximated equivalent histological slides, to show sectional, osteological morphology.

(c) Osteology

Osteological features of cranial joints in skeletonized, similar-sized *A. mississippiensis* specimens (table 1) were photographed under normal light in the University of Missouri Vertebrate Collections (MUVC) with either a Nikon D90 DLSR or Dino-Lite Edge.

3. Results

(a) Jaw joint

The jaw joint (quadrate–articular) is composed of the relatively flat, saddle-shaped quadrate articular surface and the deeply socketed, bicotylar fossa of the articular (figure 1*c–e*). Both these bones are endochondral and therefore are capped by smooth calcified cartilage that ends at clear tidelines along the periphery of the joint's articular surface. The surrounding bony tissue of the joint is marked by rugosities and discolorations on the distal quadrate and proximal articular that border a clear tideline marking the edge of the calcified cartilage and articular surfaces (following [28]; figure 1*c–e*). The elements also bear pronounced scars for the lateral collateral ligament on the lateral surface and intracapsular siphonial pneumatic diverticula on the caudal surface of the quadrate, all of which demarcate the joint capsular attachments from the surrounding muscular and integumentary domains of the bones (figure 1*c*). Despite the various structures that leave osteological correlates around the joint capsule of the jaw joint, correlates of the capsule itself are not uniform nor always clearly identifiable. Axial CT data show that during *Alligator* ontogeny, the jaw joint changes from a rather flat, incongruent bony articulation to a well-developed, saddle-shaped joint bounded by a large lateral flange of the surangular (figure 2*b,f,j*).

Histology confirms the synovial nature of the jaw joint in all three examined ontogenetic stages (figure 2*c,d,g,h,k,l*). The hatchling, juvenile and adult alligators all display the four criteria of a synovial joint: articular cartilage, a synovial cavity, a synovial membrane and a fibrous joint capsule (figure 2*d,h*). In the hatchling, three layers can be seen in the articular cartilage: a deep hypertrophic calcified cartilage layer, a proliferative zone with columnar orientation of the chondrocytes, and a thicker layer of hyaline cartilage (figure 2*d*). The quadrate also shows a fourth cartilaginous layer that appears more fibrous than the previous ones (figure 2*d*).

In the juvenile and adult specimens, the demarcations between the different types of cartilage are more evident: they still possess a deep layer of hypertrophic calcified cartilage, but no longer show any zone of proliferation (figure 2*h,l*). Instead there is a thin zone of hyaline cartilage, followed by a thicker zone of fibrocartilage (with elongated chondrocytes organized into rows; figure 2*h,l*). The quadrate shows a fourth layer of thick dense connective tissue (figure 2*h,l*), which might have originated from the fibrous layer described in the hatchling (figure 2*d*). This fibrous layer was also reported in the jaw joint of *Crocodylus porosus* [16].

(b) Otic joint

The otic joint is made of the quadrate and the squamosal, respectively, an endochondral and a membrane bone. Morphologically, the otic process of the quadrate is reduced, elongated and condylar (figure 1*f,g*). It articulates with a smooth and shallow cavity on the squamosal (figure 1*h*). The exposed calcified cartilage surface is smooth save small erosion pits revealing subchondral bone, and no clear fibrous capsule scar is discernible, particularly in younger individuals (figure 1*f,g*). Axial sections of CT data show the joint becomes more congruent during ontogeny, although the otic process assumes a flat articular head rather than a curved one, whereas the squamosal develops a shallow fossa to receive the quadrate (figure 3*b,f,j*).

Histological analyses show that the otic joint of the American alligator is not a synovial joint; indeed, in all three stages, it lacks a synovial cavity and a synovial membrane but has a fibrous connection melding with the epiphyseal cartilage of

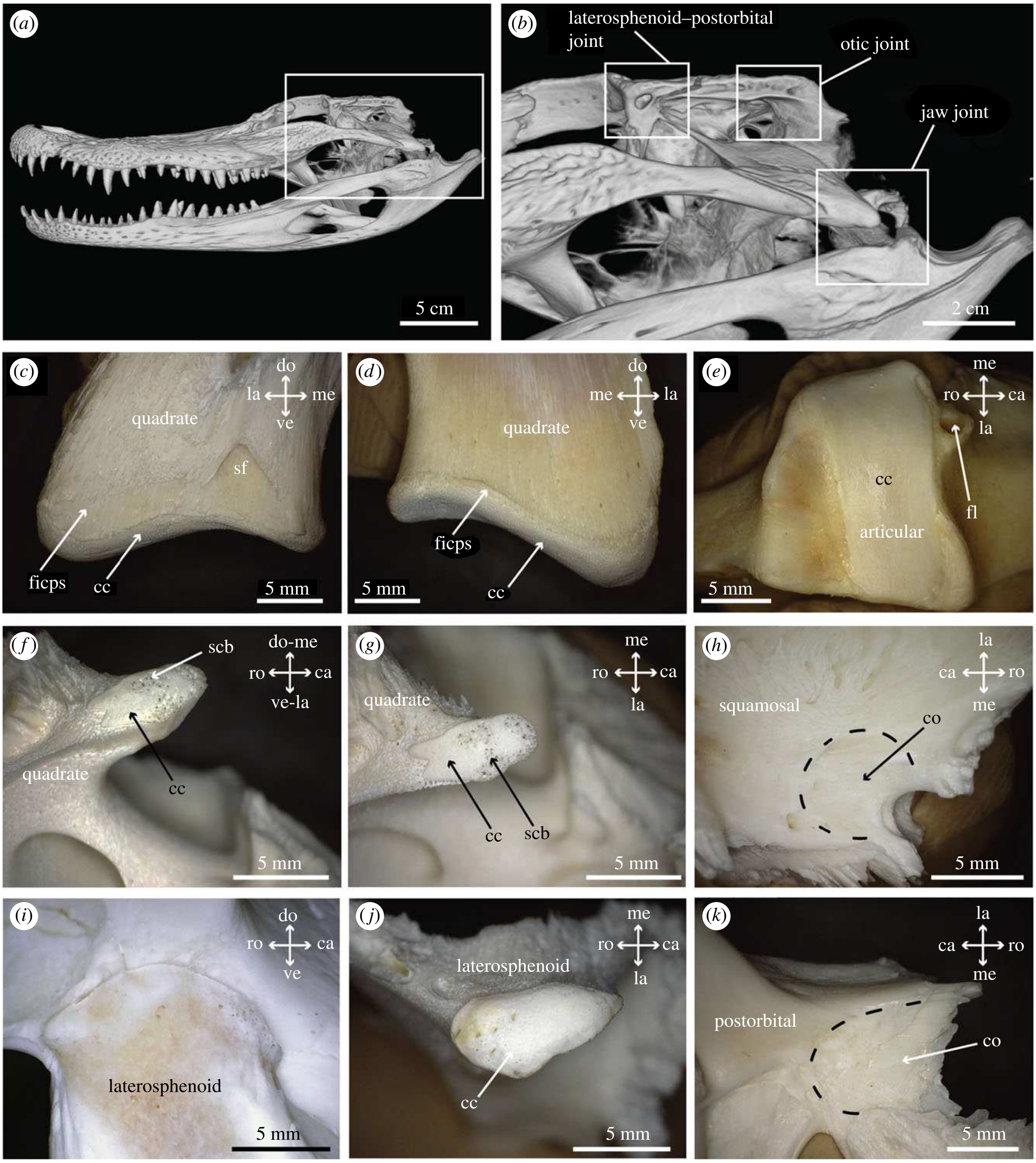

Figure 1. Three-dimensional micro-computed tomographic (μCT) reconstructions and osteological photographs of condylar cranial joints in American alligators. (*a*) Three-dimensional CT reconstruction in an adult alligator in left lateral view (*b*) close-up of the white box in (*a*) shows the three condylar joints of interest. (*c*) Caudal view of left quadrate shows osteological correlates of jaw joint capsule; (*d*) rostral view of left quadrate showing osteological correlates of jaw joint capsule; (*e*) dorsal view of left articular showing articular surface of jaw joint; (*f*) dorsolateral view of the left otic process of quadrate; (*g*) dorsal view of left otic process of quadrate showing the articular surface; (*h*) ventral view of left squamosal showing the shallow cotyle for the otic process; (*i*) ventrolateral view of left capitate process of laterosphenoid showing articulation with postorbital; (*j*) dorsal view of left capitate process showing the articular surface of laterosphenoid; (*k*) ventral view of the left postorbital shows the shallow cotyle of the LS–PO joint. Specimen numbers: MUVC-AL721 in (*a*,*b*); MUVC-AL806 in (*c*–*h*) and in (*k*); MUVC-AL008 in (*i*); MUVC-AL 805 in (*j*). ca, caudal; cc, calcified cartilage; co, cotyle; do, dorsal; ficps, fibrous capsule scar; fl; fulcrum; la, lateral; me, medial; ro, rostral; scb, subchondral bone; sf, siphonium foramen; ve, ventral.

the quadrate and surface of the squamosal thus bridging the two bony elements (figure 3*d*,*h*,*l*). In the hatchling, the otic process of the quadrate possesses a cartilage cone with a layer of hypertrophic calcified cartilage, a proliferative zone and a layer of hyaline cartilage (figure 3*d*). This superficial layer blends into a thin layer of loose connective tissues, directly in contact with the squamosal bone (figure 3*d*). In the juvenile, these three cartilaginous layers can also be seen, but the superficial hyaline cartilage of the hatchlings appears to have turned into a fibrocartilage layer (figure 3*h*). In the adult, only two cartilaginous layers are visible: a calcified layer and fibrocartilage (figure 3*l*). The fibrous layer in

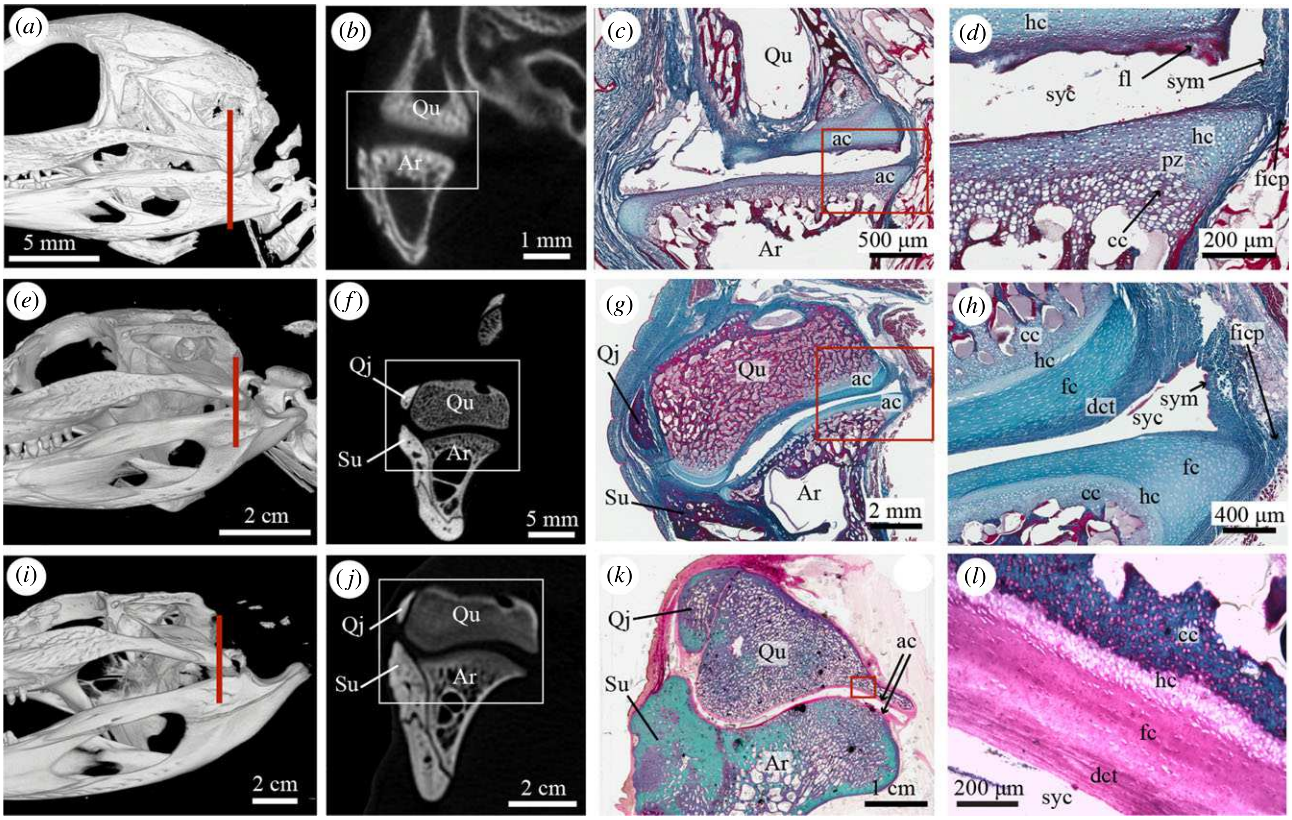

Figure 2. Micro-computed tomography and histology of the synovial jaw joint of hatchling (*a–d*), juvenile (*e–h*) and adult American alligator (*i–l*) demonstrate articular cartilage-covered joint surfaces and a synovial cavity bounded by a fibrous joint capsule. Three-dimensional CT reconstructions are shown in a hatchling (*a*), juvenile (*e*) and an adult alligator (*i*). Red lines indicate plane of CT and histological sections in (*b,f* and *j*), respectively. Associated thin-sections from the white boxes in (*b,f* and *j*) are shown, respectively, in (*c,g* and *k*) followed by higher magnifications in (*d,h,l*), respectively. Specimen numbers: OUVC10606 in (*a,b*), MUVC-AL050 in (*c,d*), MUVC-AL623 in (*e,f*), MUVC-AL075 in (*g,h*), MUVC-AL721 in (*i,j*), MUVC-AL039 in (*k,l*). ac, articular cartilage; Ar, articular; cc, calcified cartilage; dct; dense connective tissue; fc, fibrocartilage; ficp, fibrous capsule; fl, fibrous layer; hc, hyaline cartilage; pz, proliferative zone; Qu, quadrate; Qj, quadratojugal; Su, surangular; syc, synovial cavity; sym, synovial membrane.

contact with the squamosal gets denser through ontogeny (figure 3*h,l*).

This joint organization is similar to that reported in the 'syndesmodial' otic joint (between quadrate and squamosal and otoccipital) in lepidosaurs [31,32] but is very different from the clear synovial characteristics of the otic joint of birds (e.g. in the chick or mallard ducks; [17,18]). The presence of a cartilage cap on the quadrate but dense connective tissue on the opposing side of the joint could be classified as a mix of a syndesmosis and synchondrosis depending on the investigator's frame of reference. As in lepidosaurs, but unlike the squamosal portion of the otic joint of birds [17], the squamosal in *Alligator* did not possess secondary cartilage (i.e. cartilage that arises from the periosteal stem cells of membrane bones under mechanical stimuli, [33]), nor did it possess any clear periosteum near the joint.

(c) Laterosphenoid–postorbital joint

This joint is largely comprised the laterosphenoid (figure 1*i,j*) and the postorbital (figure 1*k*), respectively, an endochondral and a membrane bone. A narrow process of the quadrate and quadratojugal also extend rostrodorsally to variably articulate with these two elements. Morphologically, the pyramidal laterosphenoid extends the head of its capitate process into a shallow cavity on the ventral surface of the postorbital (figure 1*i–k*; [3]). As in the otic process of the quadrate, the surface of the capitate process of the laterosphenoid is capped by smooth calcified cartilage, and no clear fibrous capsule scar is discernible, although nearby bony signatures of muscle attachments and larger fibrous attachments are present (figure 1*i,j*). Axial CT data of the LS–PO joint show the laterosphenoid capitate process begins as a rather flat structure that develops into a rounded, condylar head, whereas the overlying postorbital cotyle becomes more concave (figure 4*b,f,j*).

Histology shows that the LS–PO joint is not synovial because it lacks a synovial cavity and a synovial membrane in all three ontogenetic stages (figure 4). Similar to the fibrous capsule of the otic joint, layers of fibrous tissues connect the epiphysis of the capitate process with the overlying connective tissue of the postorbital (figure 4). In the hatchling, the organization of epiphyseal cartilage is similar to that described for the otic joint, except that the outermost hyaline cartilage layer possesses fusiform chondroblasts (figure 4*d*). The organization of the joint in the juvenile is also virtually identical to that described for the otic joint of that same stage, however, the outermost fibrocartilaginous layer is relatively thicker (figure 4*h*). In the adult, thin layers of calcified cartilage, fibrocartilage and dense fibrous connective tissues are visible. No secondary cartilage was found on the postorbital. These data show that the common assumption that this joint is synovial in all archosaurs is incorrect, instead it is a combination of a syndesmosis and a synchondrosis in American alligators.

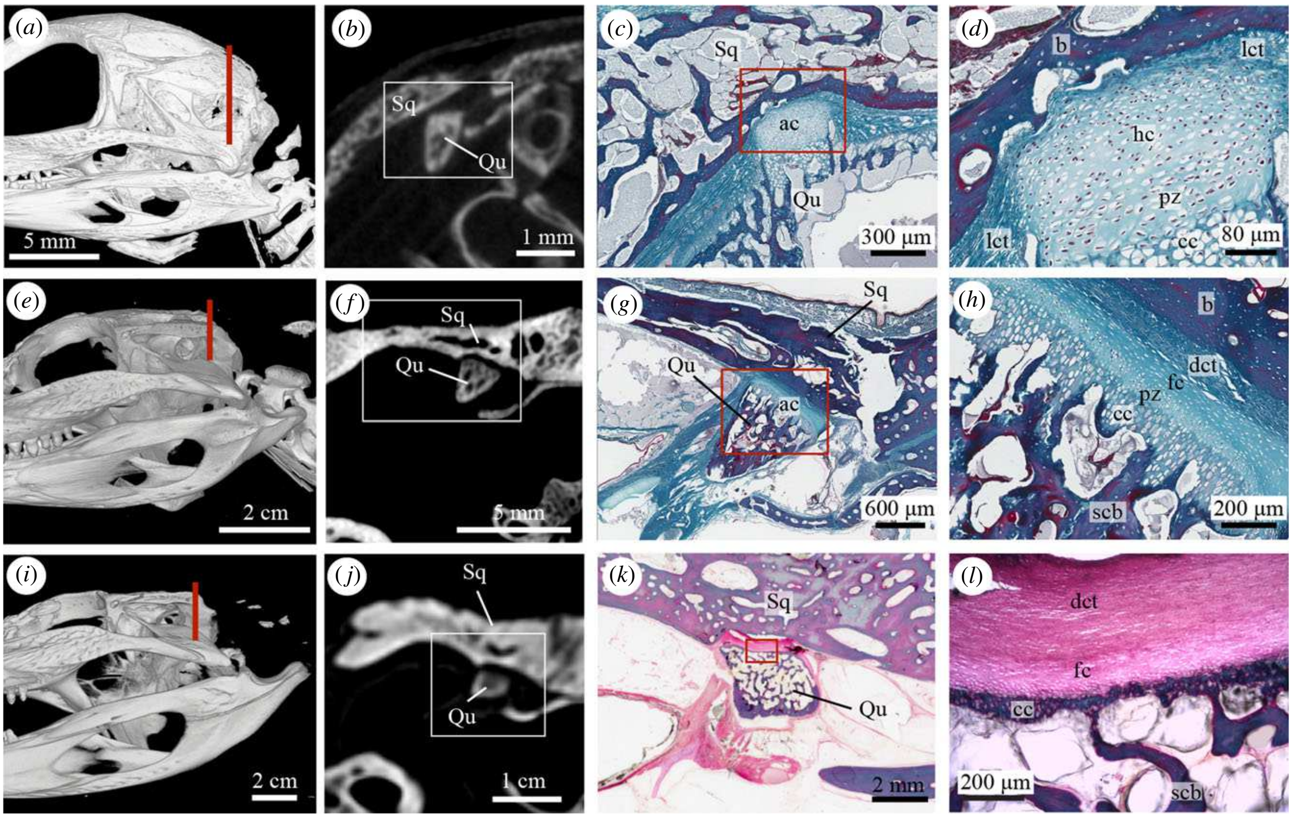

Figure 3. Micro-computed tomography and histology of the non-synovial otic joint in hatchling (*a–d*), juvenile (*e–h*) and adult American alligator (*i–l*) demonstrate the unique morphology of the articulation between the condylar otic process of the quadrate capped with primary articular cartilage, and the fibre-filled cotyle of the squamosal. Three-dimensional CT reconstructions are shown in a hatchling (*a*), juvenile (*e*) and an adult alligator (*i*). Red lines indicate planes of CT and histological sections in (*b*,*f* and *j*), and (*c*,*g* and *k*), respectively, followed by higher magnifications in (*d*, *h*, and *l*). Specimen numbers: OUVC10606 in (*a*,*b*), MUVC-AL050 in (*c*,*d*), MUVC-AL623 in (*e*,*f*), MUVC-AL075 in (*g*,*h*), MUVC-AL721 in (*i*,*j*), MUVC-AL039 in (*k*,*l*). ac, articular cartilage; b, bone; cc, calcified cartilage; dct; dense connective tissue; fc, fibrocartilage; hc, hyaline cartilage; lct, loose connective tissue; pz, proliferative zone; Qu, quadrate; scb, subchondral bone; Sq, squamosal.

4. Discussion

(a) Implications for functional inferences of cranial kinesis in fossil archosaurs

Archosaurs and other reptiles have seemingly more complicated articulations among their cranial bones compared with those reported in mammals. Mammalian bias creeps into our understanding of reptile condylar joints, where it is assumed that all of them are synovial, as are the similarly shaped, largely appendicular joints of mammals. Such morphological assumptions then errantly support inferences of function, such as cranial kinesis in the fossil record (e.g. at the otic joint of many dinosaurian clades; see [3] for a review).

Among all the presumably synovial joints investigated here in the American alligator (figure 1), only the jaw joint was truly synovial and displayed the four histological criteria described in the Introduction (figure 2), whereas both the otic and LS–PO joints lacked a synovial cavity (figures 3 and 4). These results have important implications for functional and structural inferences of cranial kinesis in non-avian dinosaurs because they show that an articular surface presenting a condylar morphology is not necessarily synovial, nor is it necessarily kinetic since the otic and the LS–PO joints are both presumably immobile in *Alligator*. Our findings suggest that osteological and morphological examination of skull bones in fossil taxa that are not accompanied by microstructural analyses (i.e. histology and/or high-resolution µCT scanning) may not provide adequate data to accurately infer the presence of a synovial joint and thus, inferences of mobility and function. Of course, osteological examination is standard, invaluable practice in vertebrate morphology and despite the shortcomings of µCT imaging only offering details of mineralized tissues at often thicker slices than histology, these three methods remain complementary and each modality offers unique insights into the biology of skeletal tissues. New advances, including easier access to higher-resolution X-ray tomography, synchrotron tomography and new contrast agents for soft tissues (e.g. DiceCT [34]) will help advance our understanding of cranial joint structure and evolution.

Non-synovial, fibrous joints may still be mobile (such as mandibular symphyses; [5]), but arguably in a much more limited context compared with synovial joints. Similar to findings of variation in muscular osteological correlates [35], we show that osteological correlates of joints should also be used with caution, as the osteological correlates of the jaw joint capsule (i.e. the only true synovial joint of our sample) were not always uniform nor always clearly identifiable (figure 1). Regardless, our histological and morphological data on the condylar joints in the head of *A. mississippiensis* complicate inferences of joint function and structure in the skulls of fossil archosaurs. Moreover, when incorporated with joints of modern birds [17], these data can help trace the evolution of joint structure, cranial kinesis and novel joint formation within archosaurs.

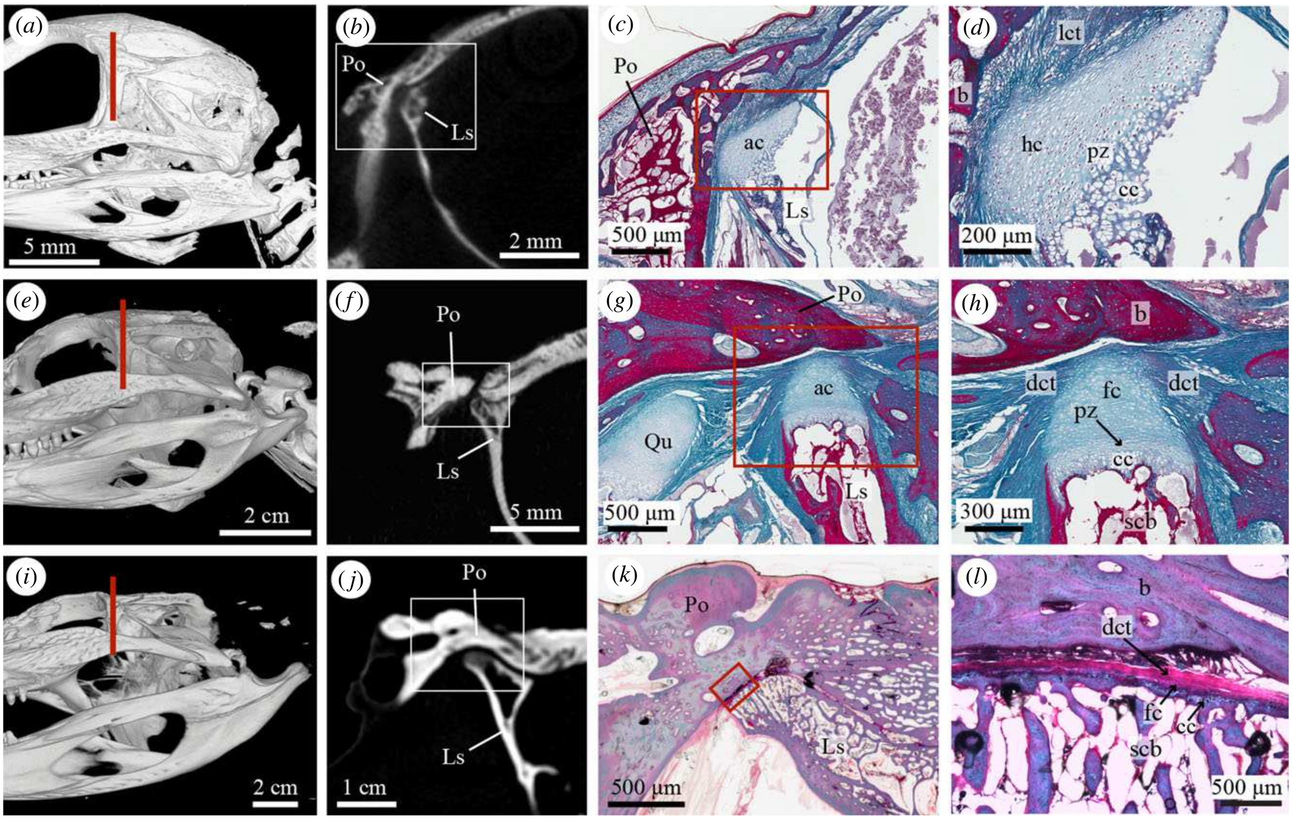

Figure 4. Micro-computed tomography and histology of the non-synovial laterosphenoid–postorbital joint in hatchling (*a*–*d*), juvenile (*e*–*h*) and adult American alligator (*i*–*l*) demonstrate the unique morphology of the articulation between the condylar capitate process of the laterosphenoid capped by primary articular cartilage, and the fibre-filled cotyle of the postorbital. Three-dimensional CT reconstructions are shown in hatchling (*a*), juvenile (*e*) and an adult alligator (*i*). Red lines indicate planes of CT and histological sections in (*b*,*f* and *j*), and (*c*,*g* and *k*), respectively, followed by higher magnifications in (*d*,*h* and *l*). Specimen numbers: OUVC10606 in (*a*,*b*), MUVC-AL050 in (*c*,*d*), MUVC-AL623 in (*e*,*f*), MUVC-AL075 in (*g*,*h*), MUVC-AL721 in (*i*,*j*), MUVC-AL039 in (*k*,*l*). ac, articular cartilage; b, bone; cc; calcified cartilage; dct; dense connective tissue; fc, fibrocartilage; hc, hyaline cartilage; lct, loose connective tissue; Ls, laterosphenoid; pz, proliferative zone; Po, postorbital; Qu, quadrate; scb, subchondral bone.

(b) Insights into the Archosaurian otic joint and the evolution of avian cranial kinesis

Histological analysis of the otic joint of the American alligator provides important insights into its evolution within Archosauria. In most birds, this joint is considered highly mobile and histological examination in some avian species revealed that it is constructed with a synovial cavity and secondary articular cartilage on the squamosal [17–19,36], two structures that are absent from this same joint in the American alligator (figure 3). Results presented here in the latter species are surprising, because the otic joint has been assumed and hypothesized to be apomorphically synovial in some lepidosaurs [6] and plesiomorphically synovial in archosaurs, or even in all diapsids [3]. However, more recent histological data on this joint in gekkottan and lacertids showed that it is in fact a syndesmosis, at least in these taxa [31,32], and all of this suggests that having a kinetic, synovial otic joint might actually be an apomorphy of birds, and perhaps of non-avian dinosaurs. Many more data are necessary to test this evolutionary hypothesis, such as histological analyses of this joint in chelonians, and fossil archosaurs and archosauriformes, including non-avian dinosaurs. Without additional data, a synovial otic joint in basal archosaurs, followed by a loss of synovial cavity in pseudosuchia (i.e. a reversal) cannot be ruled out.

(c) The absence of secondary cartilage in *Alligator* and novel joint formation in extant archosaurs

Secondary articular cartilage was not found on the squamosal nor postorbital of the American alligator (figures 3 and 4). It remains unclear whether this absence in extant crocodilians is due to (i) phylogeny [37], (ii) the disappearance of the periosteum (because no clear periosteum along the squamosal or postorbital could be identified; also see [38]), (iii) the absence of a proper mechanical environment triggering the differentiation of periosteal stem cells into chondroblasts rather than osteoblasts (see [33,39,40]) or (iv) a combination of some or all of those factors. The ability or inability to form secondary articular cartilage on membrane bones within extant archosaurs is important to discuss, because when novel kinetic articulations evolve in birds (e.g. the palatobasal joint or the craniofacial hinge of anseriforms), these novel joints are accompanied by the formation of secondary cartilage and a synovial cavity [17]. We recently proposed that secondary cartilage played an important role in the evolution of avian cranial kinesis, by allowing the formation of kinetic synovial joints not only within chondrocranial elements, but also within the dermatocranium, as well as mediating novel, derived articulations between elements (i.e. 'secondary articulations' *sensu* [17,41]). In extant crocodylians, when novel articulations appear, such as the pterygomandibular joint (a putative second jaw joint, [42]), they do not form secondary cartilage but are instead covered by a thick pad of dense irregular connective tissue [42], identical to the connective tissue layers described here on the quadrate (figure 2*h*,*l*). These tissues, although not cartilaginous, perhaps act similarly to cushioning articular cartilages. These data suggest that the two clades of extant archosaurs form novel articulations in very different ways, making it challenging to infer accurate joint tissue organization in non-avian dinosaurs. Lastly, these findings show how important it is to examine the cranial joints of fossils not only at the osteological level, but also with histological and/or other high-definition microstructural techniques.

5. Conclusion

— The otic and LS–PO joints have been assumed to be synovial in extant crocodylians based on their elongated, condylar morphology. Histological examination reveals that they are not synovial, but instead show a combination of a fibrous syndesmosis and a cartilaginous synchondrosis. This means that a condylar morphology does not necessarily indicate the presence of a synovial joint, nor does it imply kinesis. This is an important finding for evolutionary biologists and palaeontologists that focus on structural and functional inferences of kinesis in fossil taxa.

— This finding also means that osteological data alone are not necessarily enough to fully identify joint structure or to make subsequent functional inferences of cranial kinesis in fossil archosaurs. Instead, morphological observations should be paired with microstructural analyses, such as histology or higher-resolution μCT and synchrotron imaging when the former technique is not an option. If framed within an adequate phylogenetic framework, solid hypotheses and inferences of cranial kinesis in fossils can still be made, but microstructural analyses are invaluable to accurately identify specific tissue types and make functional inferences of cranial kinesis in fossilized or osteological remains.

— Our histological data from the non-synovial otic joint of the American alligator suggest that a synovial otic joint may be apomorphic for birds, if not also some still unclear, more inclusive clade of non-avian dinosaurs. However, it equally suggests that synovial otic joints could have been lost within pseudosuchian evolution, prior to the origin of crown-group crocodylians.

— Lastly, the comparisons of articulations in crocodylians and birds suggest that these two groups forming the extant phylogenetic bracket of non-avian dinosaurs build novel, kinetic cranial joints in different ways, with different tissues: secondary cartilage for birds and thick layers of dense connective tissues for crocodylians. These clade-specific differences give insights into the evolution of kinesis among archosaurs, and avian cranial kinesis among their dinosaurian ancestors.

Data accessibility. The data supporting the conclusions of this manuscript are included within the article. CT data are publically available from the Dryad Digital Repository at: http://dx.doi.org/10.5061/dryad.s56rq [43] for MUVC AL623 and MUVC AL721; http://dx.doi.org/10.5061/dryad.7h853 [44] for OUVC10606.

Authors' contributions. A.M.B., C.M.H. conceived the project; A.M.B., C.M.H. collected the data; A.M.B., C.M.H. wrote the manuscript. All authors read and approved the final manuscript.

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