

Early evolution of the biological bird: perspectives from new fossil discoveries in China

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Abstract New discoveries of fossil birds belonging to the Jehol Biota uncovered from Lower Cretaceous lacustrine deposits in northeastern China continue to greatly enrich our understanding of the first major avian radiation. The exceptional preservation of some fossils provides a rare chance to discuss many biological issues that are usually impossible to address in paleontological studies, such as: the ossification pattern of the sternum in the extinct group *Enantiornithes*, which is unlike that of modern birds and all other *archosaurs*; the discovery of preserved crop, gizzard, and intestinal contents in several clades which suggest that a near-modern digestive tract including specialized crop morphologies evolved early during avian evolution; and the rare preservation of ovarian follicles which support hypotheses that the right ovary was lost in Aves due to the limitations of powered flight. Together, these data allow a partial reconstruction of the biology of Aves very close to its origin. While no skeletal or integumentary features are recognized to define Aves, we identify two possible soft tissue features that may biologically define Aves relative to other amniotes: the presence of a crop and the loss of the right ovary.

Keywords Jehol Biota · Mesozoic birds · Avian biology · Definition of Aves

Introduction

Our understanding of the early evolution of Aves has grown exponentially over the past decades mostly as a result of the spectacular discoveries that have steadily poured out of China since the late 1980s (Hou et al. 1995; Zhou et al. 1992, 2003; Zhou and Hou 2002; Zhou and Zhang 2002, 2006b). The Lower Cretaceous Jehol Biota preserves the second oldest definitive avian fossils after *Archaeopteryx* and also the most diverse Cretaceous avifauna known to science (Zhou 2006; Zhou and Zhang 2006b). Every Early Cretaceous avian clade is represented: the Jeholornithiformes, a clade of long boney-tailed birds only more derived than *Archaeopteryx*; the two basal pygostylian clades (birds whose abbreviated boney tail ends in a compound element, the pygostyle) Sapeornithiformes and Confuciusornithiformes; and the oldest record of the two ornithothoracine clades, Enantiornithes and Ornithuromorpha (Zhou and Zhang 2006b). Neornithes, which includes all living birds, is nested within the latter clade (Chiappe 1995). Jeholornithiformes and the two pygostylian clades have only been collected in the Jehol Biota and are potentially endemic (Zhou and Zhang 2006b). The Jehol Biota preserves the greatest diversity of enantiornithines and ornithuromorphs of any avifauna, accounting for nearly half of all recognized Cretaceous species (O'Connor et al. 2011a; Zhou and Zhang 2006b).

Thousands of largely complete and articulated specimens have been unearthed, revealing unprecedented data on the skeletal morphology and diversity of basal birds, yet these data have failed to reveal a pattern in the acquisition of derived 'avian' skeletal features. Instead, the early evolution of birds has often been described as mosaic: confuciusornithiforms (including *Confuciusornis* and *Eoconfuciusornis*) represent the oldest beaked avian clade but

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have a diapsid skull with a fully formed postorbital bar (Chiappe et al. 1999; Zhang et al. 2008a) whereas the postorbital is reduced in *Archaeopteryx*; *Sapeornis*, like *Archaeopteryx*, has no sternum despite being the largest Early Cretaceous bird and the presence of well-developed sterna in all other avian clades (Zheng et al. 2014b); and *Jeholornis* has a boney-tail longer than that of *Archaeopteryx* in both overall length and number of caudal vertebrae (Zhou and Zhang 2003). Atypical of most fossils, specimens from the Jehol Biota commonly preserve feathers (Zhang et al. 2006) and in some cases also the melanosomes responsible for their color (Zhang et al. 2010); more rarely, other soft tissues such as ovarian follicles are preserved (Zheng et al. 2013). Ingested contents are found preserved in nearly every segment of the alimentary canal (Zheng et al. 2014a; Zhou et al. 2004). The exceptional preservation of numerous young juvenile enantiornithines and even one embryo reveal aspects of development such as strategy and ossification patterns (Chiappe et al. 2007; Zhou and Zhang 2004). Together, these data allow a partial reconstruction of the biology of basal birds.

The Jehol Biota has also produced basal members of derived maniraptoran clades considered to be close relatives of birds such as the oviraptorosaur *Caudipteryx*, the dromaeosaurid *Microraptor*, and the troodontid *Mei* (Ji et al. 1998; Xu and Norell 2004; Xu et al. 2003). Most phylogenetic analyses consider Deinonychosauria, the clade formed by Troodontidae and Dromaeosauridae, to form the sister taxon to Aves; together with the Scansoriopterygidae, these taxa form Paraves (Turner et al. 2012; Xu et al. 2010a). The sister group to Paraves, Oviraptorosauria, has been considered in the past by some to be more closely related to Aves, and several authors have considered this group to be flightless birds (Elzanowski 1999; Lü et al. 2002; Maryanska et al. 2002). The enigmatic Middle to Late Jurassic Scansoriopterygidae has been resolved as a basal avian clade (Zhang et al. 2008b), the sister group to Aves (together forming Avialae) (Xu et al. 2010a), and a primitive lineage of oviraptorosaurs (O'Connor and Sullivan 2014). Although there is no agreement on the phylogenetic position of this taxon, which is obfuscated by the poor preservation of the entirely juvenile and subadult described material (O'Connor and Sullivan 2014), hypotheses all place this taxon in a fairly derived position in the maniraptoran tree. Only one possible scansoriopterygid has been named from the Jehol Biota, *Zhongornis haoae*, which was originally described as a basal bird (Gao et al. 2008; O'Connor and Sullivan 2014). Representatives of these clades from the Jehol Biota also preserve rare indicators of biology such as feathers, stomach contents, soft tissues, and behaviour, helping to narrow the phylogenetic bracket for the origin of many

derived avian biological features by elucidating the condition in the closest relatives to birds.

Aves is the clade formed by common ancestor of *Archaeopteryx* and Neornithes (crown group birds). Some researchers prefer to use Avialae (=Aves) and Aves (=Neornithes) (Gauthier, 1986); however, we find this phylocode terminology does not clarify phylogenetic issues as was originally proposed (Nixon et al. 2003), and, since basal forms like *Confuciusornis* and *Enantiornithes* are still clearly birds (L. Aves; causing particular confusion in Latin languages), we find the classical terminology used here preferable. Currently, Aves is without a character-based definition; the last notable attempt—more than half a century ago—employed three skeletal features (the presence of a furcula, retroverted pubes, and a reversed hallux) and the presence of feathers (de Beer 1954). However, these features no longer define Aves, being either present in non-avian dinosaurs (furcula, feathers) or absent in basal-most birds (retroverted pubis, reversed hallux) (Mayr et al. 2005; Witmer 2002). Living birds are highly modified compared to other amniotes with numerous biological features that make them unique among extant animals (Gill 2007). Therefore, it may be possible to use some of these differences in order to define Aves. The Jehol Biota provides data regarding the biology of dinosaurs across the avian transition, revealing when some derived 'avian' traits arose in the maniraptoran lineage. First, the relevant biological information from derived maniraptorans is summarized. Then, recent breakthroughs in our understanding of the biology of basal birds are reviewed in three regards: development of the sternum; the alimentary tract; and reproduction. We synthesize this information in order to elucidate the plesiomorphic avian condition and put forward the first attempt at a potential biological definition for Aves.

Institutional abbreviations: IVPP, Institute of Vertebrate Paleontology and Paleoanthropology, Beijing, China; STM, Shandong Tianyu Museum of Nature, Pingyi, China.

Biology of derived Jehol maniraptorans

Oviraptorosaurs from the Jehol Biota reveal that, at this point in evolution, theropods experienced ontogenetic changes in their plumage, similar to living birds (Xu et al. 2010b). Gizzard stones are preserved in several specimens of *Caudipteryx* indicating that oviraptorosaurs had a two-part stomach and a ventriculus specialized for grinding (Ji et al. 1998; Zhou et al. 2000). Deinonychosaurians from the Jehol Biota do not preserve gastroliths, although several specimens of *Microraptor* preserve stomach contents including the remains of an enantiornithine bird, mammals, and fish; such a varied diet suggests this taxon was an

opportunistic predator (O'Connor et al. 2011b; Xing et al. 2013). Two specimens of the Jehol troodontid *Mei long* are preserved in an avian style sleeping posture (Gao et al. 2012; Xu and Norell 2004) and, like *Archaeopteryx*, members of this clade do not have a sternum (Zheng et al. 2014b). Based on the largest published dataset of any single dinosaur taxon (*Anchiornis*, $n = 229$), troodontids are also considered to lack a cartilaginous sternum (O'Connor et al. 2014b; Zheng et al. 2014b). Although most dromaeosaurids appear to have paired medially unfused sternal plates (e.g., *Sinornithosaurus*) (Norell and Makovicky 2004), the median suture is fully closed in adult specimens of *Microraptor gui* forming a true sternum (Xu et al. 2003). Although Jehol oviraptorosaurs have medially unfused sternal plates without a well-developed medial articulation (e.g., *Caudipteryx*) (Zhou and Wang 2000), a medially fused sternum is also present in at least one Late Cretaceous oviraptorid (*Ajancingenia*) (Osmólska et al. 2004).

Basal bird biology

Sternal development: Enantiornithes

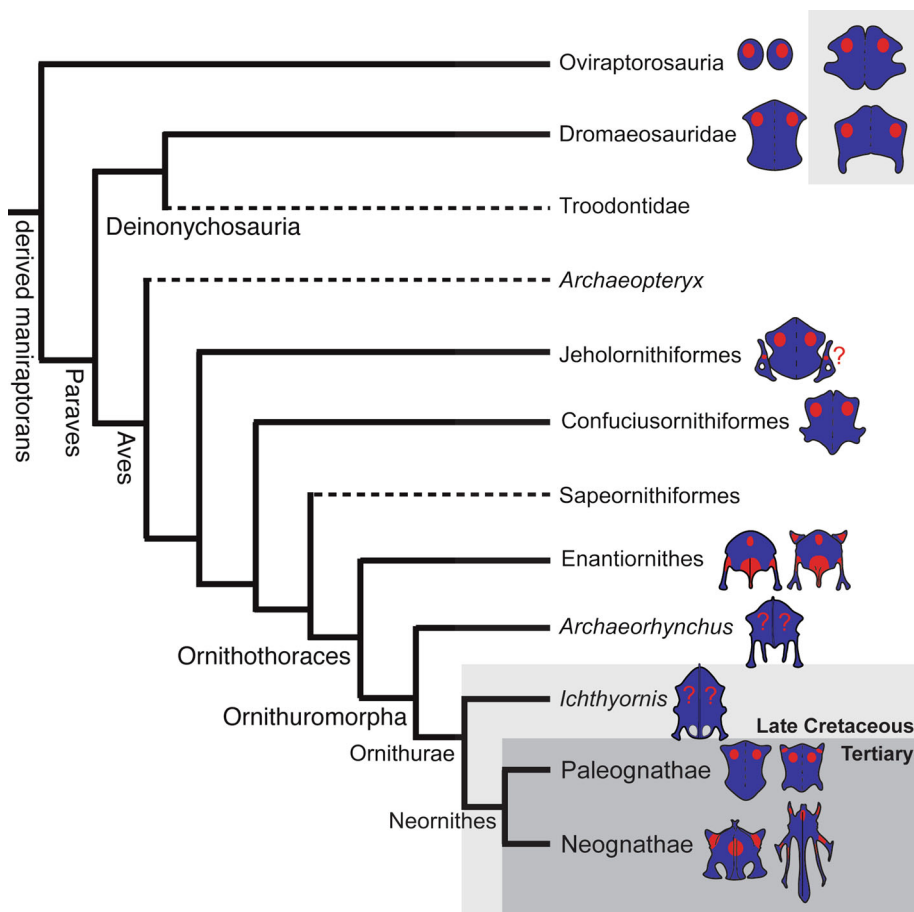
Enantiornithines are inferred to have a developmental strategy that is super precocial based on the discoveries of well ossified hatchlings and late stage embryos with rectrices that suggest enantiornithines were volant immediately or soon after hatching (Chinsamy and Elzanowski 2001; Zhou and Zhang 2004); the only super-precocial living birds are the primarily ground-dwelling megapodes, whereas enantiornithines were considered volant arboreal birds. Locomotor activity is known to slow growth and flight is the most physically demanding form of locomotion (Gill 2007; Starck and Ricklefs 1998), thus slow growth would be predicted in the Enantiornithes. This is confirmed through osteohistological analysis and further supported by the numerous young juvenile specimens that have been collected in the Jehol Group, which together hint at a very protracted juvenile period of ontogeny (Chiappe et al. 2007; Chinsamy et al. 1995; O'Connor et al. 2014a). These juveniles preserve the ossification sequences of compound elements such as the carpometacarpus, tarsometatarsus and sternum (Chiappe et al. 2007). In particular, the sternum reveals a pattern unique among archosaurs, consisting primarily of a caudal median ossification centre with additional contribution from a proximal median centre and a bilateral pair that forms the lateral trabeculae (Zheng et al. 2012). This pattern is very different from the typical archosaur condition in which the sternum forms from a bilateral pair of elements that medially fuse in the adult phenotype of some derived maniraptorans (oviraptorosaur

Ajancingenia, dromaeosaurid *Microraptor*) and basal birds (*Confuciusornis*, *Jeholornis*) (Zheng et al. 2012) (Fig. 1). The sternum is one of the last elements to ossify (Starck 1993) and a midline suture persists until late in ontogeny in basal birds (*Confuciusornis*, *Jeholornis*) and the extant flightless ratites (Von Blötzheim 1958; Zheng et al. 2012). The median pattern of ossification observed in enantiornithines may have served to reinforce the sternal midline at an earlier ontogenetic stage, helping to resist compression forces induced by volant activity in young juveniles during their protracted ontogeny.

Alimentary tract

A large number of specimens preserving ingested items allow for a partial reconstruction of the alimentary tract. These specimens further represent a good portion of the known phylogeny, thus revealing evolutionary trends (Fig. 2). The holotype specimen of *Jeholornis prima* preserves a large number of seeds in the stomach, which lead authors to infer the presence of a large crop (Zhou and Zhang 2002). Several specimens of *Sapeornis* preserve seeds in a ventrally located crop as well as gizzard stones in the ventriculus (Zheng et al. 2011). This is consistent with observations from living herbivorous birds in which the crop forms a distinct ventrally located pouch located just proximocranial to the thoracic girdle (Gill 2007). The Jehol ornithuromorphs preserve the greatest wealth of direct evidence: numerous specimens of *Yanornis* have been collected with fish bones in the crop and ventriculus and one specimen preserves sand impacted in the intestines (Zheng et al. 2014a); *Piscivoravis* has macerated fish bones in the ventriculus (Zhou et al. 2013a); several specimens of *Archaeorhynchus* preserve a large cluster of small gizzard stones (Zhou et al. 2013b; Zhou and Zhang 2006a); several specimens of *Iteravis* have a small number of large gizzard stones (Zhou et al. 2014); and *Hongshanornis* preserves a seed-filled crop and gizzard stones in the ventriculus (Zheng et al. 2011). These specimens together indicate the presence of an essentially modern alimentary tract with a specialized crop diversified into several morphologies and a two-part stomach with the ventriculus adapted for grinding (Zheng et al. 2014a). Differences in crop and gizzard stone morphology indicate ornithuromorphs had specialized their digestive tract to handle a diversity of diets. Furthermore, specimens of *Yanornis* preserve both whole fish and macerated fish bones in the crop indicating that peristalsis—the complex system of muscle contractions responsible for bidirectional movement between the oesophagus and stomachs—was already in place (Zheng et al. 2014a). This increases the flexibility of the avian digestive system, allowing birds to regurgitate hard-to-

Fig. 1 Simplified cladogram of derived maniraptoran relationships depicting relative sternal ossification patterns, which indicate there are no shared sternal features at the base of the avian clade. *Dashed line* indicates where sternal plates are absent. *Light grey* indicates Late Cretaceous; *darker grey* indicates Tertiary. [Color for online/pdf only]



digest items (in the form of pellets), and further increasing the efficiency of the system by decreasing weight and overall gut residency times.

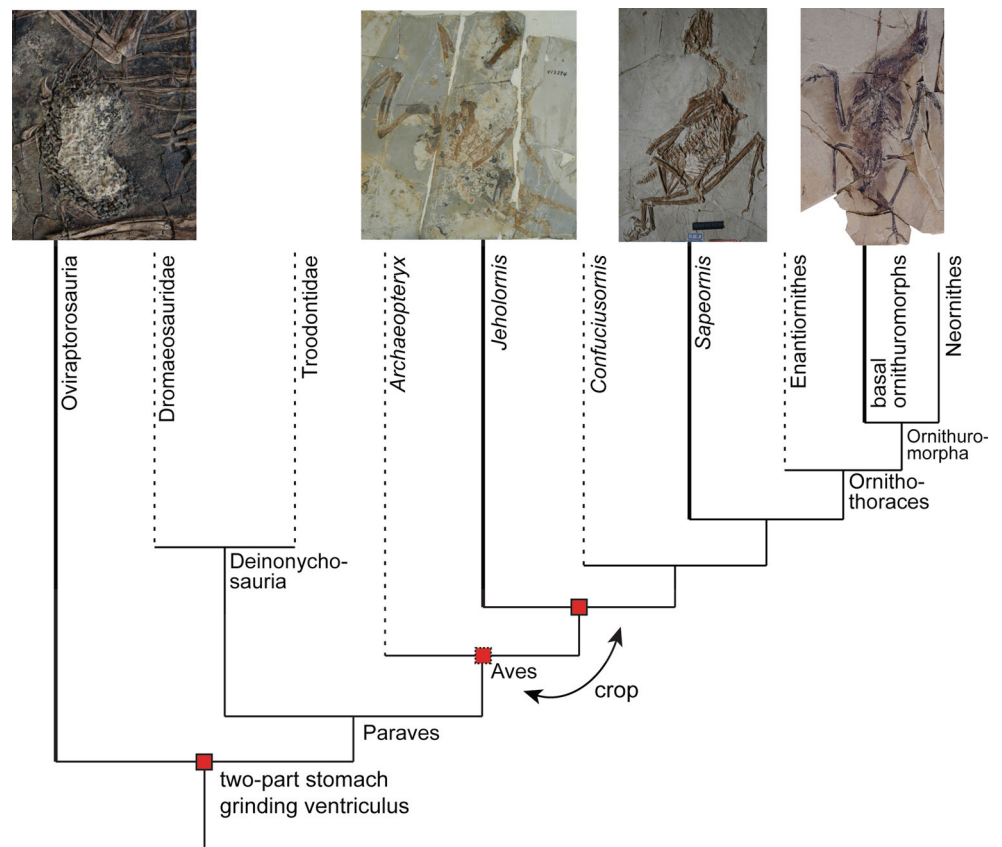
Confuciusornithiformes and Enantiornithes are completely lacking in regards to direct evidence of their diet. One specimen of *Confuciusornis* preserves a small cluster of fish bones near the neck that have been interpreted as a pellet (Dalsätt et al. 2006); however, the singularity of this evidence in light of the over 1000 known specimens suggest that this interpretation may be incorrect. One enantiornithine specimen preserves a few small stones in the abdomen, interpreted as rangle—stones ingested by predatory birds to help clean their alimentary tract (Li et al. 2014); again, support for this interpretation is lacking and the only clues of diet in Jehol enantiornithines are dental and rostral morphology (O'Connor and Chiappe 2011). Tooth morphology in particular is diverse and suggests a range of food items; however, the absence of evidence for a crop or two-part stomach is perplexing given that these features are clearly present in *Sapeornis* and ornithuromorphs and enantiornithines fall within this phylogenetic bracket. This may suggest major trophic partitioning between Jehol clades. Notably, despite their modern alimentary tract ornithuromorphs retain teeth—in fact,

Yanornis martini has the most hypertrophied dentition of any bird in the Jehol fauna. However, birds with a ventriculus adapted for grinding and an herbivorous diet do show trends in tooth reduction (e.g., *Sapeornis*, *Hongshanornis*, *Archaeorhynchus*, *Iteravis*) (Zheng et al. 2011; Zhou et al. 2014).

Reproductive system

Several Jehol birds preserve what appears to be the soft tissue impression of mature ovarian follicles (O'Connor et al. 2013a; Zheng et al. 2013). Although a controversial interpretation, no feasible alternative or well-supported counter-argument has been proposed (Mayr and Manegold 2013; O'Connor et al. 2013b). A total of seven specimens have been described preserving follicles: one *Jeholornis* and six enantiornithines. These specimens are all primarily in dorsal or ventral view and preserve the cluster of follicles entirely on the left side of the body, consistent with living birds in which the right ovary has been lost (Zheng et al. 2013). Follicles are proportionately larger in enantiornithines compared to *Jeholornis*, which in turn has a greater number of follicles (Fig. 3). The enantiornithine

Fig. 2 Relationships between specimens preserving evidence of the morphology of the alimentary canal: *Caudipteryx* (Oviraptorosauria) IVPP V12430 preserves gizzard stones; *Jeholornis* (Aves) IVPP V13352 preserves seeds indicative of a crop; *Sapeornis* (Pygostylia) STM15-15 preserves both seeds in the crop and gizzard stones; *Yanornis* (Ornithuromorpha) STM9-15 preserves fish in the crop and ventriculus. The crop is either a synapomorphy of Aves or *Jeholornis* + all more derived birds. *Dashed line* indicates no data. [Color for online/pdf only]



specimens further encapsulate a diversity of taxa and similar to living birds reveal a spectrum of follicle to body size ratios which are inversely related to the number of preserved follicles, also consistent with the evolution of increasing k-selected reproductive strategies across Theropoda and paralleling evolution in Neornithes (O'Connor et al. 2013a; Zheng et al. 2013).

Discussion

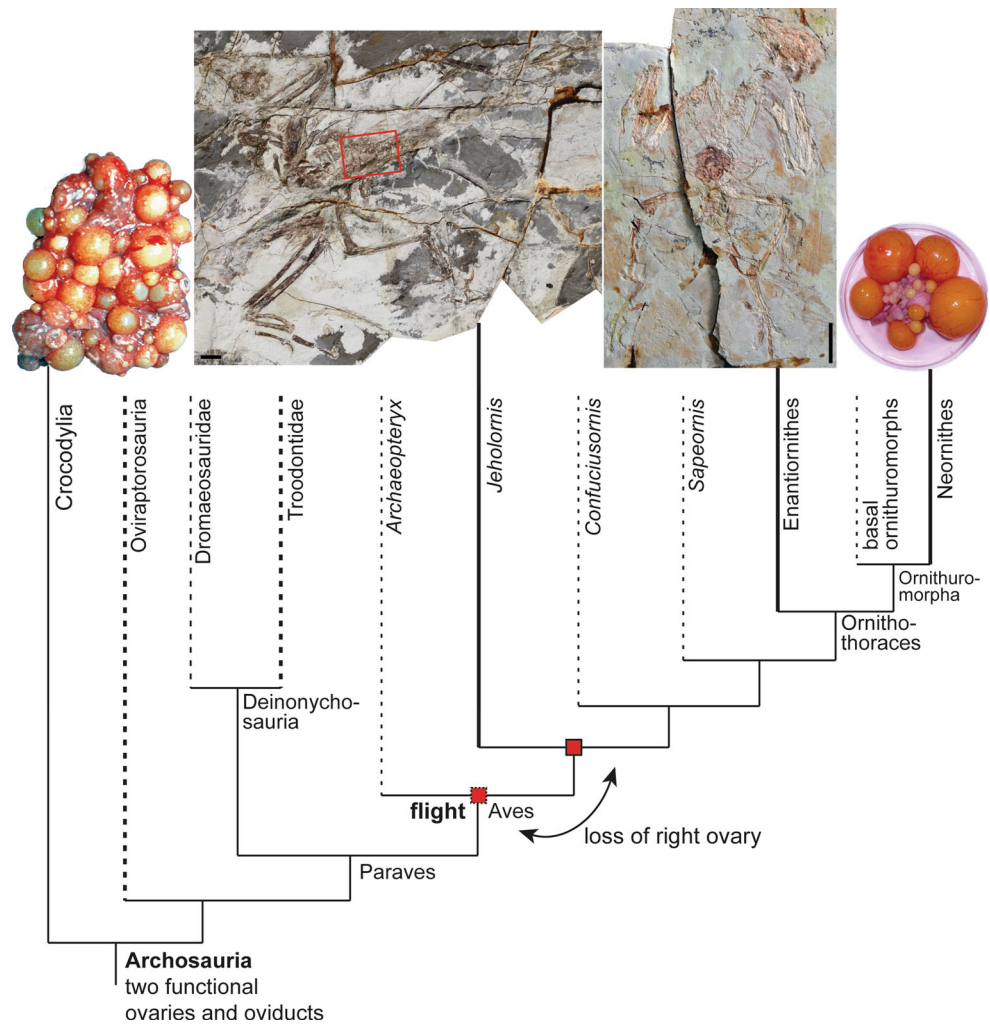
The exceptional preservation of the Jehol Biota provides a taphonomic window into the biology of the second oldest avifauna (Zhou et al. 2003). Living birds are highly modified compared to other groups of amniotes and this is commonly attributed to the evolution of flight, the most physically demanding form of locomotion, dictating that birds must be light weight and highly efficient (Gill 2007). This has resulted in numerous modifications not just to the skeleton but also to the biology of birds, producing modifications in the breathing and feeding mechanisms, reproductive system, growth strategy, and others. Crocodylia represent the only other living clade of archosaurs and, together with Neornithes, provide a phylogenetic bracket for studying the biology of extinct dinosaurs. Numerous avian features are known to have evolved

outside of Aves (e.g. asymmetrical pennaceous feathers, brooding, asymmetrical eggs) (Grellet-Tinner and Chiappe 2004; Xu et al. 2003), but others are apparently absent in basal birds (e.g. keeled sternum, rapid growth) (O'Connor et al. 2014a; Zheng et al. 2014b). Below, we discuss derived maniraptoran biology in three aspects (sternal development, digestion, and reproduction) in the context of the crocodylian and neornithine condition, and make inferences regarding the evolution of these features in early birds in order to identify features that may potentially define the avian clade.

Sternum

The crocodylian sternum consists of two medially articulating cartilaginous plates, whereas the neornithine sternum is one of the most complex elements in the avian skeleton, as well as one of the largest and most characteristic with its deep ventral keel and numerous caudal trabeculae. However, a sternum was absent in the basal birds *Archaeopteryx* and *Sapeornis*, and thus the origin of the avian sternum is unclear and the plesiomorphic condition is unknown (Zheng et al. 2014b). In basal birds, the sternum, where present, is formed by two medially articulating plates as in closely related non-avian dinosaurs (e.g. dromaeosaurids, oviraptorids), that fuse late in

Fig. 3 A simplified cladogram of archosaur relationships showing data regarding reproductive system. A single ovary is regarded as an autapomorphy of Aves. *Bold dashed lines* indicate indirect evidence from preserved eggs that suggest two functional ovaries and oviducts; *normal dashed lines* indicate absence of evidence. Crocodylia is represented by *Alligator mississippiensis*—note that mature follicles are nearly equal in size (one of two ovaries; image provided by L. Guillette and reproduced with permission); *Jeholornis* is represented by STM2-51; Enantiornithes is represented by an indeterminate specimen STM10-12; Neornithes is represented by *Gallus*—note the strong degree of follicular hierarchy in the maturing follicles (image provided by A. Johnson and reproduced with permission) [Color for online/pdf only]



ontogeny (*Jeholornis*, *Confuciusornis*); late stage fusion is also documented in some non-avian theropods (e.g. *Ajancingenia*, *Microraptor*) (Osmólska et al. 2004; Xu et al. 2003). This pattern is also present in extant flightless paleognaths, the ratites (Von Blötzheim 1958). Jehol enantiornithines, however, reveal a pattern previously undocumented among archosaurs. Instead of being formed by medially articulating plates, the sternum is formed primarily by proximodistally arranged median ossification centers (Zheng et al. 2012). Therefore, despite the importance of the boney sternum in living birds, this skeletal feature fails to provide any synapomorphies or autapomorphies for Aves as a whole (Fig. 1).

Digestion

Compared to neornithines, the crocodylian oesophagus is unmodified and only a single stomach, the ventriculus, is present; Neornithines have an expanded oesophageal pouch called the crop and a two-part stomach—the proventriculus

and ventriculus (Gill 2007). Although gastroliths have been found in the stomachs of crocodylians, these are not true gizzard stones and the ventriculus is unmodified for grinding (Wings 2007). A two-part stomach apparently evolved outside Aves, as evidenced by the presence of gizzard stones in basal oviraptorosaurs (Ji et al. 1998). However, the crop is so far only known in Aves. Given the phylogenetic bracket for this feature, considered present in the basal *Jeholornis* (Zhou and Zhang 2002), we suggest the presence of a crop may be an autapomorphy of Aves (Fig. 2). Unfortunately, the presence of a crop cannot be determined in *Archaeopteryx*. Although morphologically simple in many living birds, the crop is capable of expanding enormously to carry large prey items or great quantities of food. This is considered to have evolved in order to allow birds to swallow prey items whole in the absence of teeth and thus the ability to orally process food (Gill 2007); alternatively, it is attributed to the need to gather large amounts of food quickly, to minimize time spent exposed to danger while foraging in the open (Zheng

et al. 2011). However, a crop is clearly present in many toothed Early Cretaceous birds invalidating the first hypothesis (Zheng et al. 2014a); one specimen of *Yanornis* preserves two whole fish in the crop indicating that, despite its hypertrophied dentition, it did not orally process food. Although the second hypothesis cannot be tested, an alternative is presented here: the crop evolved due to the decrease in body size that occurred at the dinosaur–avian transition (Turner et al. 2007). As body size decreased, so did the size of the abdominal cavity; this was also coupled with an increase in rigidity of the ventral body wall due to the increase in size of the pectoral muscles (and sternum where present). Furthermore, the physical demands of flight suggest an increase in oxygenic and caloric demands (Gill 2007). Increased demands for oxygen presumably increased the size of the respiratory system, further limiting the space in the abdominal cavity (although the respiratory system also invaded the skeleton, saving space and decreasing bone weight). Because of the limited storage capabilities of the two specialized stomachs, in order to satisfy the increased metabolic demands of flight, food now had to be stored outside the abdominal cavity, necessitating the evolution of the crop. The oesophagus is not ventrally bounded by bone and is free to expand to many times its normal size, thus allowing birds to gather large amounts of food despite their small size and crowded abdominal cavity.

The feeding mechanism is the combination of the feeding apparatus, the jaws and associated musculature, and the alimentary tract (Gill 2007). Although basal ornithuromorphs have essentially a modern alimentary canal, a primitive feeding apparatus obviously persisted—teeth are present in the Late Cretaceous ornithurine birds, *Ichthyornis* and *Hesperornis* (Marsh 1880). The loss of teeth is commonly attributed to the evolution of flight in order to reduce overall weight as well as the weight of the skull, thus affecting the centre of gravity (Gill 2007). However, given that teeth persist for over 85 My of avian evolution, flight was clearly not a limiting factor. However, where a grinding gizzard is present, dentition is reduced (Zheng et al. 2011). This supports hypotheses that these two features—teeth and a grinding ventriculus—are redundant (Louchart and Viriot 2011). This indicates that evolutionary trends in tooth morphology are diet-related; the diversity of tooth morphologies in Jehol ornithuromorphs, including edentulous forms, represent diet specific specialization rather than broad evolutionary trends. There is comparatively little tooth reduction in Early Cretaceous enantiornithines (O'Connor and Chiappe 2011), consistent with the complete absence of evidence for a grinding gizzard. Confuciusornithiforms represent more of a puzzle—this edentulous clade, the oldest beaked birds (Hou et al. 1995), preserve no direct evidence of a grinding gizzard or

an herbivorous diet. Given the large number of specimens, this is considered a true absence. Notably, they also have a complete postorbital bar indicating that the massive skull was rigid, and akinetic (Chiappe et al. 1999; Zhang et al. 2008a).

Reproduction

The reproductive strategies of the two clades of living archosaurs are highly disparate. Crocodylians have large clutches of small, symmetrical eggs with a single crystallographic layer; they bury their eggs during incubation and have minimalistic roles as parents (Huchzermeyer 2003). Like all other amniotes, crocodylians have two functional ovaries and oviducts. Living birds are unique in that they typically only have a single functional ovary and oviduct—the left. The loss of the right ovary is commonly attributed to flight (Gill 2007). Because of their higher basal metabolic rate, neornithines undergo rapid vitellogenesis; because the process of yolk deposition begins in the first follicle that will ovulate, living birds show a high degree of follicular hierarchy, whereas crocodylians, with their low basal metabolic rate, have extended periods of vitellogenesis producing a very low follicular hierarchy (Huchzermeyer 2003) (Fig. 3).

Some aspects of maniraptoran reproduction have a long history of study through the discoveries of fossilized eggs and nests. These specimens reveal that asymmetrical eggs with three crystallographic layers evolved outside Aves among derived non-avian maniraptorans (Buffetaut et al. 2005; Grellet-Tinner and Chiappe 2004). An exceptional specimen of oviraptorid from the Late Cretaceous of Mongolia was discovered preserving two eggs between the pubes, interpreted as evidence that two functional oviducts and ovaries were still present in this clade (Sato et al. 2005). The paired structure of eggs in the nests of Late Cretaceous troodontids suggests two functional ovaries and oviducts were also present in this clade (Varricchio et al. 1997). Soft tissue aspects of the reproductive system have for obvious reasons otherwise remained elusive until the recent discovery of mature ovarian follicles preserved in several Jehol birds. The presence of two ovaries in the derived maniraptoran clade Oviraptorosauria (Sato et al. 2005) and potentially Troodontidae as well (Varricchio et al. 1997), and only a single ovary in the basal long boney-tailed bird *Jeholornis*, only more derived than *Archaeopteryx*, indicates the loss of the right ovary occurred very close to the dinosaur-avian transition (Zheng et al. 2013). This is consistent with the hypothesis that the right ovary was lost due to the physical constraints of flight. Notably, the holotype of *Compsognathus longipes* also apparently preserves ovarian follicles (Griffiths 1993,

1999); however this specimen is preserved in lateral view and the presence of a single versus paired ovaries cannot be determined (O'Connor et al. 2013a). Two would be expected given the phylogenetic position of this taxon outside Maniraptora (Turner et al. 2012). With the loss of the right ovary known to have occurred very near the advent of Aves, we propose that the presence of a single ovary may define the avian clade (Fig. 3). However, unlike living birds, Early Cretaceous taxa show a minimal follicular hierarchy similar to crocodylians and consistent with the lower metabolic rate inferred through osteohistology (Chinsamy et al. 1995; O'Connor et al. 2014a; Zheng et al. 2013). Given that the derived growth strategy present in most neornithines evolved within Ornithuromorpha (Chinsamy et al. 1995), we suspect that follicular hierarchy comparable to living birds may also be restricted to derived members of this clade.

Conclusions

We have explored three different aspects of basal bird biology: the reproductive system, alimentary canal, and one element of the skeletal system—the sternum. As with previous studies that have attempted to use integumentary or skeletal features to define Aves, the sternum provides no features unique to Aves as a whole, and this element may even have been plesiomorphically absent. However, when we broaden our scope beyond the primarily skeletal perspective and consider what it means to be biologically avian, we identify two potential autapomorphies for the clade. Although with no data from *Archaeopteryx* or the immediate sister-taxon to Aves (as yet unknown), we cannot confirm with absolute certainty whether these traits are truly limited to birds. Given that the phylogenetic position of *Archaeopteryx* is somewhat controversial, the former issue may prove irrelevant. Increased clarity regarding the phylogenetic relationships of derived maniraptorans and new discoveries will surely elucidate these issues. Given that the rate of discovery in the Middle–Late Jurassic and Early Cretaceous deposits in China shows little sign of waning, and recognizing that conclusions based on the current data are highly susceptible to modification with new data, we consider this first biological definition for Aves an initiation point from which to expand, as does our understanding.

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