

Archaeorhynchus preserving significant soft tissue including probable fossilized lungs

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We describe a specimen of the basal ornithuromorph *Archaeorhynchus spathula* from the Lower Cretaceous Jiufotang Formation with extensive soft tissue preservation. Although it is the fifth specimen to be described, unlike the others it preserves significant traces of the plumage, revealing a pintail morphology previously unrecognized among Mesozoic birds, but common in extant neornithines. In addition, this specimen preserves the probable remnants of the paired lungs, an identification supported by topographical and macro- and microscopic anatomical observations. The preserved morphology reveals a lung very similar to that of living birds. It indicates that pulmonary specializations such as exceedingly subdivided parenchyma that allow birds to achieve the oxygen acquisition capacity necessary to support powered flight were present in ornithuromorph birds 120 Mya. Among extant air breathing vertebrates, birds have structurally the most complex and functionally the most efficient respiratory system, which facilitates their highly energetically demanding form of locomotion, even in extremely oxygen-poor environments. *Archaeorhynchus* is commonly resolved as the most basal known ornithuromorph bird, capturing a stage of avian evolution in which skeletal indicators of respiration remain primitive yet the lung microstructure appears modern. This adds to growing evidence that many physiological modifications of soft tissue systems (e.g., digestive system and respiratory system) that characterize living birds and are key to their current success may have preceded the evolution of obvious skeletal adaptations traditionally tracked through the fossil record.

Aves | Ornithuromorpha | respiration | lungs | Jehol

Although soft tissue preservation in fossils is considered extremely rare, during the last three decades, thousands of specimens of feathered dinosaurs and birds have been collected from Late Jurassic and Early Cretaceous deposits primarily in China, recording numerous integumentary structures (1), variations in plumage (2), feather color (3), and even the dermal structures of the wing (4, 5). Preservation of internal soft-tissue structures is far less common. However, exceptional discoveries of preserved soft tissues in Jehol birds have already revealed aspects of the digestive tract (6), reproductive organs (7), tendons/ligaments of the foot (8), and the internal structure of the propatagium (5).

Neornithines have a highly efficient respiratory system with unidirectional airflow, lungs supplemented by air sacs, and a highly pneumatized skeleton (9). Although recent evidence suggests that unidirectional airflow is plesiomorphic to Archosauria (10, 11), the evolution of the avian form of respiration aided by air sacs within the Dinosauria has been the subject of great interest (12–14). Soft tissue traces being unlikely to fossilize, inferences regarding respiratory function have previously relied entirely on osteological features such as pneumatization (14) of the skeleton and the morphology of the uncinat processes on the thoracic ribs (15, 16). Here we report a plausible occurrence

of preserved traces of lung tissue in a stem bird, occurring in a specimen of the basalmost ornithuromorph *Archaeorhynchus spathula* (STM7-11; Shandong Tianyu Museum of Nature, Pingyi, China; Fig. 1) from the Early Cretaceous Jehol Lagerstätte in northeastern China. This Lagerstätte has produced more exceptional specimens of early birds and dinosaurs preserving soft tissue than any other (1, 6, 7), a taphonomic phenomenon likely exaggerated by the enormous volume of collected specimens. Although this is the fifth specimen of *Archaeorhynchus* to be described (17–19), this specimen is distinct in that it preserves extensive soft tissue, which includes informative feather remains. The identification of unusual soft tissue traces in *Archaeorhynchus* STM7-11 as lungs is supported by topographical location and gross anatomical observations mainly acquired by SEM analysis.

Results

Purported Lung Tissue. Preserved primarily in the counterslab of *Archaeorhynchus* STM7-11 (*SI Appendix, Supplementary Materials* for taxonomic referral) rostral to the ventriculus is a speckled white material that has not been previously observed in any Jehol specimen. In the ventral slab, the material forms two distinct nearly symmetrical lobes slightly displaced to the right side of the

Significance

Archaeorhynchus spathula is a basal member of the Ornithuromorpha, the lineage that includes neornithines. Although this is the fifth reported specimen, unlike the others it preserves significant soft tissue, revealing a tail morphology previously unknown in Mesozoic birds and an exceptional occurrence of fossilized lung tissue. This identification is based on topographical location and anatomical features revealed mainly by scanning electron microscopy. A structure similar to that in neornithines indicates that the 120-Ma-old *Archaeorhynchus* may have been capable of acquiring the large quantities of oxygen needed to support powered flight. Skeletal features related to respiration remain primitive, supporting inferences that many physiological adaptations preceded skeletal changes during the evolution of the anatomically modern bird.

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Fig. 1. *Archaeorhynchus* STM7-11 (main slab, dorsal view) preserving integument (millimeter scale visible in Lower lefthand corner). Small rectangles indicate autapomorphies of *Archaeorhynchus*, enlarged for detail [spatulate edentulous dentary (10× magnification); coracoid with wide omal margin and blunt procoracoid (3× magnification); metatarsal I and digit I reduced (3× magnification)]. ac, acrocoracoid process; mtl, metatarsal I; pr, procoracoid process. Numbers refer to the six rectrices on the *Right* side of the body. Image courtesy of J. Zhang (Institute of Vertebrate Paleontology and Paleoanthropology, Beijing).

body (Fig. 1 and *SI Appendix*, Fig. S1). In the dorsal slab, the thoracic ribs are deeply embedded within and overlapped by remains of the white material (Fig. 2 *A* and *B*), confirming that the substance is located within the thoraco-abdominal (coelomic) cavity. Furthermore, the deep insertion of the ribs resembles the condition in modern birds, in which the lungs bear costal and vertebral impressions that accommodate parts of these bones. The paired morphology of the structures, as well as their craniodorsal location in the body cavity and association with the rib cage (Fig. 2 *D* and *E*), are consistent with their identification as the lungs, based on comparison with modern birds (20, 21). In birds, cranial

to the ventriculus (the muscular stomach) anatomically lies the proventriculus (the glandular stomach); this organ is small and lacks any paired structure, and in small birds, it is not used to store food (the function of the esophageal crop). The fine-grained appearance of the white material further suggests it is not food. Interpretation of the substance as triturated food remains pushed forward within the alimentary canal are unlikely, given that the gastrolith mass is largely undisturbed (Fig. 1), suggesting also that the alimentary canal had not ruptured, and given that no complete gastral mass in any Jehol specimen is preserved in a similar white material or any material distinct from the matrix itself. The large

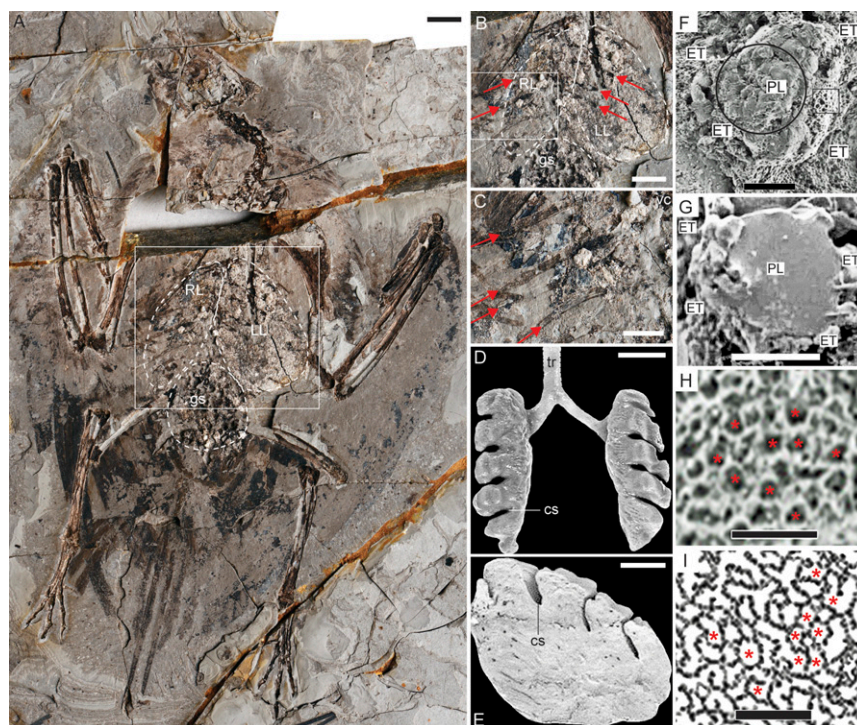


Fig. 2. Comparison of the macroscopic and microscopic lung morphology in *Archaeorhynchus* STM7-11 and living birds: (A) counterslab of STM7-11 showing the ventral aspect (medial surface of ribs exposed): plausible lung tissue is located in the boxed area (enlarged in B). (Scale bar, 1 cm.) (B) Close-up of lungs with arrows indicating impressions made by the ribs. (Scale bar, 1 cm.) Boxed area is enlarged in C. (C) Close-up of the boxed area in B showing the costal impressions (red arrows) on the presumptive right lung. (Scale bar, 5 mm.) (D) Dorsal view of the embryonic lungs of the ostrich (*Struthio camelus*) showing deep costal sulci (rib impressions). (Scale bar, 3 cm.) (E) A longitudinal slice of the embryonic lung of the domestic fowl (*Gallus gallus domesticus*) showing deep costal sulci (rib impressions), (Scale bar, 1 mm.) (F) SEM image of sample '2' from the lung preserved in the counterslab showing presumptive parabronchus of the fossilized lung. The parabronchial lumen is surrounded by parenchyma (gas exchange tissue)-like tissue. (Scale bar, 10 μm .) (G) Latex cast preparation of the lung of the domestic fowl showing a parabronchus that consists of a parabronchial lumen (PL) surrounded by exchange tissue (ET). (Scale bar, 250 μm .) (H) Close up of boxed area in F showing presumptive gas exchange tissue with minuscule air space-like cells (some of which are indicated by asterisks) that resemble the air capillaries of the lung of the modern avian lung. (Scale bar, 5 μm .) (I) Exchange tissue of the lung of the ostrich showing air capillaries (asterisks), the terminal gas exchange units. (Scale bar, 20 μm .) cs, costal sulci; ET, parenchyma-like tissue for gas exchange; PL, probable parabronchial lumen; RL, right lung; tr, trachea; vc, location of the vertebral column.

area occupied by the soft tissue in STM7-11 is inconsistent with the proventriculus, which is typically small and too far caudal to be in the crop, contents of which are preserved whole (22). Furthermore, the purported lung tissue does not resemble stomach contents preserved in the Jehol birds *Jeholomis* (23) and *Yanornis* (6), which are distinct from the traces preserved in STM7-11 in color and position and lack a paired appearance. *Archaeorhynchus* IVPP V20312 preserves a dark organic/carbonized mass proximal to the gastral mass (19); the position, smaller size, and singular appearance of the remains are consistent with possible identification as traces of the contents of the proventriculus. If the substance in STM7-11 was interpreted as food remains, then both the intimate association with the ribs and the paired morphology would be difficult to explain. The only other organs found in this part of the body are the heart and the liver, but these are smaller structures and not paired organs such as the lungs (24). Although the bilobed appearance of the avian liver could possibly lead to a paired appearance, the tissue in question lacks the reddish color associated with fossilized liver tissue, resulting from its high iron content (25, 26).

SEM Analysis. Twenty-two samples were observed using SEM (*SI Appendix, Supplementary Materials*). Of the 12 taken from the purported lung tissue (*SI Appendix, Figs S2 and S3*), seven reveal structures that resemble parabronchi (airways, tertiary bronchi; Fig. 2 F and G) and a parenchymatous region that consists of closely packed minuscule air cells (Fig. 2H); the spaces have an

average diameter of $\sim 3 \mu\text{m}$ (Fig. 2 H and I) and correspond with the air capillaries of the lungs of some of the very small modern birds (20, 27). This structure has not been observed in any other known sample taken from a Jehol vertebrate. Albeit minuscule in size, the air capillaries of the avian lung are very strong (28–32). Structural interdependence between the air and the blood capillaries and presence of type IV collagen in the basement membrane of the blood–gas barrier (33, 34) are some of the features that may explain how and why the seemingly delicate structures may have been well-preserved in the fossilized lung. The microscopic characteristics of the preserved substance correspond to those of the lung, but totally differ from those of the liver (Fig. 2H). An energy dispersive X-ray spectrometer (EDS) fails to distinguish the lung tissue from the matrix (*SI Appendix, Fig. S4*). The two samples taken from the wing feathers do not reveal any visible melanosomes (samples S15 and S16). Bacteria-like elongate mono-organelles longer than eumelanosomes, and lacking organization, are visible in two samples (samples S4 and S7; *SI Appendix, Fig. S5*).

Stomach Contents. STM7-11 preserves ~ 100 gastroliths in a cluster in the abdominal cavity, similar to the complete masses preserved in *Archaeorhynchus* IVPP V17091, IVPP V17075, and IVPP V20312. As in previously described specimens (17–19), these are interpreted as gizzard stones. The cluster is preserved mostly in the main slab, consistent with the more ventral position of

the ventriculus in the abdominal cavity (Fig. 1 and *SI Appendix*, Fig. S1).

Plumage. The carbonized remains of feathers surround the skeleton (Fig. 1). The feathers are nearly entirely preserved in the main slab, although clear impressions are preserved in the counterslab. Crural feathers are notably absent, as in other Early Cretaceous ornithomorphs. Body feathers, best preserved in the capital tract, on the neck and right shoulder, appear to be without a rachis, having a wispy, hair-like appearance, as in other Jehol birds (e.g., enantiornithines, *Jeholornis*, *Confuciusornis*) (1, 35). They appear to consist of a bundle of filaments basally attached for a third of their length (Fig. 3B). The wings are folded inward, and the feathers are preserved primarily as amorphous carbon (confirmed through SEM), revealing few details of their morphology (Fig. 1). The asymmetrical primaries have a maximum estimated length of 110 mm, similar to estimates based on IVPP V14287 and IVPP V17091. A row of short (12–13 mm) symmetrical secondary covert feathers is preserved on the right wing attaching to the ulna; in this mode of preservation, the rachis and barbs appear the same thickness (Fig. 3C). The proximal ends of pennaceous feathers are visible projecting off the right alular digit in the counterslab, indicating an alula was present.

Twelve rectrices are preserved: 10 shorter rectrices forming a tail fan and two medial elongate pinfeathers (Fig. 1 and *SI Appendix*, Fig. S6). Based on length and comparison with the right half of the tail fan, we consider that two of the left rectrices are missing. The tail feathers range from 44 to 31 mm, so that the

caudal margin is gently graded. The tail fan defines an 80° arc around the pygostyle and caudal vertebrae, and the individual feathers do not overlap, whereas in all other known Jehol bird specimens preserving fan-tails, the feathers overlap, forming a single surface but defining a smaller angle (36). Visible in the well-preserved outer right rectrices (Fig. 3A), the rachises preserve a longitudinal medial stripe. Although these rectrices are well preserved in both slabs, this feature is only clearly visible in the main slab, which preserves the ventral surface. The caudal margins of the individual rectrices are rounded. The vanes in the outer rectrices are strongly asymmetrical, with the medial vane being five times the width of the lateral vane. The degree of asymmetry decreases from the outer to inner rectrix, with the innermost rectrices appearing roughly symmetrical, as observed in the aerodynamic tail fans of living birds (37). The elongate central feathers are incomplete; their maximum preserved length is 50 mm on both sides. No barbs can be confidently associated along the ribbon-like preserved portions of these elongate feathers, which may suggest these feathers were similar to the rachis-dominated feathers in *Confuciusornis* and some enantiornithines (38).

Discussion

Lungs. After birds attained the capacity for powered flight, they dispersed widely, and consequently underwent a notable adaptive radiation that ultimately resulted in Aves being the most speciose of the amniote clades. Among vertebrates, birds have structurally the most complex, and functionally the most efficient, respiratory system: the lung-air sac system (20, 21, 39, 40), which is

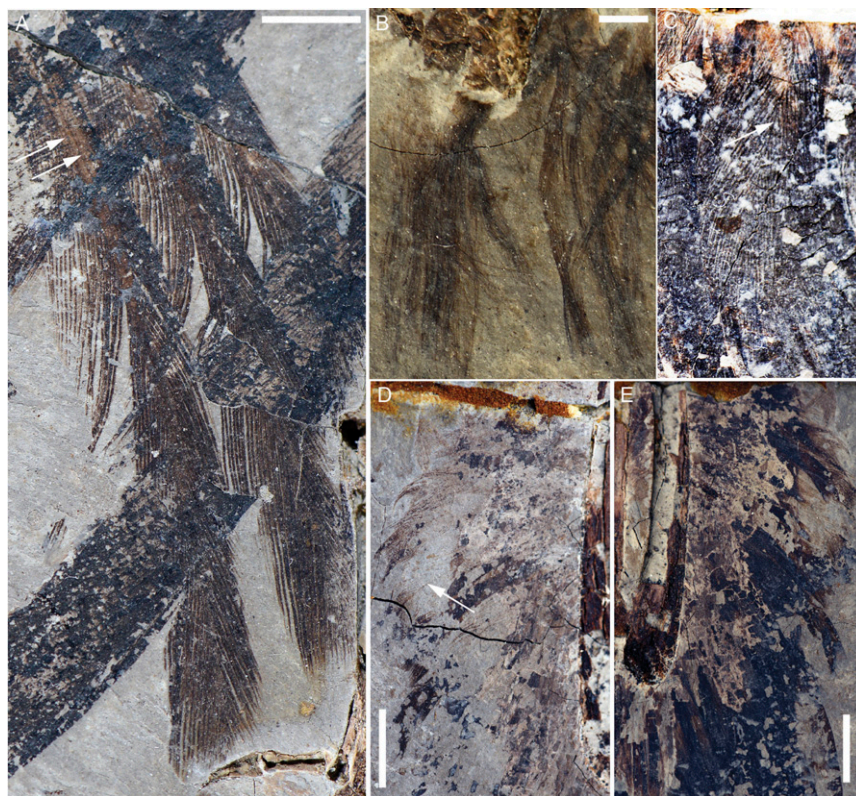


Fig. 3. Details of the feathers preserved in *Archaeorhynchus* STM7-11. (A) details of rectrices forming the tail fan showing the medially striped rachis (white arrows indicate edge of rachis and medial stripe). (Scale bar, 5 mm.) (B) Apparently rachis-less body feathers covering the neck. (Scale bar, 1 mm.) (C) Pennaceous secondary covert of the right wing preserved as an impression in the carbon (white arrow indicates narrow rachis). (Scale bar, 1 mm.) (D) Pennaceous secondary coverts of the left wing (counterslab; arrow indicates rachis). (Scale bar, 5 mm.) (E) Pennaceous secondary coverts of the left wing (main slab). (Scale bar, 5 mm.) (Scale bars in A, D, and E equal 5 mm, and in B and C they equal 1 mm.) Image courtesy of J. Zhang (Institute of Vertebrate Paleontology and Paleoanthropology, Beijing).

capable of supporting their highly energetically demanding form of locomotion (powered flight), even in extremely oxygen-poor environments (e.g., for the bar-headed geese, which fly over the Himalayas). Although certain morphological similarities exist between the lungs of birds and those of other reptiles (41, 42), important questions on how, when, and why the modern avian lung evolved remain unclear (43).

Unusual traces of paired soft tissue structures preserved in *Archaeorhynchus* STM7-11 are, largely by exclusion, identified as plausible remnants of the lungs (Fig. 2), a deduction supported by topographical location and gross morphological and microscopic lines of evidence. In fact, this is not the first known record of fossilized lungs in a vertebrate; lung remains are also preserved in a Mesozoic mammal from the 125-Ma Las Hoyas Lagerstätte (25) and several Tertiary fossil amphibians (44, 45). Because EDS failed to reveal the chemical composition of the authigenic mineral forming the lung remains, we cannot attempt to explain the taphonomic processes responsible for this preservation at this time. Although not all of the main structures of the avian respiratory system could be identified on the supposed fossil lung (e.g., the very delicate air sacs that are even less likely to fossilize), the microstructure of the preserved lung closely resembles that of modern birds. This suggests that the general structural design of the avian lung has been conserved for a very long time, being already present in the most primitive members of the Ornithuromorpha, the clade that includes Neornithes, and can be presumed to have considerable functional utility. The preserved morphology of the presumptive fossilized lungs suggests they were directly attached to the wall of the coelomic cavity as in modern birds, a feature rendering the lungs practically inflexible, a state that allowed the parenchyma to be subdivided into extremely small terminal gas exchange units (air capillaries) conferring large respiratory surface area and allowing development of an extremely thin blood–gas barrier (20, 46, 47). These specializations resulted in the large pulmonary diffusion capacities of oxygen necessary to support active flight. The external morphology of the avian respiratory system is remarkably uniform (20, 48), but internal specializations exist between lungs of volant and nonvolant species (20, 47, 49). The large energetic demands of flight, and therefore the concomitant great need for respiratory efficiency may have compelled conservation of the structural components of the avian lung once achieved. According to the morphology of the uncinata processes and gastralia, it is hypothesized that advanced nonavian maniraptorans had respiratory systems as derived as *Archaeopteryx* (16). However, it is considered unlikely that *Archaeopteryx* was capable of sustained powered flight (50), and the same skeletal indicators (uncinate processes, gastralia, sternal morphology) suggest the neornithine-like respiratory mechanism may be limited to a subset of the Ornithuromorpha. Therefore, it is possible such advanced morphology of the lung soft tissue may be limited to the Ornithuromorpha and may have contributed to the survival of their descendants through the K-T extinction event.

Plumage. Two previously described specimens (IVPP V14287 and V17091) preserve weak traces of the remiges and body feathers, revealing only an estimate of primary length (17, 18), whereas the plumage preserved in STM7-11 is nearly complete. The tail preserved in *Archaeorhynchus* STM7-11 reveals a morphology previously unknown among Cretaceous birds, but fairly common in living birds: a short, aerodynamic fan-shaped array of rectrices together with a pair of elongate, centrally located streamers. Referred to as a pintail (51), this morphology is present in both dimorphic and nondimorphic extant species (e.g., the Momotidae, both genders; *Dicrurus paradiseus*, the greater racket-tailed drongo, both genders; *Trochilus polytmus*, the red-billed streamer tail, males; *Oreatus underwoodii*, the booted racket-tail, males) making it impossible to determine sex from a single specimen without

additional evidence. Although much focus is put on the sexually driven selection for elaborate tail morphologies in some birds, most living birds have tail morphologies, the primary function of which is to supplement the wing apparatus in flight (aerodynamic function) (52). However, tail plumage in each species is not under just one selective pressure, and the pintail morphology serves both clear aerodynamic and ornamental functions; although the medial tail feathers are elongated under sexual selection, the incurred drag is relatively low, and such tails are associated with minimal cost (53).

With the exception of *Jeholornis* (54) among Cretaceous birds, most tail morphologies appear to be decisively either ornamental or aerodynamic in primary function [although by limiting barbs to the distal end of elongate tail feathers, i.e., the racket-plume morphology observed in *Confuciusornis* and some enantiornithines, the aerodynamic cost of this ornament is reduced (55), and conversely the rounded caudal margin of ornithuromorph tail fans is considered relatively more ornamental than a straight caudal margin] (56). In all Jehol ornithuromorphs for which tail plumage is clearly preserved, the tail shape clearly has aerodynamic function (i.e., having a morphology that would impart forces to the body from airflow), formed by an overlapping array of rectrices that create a surface capable of generating lift (52, 57–60). The tail preserved in STM7-11 provides unequivocal evidence of ornamental tail feathers in a Jehol ornithuromorph. However, unlike the paired ornamental feathers that form the entire tail in other Early Cretaceous birds (*Confuciusornis*, enantiornithines, potentially *Iteravis*), in *Archaeorhynchus* these ornamental feathers are combined with a short, graded, aerodynamic fan in a pintail arrangement (Fig. 1). Although the short feathers do not overlap forming a cohesive surface, we consider this a taphonomic artifact, an interpretation supported by the wider angle formed by the rectrices in STM7-11 relative to other Jehol specimens in which the tail feathers are preserved, forming an airfoil (36). The increasing asymmetry observed in the inner to outer rectrices in STM7-11 also characterizes living birds with aerodynamic tail fans, further supporting interpretations that these shorter tail feathers overlapped *in vivo*.

Preservation in previously described Early Cretaceous ornithuromorphs precluded an accurate estimate of the complete tail; *Yixianornis* IVPP V13631 preserves at least eight (59), and *Hongshanornis* DNHM2945/6 is reported to have a minimum of 10 (57). The aerodynamic tail fan of *Archaeorhynchus* would have consisted of 12 rectrices, as in most living birds. The *Archaeorhynchus* tail fan is proportionately shorter and more deeply graded than that present in *Piscivoravis*, *Yixianornis*, *Yanornis*, and *Hongshanornis* (36), further adding to the diversity of recognized tail shapes exploited by Early Cretaceous birds.

Materials and Methods

The specimen was observed under a binocular microscope and sampled manually for SEM analysis in two rounds (*SI Appendix, Figs S2 and S3*). In the preliminary round, three samples were collected from the counterslab: one from the surrounding matrix and two from the purported lung tissue (*SI Appendix, Fig. S3*). The uncoated samples were investigated under a SEM (Leo1530VP), with a Variable Pressure Secondary Electron Detector in a low vacuum condition with a higher accelerating voltage (20 keV). An EDS was used for selected point analysis of elemental compositions. All EDS data of the uncoated samples were collected at 20 keV accelerating voltage, at a working distance of 10 mm and for 120 seconds. After positive results in the first round of sampling, a second batch of 19 samples was collected. Sixteen samples are from the main slab: seven from the lungs, three from areas directly surrounding the lung tissue, three from the gastral mass, two from feathers, and one from the distal radius (*SI Appendix, Fig. S2*). Three additional samples were collected from the counterslab representing the left lung, matrix, and carbonized remains below the left femur (*SI Appendix, Fig. S3*). One sample could not be imaged.

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- Zhang F, Zhou Z, Dyke GJ (2006) Feathers and 'feather-like' integumentary structures in Liaoning birds and dinosaurs. *Geol J* 41:395–404.
- Zheng X, et al. (2013) Hind wings in Basal birds and the evolution of leg feathers. *Science* 339:1309–1312.
- Zhang F, et al. (2010) Fossilized melanosomes and the colour of Cretaceous dinosaurs and birds. *Nature* 463:1075–1078.
- Navalón G, Marugán-Lobón J, Chiappe LM, Luis Sanz J, Buscalioni AD (2015) Soft-tissue and dermal arrangement in the wing of an Early Cretaceous bird: Implications for the evolution of avian flight. *Sci Rep* 5:14864.
- Zheng X-T, et al. (2017) Exceptional preservation of soft tissue in a new specimen of *Eoconfuciusornis* and its biological implications. *Natl Sci Rev* 4:441–452.
- Zheng X, et al. (2014) New specimens of *Yanornis* indicate a piscivorous diet and modern alimentary canal. *PLoS One* 9:e95036.
- Zheng X, et al. (2013) Preservation of ovarian follicles reveals early evolution of avian reproductive behaviour. *Nature* 495:507–511.
- Jiang B, et al. (2017) Cellular preservation of musculoskeletal specializations in the Cretaceous bird *Confuciusornis*. *Nat Commun* 8:14779.
- Gill FB (2007) *Ornithology* (W.H. Freeman and Company, New York), 3rd Ed, p 758.
- Farmer CG (2010) The provenance of alveolar and parabronchial lungs: Insights from paleoecology and the discovery of cardiogenic, unidirectional airflow in the American alligator (*Alligator mississippiensis*). *Physiol Biochem Zool* 83:561–575.
- Farmer CG, Sanders K (2010) Unidirectional airflow in the lungs of alligators. *Science* 327:338–340.
- Wedel MJ (2003) Vertebral pneumaticity, air sacs, and the physiology of saurpoid dinosaurs. *Paleobiology* 29:243–255.
- O'Connor PM, Claessens LPAM (2005) Basic avian pulmonary design and flow-through ventilation in non-avian theropod dinosaurs. *Nature* 436:253–256.
- Benson RB, Butler RJ, Carrano MT, O'Connor PM (2012) Air-filled postcranial bones in theropod dinosaurs: Physiological implications and the 'reptile'-bird transition. *Biol Rev Camb Philos Soc* 87:168–193.
- Codd JR, Manning PL, Norell MA, Perry SF (2008) Avian-like breathing mechanics in maniraptoran dinosaurs. *Proc Biol Sci* 275:157–161.
- Pei R, Li Q-G, Meng Q-J, Gao K-Q, Norell MA (2014) A new specimen of *microraptor* (Theropoda: Dromaeosauridae) from the lower Cretaceous of Western Liaoning, China. *Am Mus Novit* 3821:1–28.
- Zhou S, Zhou Z-H, O'Connor JK (2013) Anatomy of the Early Cretaceous *Archaeorhynchus spathula*. *J Vertebr Paleontol* 33:141–152.
- Zhou Z-H, Zhang F-C (2006) A beaked basal ornithurine bird (Aves, Ornithurae) from the Lower Cretaceous of China. *Zool Scr* 35:363–373.
- Wang M, Zhou Z-H (2016) A new adult specimen of the basalmost ornithuromorph bird *Archaeorhynchus spathula* (Aves: Ornithuromorpha) and its implications for early avian ontogeny. *J Syst Palaeontol* 15:1–18.
- Maina JN (2005) *The Design of the Lung-air Sac System of Birds: Development, Structure, and Function* (Springer Verlag, Heidelberg), p 210.
- Maina JN (2017) Pivotal debates and controversies on the structure and function of the avian respiratory system: Setting the record straight. *Biol Rev Camb Philos Soc* 92:1475–1504.
- Zheng X, et al. (2011) Fossil evidence of avian crops from the Early Cretaceous of China. *Proc Natl Acad Sci USA* 108:15904–15907.
- O'Connor JK, et al. (2018) First report of gastroliths in the Early Cretaceous basal bird *Jeholornis*. *Cretac Res* 84:200–208.
- Duncker HR (1979) Coelomic cavities. *Form and Function in Birds*, eds King AS, McLelland J (Academic Press, London), Vol 1, pp 39–67.
- Martin T, et al. (2015) A Cretaceous eutriconodont and integument evolution in early mammals. *Nature* 526:380–384.
- Dal Sasso C, Maganuco S (2011) *Scipionyx samniticus* (Theropoda: Compsognathidae) from the lower Cretaceous of Italy: Osteology, ontoenetic assessment, phylogeny, soft tissue anatomy, taphonomy and paleobiology. (Memorie della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale, Milan), p. 1–282.
- Maina JN (1984) Morphometrics of the avian lung. 3. The structural design of the passerine lung. *Respir Physiol* 55:291–307.
- Macklem PT, Bouverot P, Scheid P (1979) Measurement of the distensibility of the parabronchi in duck lungs. *Respir Physiol* 38:23–35.
- West JB, Watson RR, Fu Z (2006) The honeycomb-like structure of the bird lung allows a uniquely thin blood-gas barrier. *Respir Physiol Neurobiol* 152:115–118.
- West JB, et al. (2010) Structure-function studies of blood and air capillaries in chicken lung using 3D electron microscopy. *Respir Physiol Neurobiol* 170:202–209.
- Maina JN (2007) Spectacularly robust! Tensegrity principle explains the mechanical strength of the avian lung. *Respir Physiol Neurobiol* 155:1–10.
- Maina JN (2007) Minutalization at its extreme best! The underpinnings of the remarkable strengths of the air and the blood capillaries of the avian lung: A conundrum. *Respir Physiol Neurobiol* 159:141–145.
- Jimoh SA, Maina JN (2013) Immuno-localization of type-IV collagen in the blood-gas barrier and the epithelial-epithelial cell connections of the avian lung. *Biol Lett* 9:20120951.
- Maina JN, Jimoh SA, Hosie M (2010) Implicit mechanistic role of the collagen, smooth muscle, and elastic tissue components in strengthening the air and blood capillaries of the avian lung. *J Anat* 217:597–608.
- O'Connor JK, Chiappe LM, Chuong C-M, Bottjer DJ, You H (2012) Homology and potential cellular and molecular mechanisms for the development of unique feather morphologies in early birds. *Geosciences (Base)* 2:157–177.
- O'Connor JK, Zheng X-T, Hu H, Wang X-L, Zhou Z-H (2017) The morphology of *Chiappeavis magnapremaxillo* (Pengornithidae: Enantiornithes) and a comparison of aerodynamic function in Early Cretaceous avian tail fans. *Vertebr Palasiat* 55:1–18.
- Baumel JJ (1987) Functional morphology of the tail apparatus of the pigeon (*Columba livia*). *Adv Anat Embryol Cell Biol* 110:1–115.
- Wang X-L, et al. (2014) Insights into the evolution of rachis dominated tail feathers from a new basal enantiornithine (Aves: Ornithothoraces). *Biol J Linn Soc Lond* 113:805–819.
- Fedde MR (1980) The structure and gas flow pattern in the avian lung. *Poult Sci* 59:2642–2653.
- Scheid P (1979) Mechanisms of gas exchange in bird lungs. *Rev Physiol Biochem Pharmacol* 86:137–186.
- Perry SF (1992) Gas exchange strategies in reptiles and the origin of the avian lung. *Physiological Adaptations in Vertebrates: Respiration, Circulation, and Metabolism*, eds Wood SC, Weber RE, Hargens AR, Millard RW (Marcel Dekker Inc., New York), pp 149–167.
- Duncker H-R (2004) Vertebrate lungs: Structure, topography and mechanics. A comparative perspective of the progressive integration of respiratory system, locomotor apparatus and ontogenetic development. *Respir Physiol Neurobiol* 144:111–124.
- Farmer CG (2017) Pulmonary transformations of vertebrates. *The Biology of the Avian Respiratory System*, ed Maina JN (Springer-Verlag, Heidelberg).
- Tissier J, et al. (2016) Synchrotron analysis of a 'mummified' salamander (Vertebrata: Caudata) from the Eocene of Quercy, France. *Zool J Linn Soc* 177:147–164.
- McNamara ME, et al. (2009) Exceptionally preserved tadpoles from the miocene of Libros, Spain: Ecomorphological reconstruction and the impact of ontogeny upon taphonomy. *Lethaia* 43:290–306.
- Maina JN, West JB (2005) Thin but strong! The dilemma inherent in the structural design of the blood-water/gas barrier: Comparative functional and evolutionary perspectives. *Physiol Rev* 85:811–844.
- Maina JN, King AS, Settle G (1989) An allometric study of pulmonary morphometric parameters in birds, with mammalian comparisons. *Philos Trans R Soc Lond B Biol Sci* 326:1–57.
- McLelland J (1989) Anatomy of the lungs and air sacs. *Form and Function in Birds*, eds King AS, McLelland J (Academic Press, London), Vol 4, pp 221–279.
- Maina JN (1989) Morphometrics of the avian lung. *Form and Function in Birds*, eds King AS, McLelland J (Academic Press, London), Vol 4, pp 307–368.
- Mayr G (2017) Pectoral girdle morphology of Mesozoic birds and the evolution of the avian supracoracoideus muscle. *J Ornithol* 158:859–867.
- Fitzpatrick S (1997) Patterns of morphometric variation in birds' tails: Length, shape and variability. *Biol J Linn Soc Lond* 62:145–162.
- Thomas ALR (1997) On the tails of birds. *Bioscience* 47:215–225.
- Balmford A, Thomas ALR, Jones IL (1993) Aerodynamics and the evolution of long tails in birds. *Nature* 361:628–631.
- O'Connor J, et al. (2013) Unique caudal plumage of *Jeholornis* and complex tail evolution in early birds. *Proc Natl Acad Sci USA* 110:17404–17408.
- Møller AP, Hedenström A (1999) Comparative evidence for costs of secondary sexual characters: Adaptive vane emargination of ornamented feathers in birds. *J Evol Biol* 12:296–305.
- Fitzpatrick S (1999) Tail length in birds in relation to tail shape, general flight ecology and sexual selection. *J Evol Biol* 12:49–60.
- Chiappe LM, et al. (2014) A new specimen of the Early Cretaceous bird *Hongshanornis longicresta*: Insights into the aerodynamics and diet of a basal ornithuromorph. *PeerJ* 2:e234.
- Zhou S, Zhou Z-H, O'Connor JK (2013) A new piscivorous ornithuromorph from the Jehol Biota. *Hist Biol* 26:608–618.
- Clarke JA, Zhou Z, Zhang F (2006) Insight into the evolution of avian flight from a new clade of Early Cretaceous ornithurines from China and the morphology of *Yixianornis grabaui*. *J Anat* 208:287–308.
- Zhou S, Zhou Z-H, O'Connor JK (2012) A new toothless ornithurine bird (*Schizoura llii* gen. et sp. nov.) from the Lower Cretaceous of China. *Vertebr Palasiat* 50:9–24.