Disassociated rhamphotheca of fossil bird *Confuciusornis* informs early beak reconstruction, stress regime, and developmental patterns

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Soft tissue preservation in fossil birds provides a rare window into their anatomy, function, and development. Here, we present an exceptionally-preserved specimen of *Confuciusornis* which, through Laser-Stimulated Fluorescence imaging, is identified as preserving a dis-associated rhamphotheca. Reconstruction of the in vivo position of the rhamphotheca validates the association of the rhamphotheca with two previous confuciusornithid specimens while calling that of a third specimen into question. The ease of dissociation is discussed and proposed with a fourth specimen alongside finite element analysis as evidence for preferential soft-food feeding. However, this proposition remains tentative until there is a better understanding of the functional role of beak attachment in living birds. Differences in post-rostral extent and possibly rhamphotheca curvature between confuciusornithids and modern birds hint at developmental differences between the two. Together, this information provides a wealth of new information regarding the nature of the beak outside crown Aves.
Early-diverging, short-tailed confuciusornithids are the earliest birds (we use birds in reference to the Avialae) known to have fully edentulous keratinous beaks1–4, which displayed great disparity in form1,4,5. Confuciusornis dui had a curved beak1 whilst beaks were straight in Confuciusornis sanctus4,6 and Eoconfuciusornis zhengi5. Confuciusornithid beaks have been treated as structurally equivalent to those of modern birds1,7,8, but it is unclear to what extent these assumptions are valid given that beaks evolved convergently in modern birds (Aves) and non-avian avialans9–12. Here we show that such traditional assumptions should be more carefully applied in light of a new Confuciusornis sanctus specimen STM 13-162 in which laser-stimulated fluorescence (LSF) imaging reveals a disassociated straight-shaped keratinous beak (rhamphotheca) (Fig. 1). Firstly, we comment on uncertainties in the in vivo location of confuciusornithid keratinous beaks and the implications this has on the interpretation of reported specimens. Secondly, we propose that Confuciusornis exhibits loose beak attachment which, likened to some modern birds, may indicate that the beak operated in a low stress regime suited for feeding on softer foods. The latter exposes the importance of more rigorous study of beak attachment in modern birds. Finally, we comment on differences in the keratinous and bony beak anatomy between confuciusornithids and modern birds which points to subtle developmental differences whose origins would be a worthwhile target of future developmental studies.

Results

Taxonomy. STM 13-162 is referred to Confuciusornithidae based on its edentulous upper and lower jaws, prominent rostral and caudal dentary fenestrae, and pedal digit I ungual being smaller than those of other digits. It is more precisely referred to Confuciusornis based on its suboval fenestra perforating the deltopectoral crest (considered absent in Eoconfuciusornis zhengi) and pedal digit I being less than half the length of pedal digit II (proportionally shorter than in Changchengornis hengdaoziensis or Yangavis confucii)13,14. The two species of Confuciusornis considered valid, C. sanctus and C. dui, are differentiated by the dentary which is only partially preserved in STM 13-162. We believe that the bone visibly entering the more rostral dentary fenestra is the remnant of the triangular ventral process of the surangular, which is absent in C. dui13. Furthermore, the dorsal process of the maxilla in STM 13-162 is more cranially-positioned than in C. dui, consistent with C. sanctus (compare Fig. 1c, d in Wang et al.13). On these grounds we refer STM 13-162 to Confuciusornis sanctus.

Description. In STM 13-162 the dorsal margins of the premaxilla and upper keratinous beak (rhamphotheca) are subparallel and separated (Fig. 1a, b). BMNH-CPH986 is an additional specimen of C. sanctus that has a disassociated rhamphotheca in a similar state of separation6, p. 156, but has not been commented on before. The rhamphothecae of IVPP V123524, IVPP V119774,5, and IVPP V115531,4 have no visible separation between the rhamphotheca and premaxilla. The rhamphotheca of STM 13-162 appears undeformed, as in BMNH-CPH9866, p. 156, IVPP V123524, and IVPP V119774,5. The extent of deformation of the rhamphotheca of IVPP V11553 could not be evaluated due to loss of the original specimen1.

Reconstruction. We reconstruct the in vivo position of the rhamphotheca in STM 13-162 in Fig. 1c by aligning the dorsal edge of the upper rhamphotheca with that of the premaxilla and then aligning the tip of the lower rhamphotheca with that of the upper one. The reconstructed rhamphotheca is straight and extends rostrally only a short distance past the premaxilla (Fig. 1c). The rostrocranial extent and dorsoventral thickness of the reconstructed rhamphotheca (Fig. 1c) is similar to the reconstructed rhamphotheca of BMNH-CPH986 (Fig. 1d) and the in situ rhamphothecae of IVPP V123524 (Fig. 1e) and IVPP V119774,5 (Fig. 1f). The rhamphotheca of IVPP V115531,4 (Fig. 1g), which may or may not be in situ, projects farther rostrally and is more curved than in STM 13-162, BMNH-CPH986, IVPP V123524, and IVPP V119774,5. All specimens except IVPP V12352 have a rhamphotheca that project caudally to around the level of the rostrocranial midpoint of the naris, without encircling it (Fig. 1c, d, f, g). It is unclear if termination of the
The rhamphotheca of STM 13-162 provides important data about how the rhamphotheca was structured in confuciusornithids. In modern birds, the beak dermis is anchored to the underlying bone via collagen fibres (Sharpey's fibres)\(^\text{15,16}\). Similar structures also anchor the cornified palatal epithelium of turtles\(^\text{17}\). This form of anchoring is present in the phylogenetic bracket of beaked reptiles and is widespread in vertebrates\(^\text{18}\), should be present in confuciusornithids. While it is possible that any collagen fibres may have decayed away after death, and that STM 13-162 and BMNH-PIH986\(^\text{6}\) p. 156 represent a more advanced stage of decay than other specimens, decay studies of modern birds show the skull detaching from the body before any parts of the skull (including the rhamphotheca) separate from one another\(^\text{19,20}\). The combination of an articulated skull and post-crania but a disassociated rhamphotheca in both STM 13-162 and BMNH-PIH986 would seem to imply that the rhamphotheca of Confuciusornis was relatively loosely attached in life. In finches, Sharpey's fibres tend to be more numerous in areas of higher stress\(^\text{17}\). This suggests that the strength of beak attachment might be a proxy for in vivo stress, with higher the functional implications of modern bird beak attachments are still not well understood. In this context, the beak of Confuciusornis may have experienced a relatively lower stress regime in life, but this will require further testing as larger fossil and living bird datasets become available. Despite its disassociation, the shape of the rhamphotheca itself does not appear to be deformed in either STM 13-162 or BMNH-PIH986 (Fig. 1b, 6 p. 156), suggesting that it was not particularly soft or malleable (contra\(^\text{6}\)).

Confirmation of a low stress regime can provide insight into the palaeoecology of Confuciusornis. Extant animals that consume softer foods tend to have jaws that are less well adapted to the high stress regimes associated with processing hard food\(^\text{21-24}\). While our preliminary finite element models cannot currently distinguish between sally-striking and generalized herbivory, they do indicate that the stress regime in the Confuciusornis mandible is incongruent with gleaning predation and oral processing of hard foods (Fig. 2). Claims that it had a strong lower jaw\(^\text{8}\) are also unsubstantiated. We therefore propose that Confuciusornis and other confuciusornithids would have preferentially taken soft foods, or that any granivory involved pecking and then processing in the gizzard, rather than oral processing (although gastroliths and seed remains seen in other Mesozoic birds\(^\text{7,25}\) are unknown in confuciusornithids, suggesting that this is unlikely). These conclusions are contingent on the taxa selected being adequate representatives of their feeding style. Future tests with larger sample sizes in each category should allow for more reliable and precise characterization of feeding in Confuciusornis. Our FEA findings are further supported by a cranial mechanical advantage calculated at 0.117 and a straight beak (Supplementary Fig. 1), which falls within the range of grabbing/gleaning, pecking/grazing, and probing uses of beak during feeding described by Fig. 5 in Nava\(\text{lon et al.}\)\(^\text{26}\). While these claims are tentative and vulnerable to taphonomic bias, we wish to highlight that the functional implications of beak attachment are worth exploring in further detail.

Comparisons between the keratinous and bony beak anatomy of confuciusornithids and modern birds provide an opportunity to better understand rhamphotheca formation in early birds. In modern birds, rhamphotheca formation is still poorly understood at a molecular level\(^\text{12,27}\). Recent studies have investigated gross morphological trends in rhamphotheca form relative to the underlying bone\(^\text{27-29}\), which presumably stems from common developmental systems. Rostrocaudal extent of the rhamphotheca, relative to homologous regions of the skull, is one of the most predictable rhamphotheca characteristics in modern birds. The caudal extension of the rhamphotheca in Confuciusornis to the
approximate level of the rostrocranial midpoint of the naris, without encircling it (Fig. 1c, d, e, g,5), resembles all but a few later-diverging avian taxa28,29 p. 34, 30. However, the relatively short rostral projection of the rhamphotheca in confuciusornithids despite their highly vascular rostra (Fig. 1c–f,5) is in stark contrast to modern birds. In avians, the rhamphotheca tends to extend farther rostrally beyond the skull with increased vascularization of the rostrum (i.e., larger premaxillary foramina)29 p. 35. As opposed to the other confuciusornithid specimens, IVPP V115531,4 has a longer rostral projection of the rhamphotheca as expected in modern birds, but a shape unlike modern birds. Rhamphotheca shape is controlled by more complex developmental factors than rostrocaudal extent12 and is in turn more difficult to quantify29,31.

The simplest metric of shape is the difference in curvature between the rhamphotheca and the premaxilla. In modern birds, the rhamphotheca is generally more curved than the premaxilla. As the premaxilla becomes more curved, the curvature of the rhamphotheca increases disproportionately29 p. 58, 31. STM 13-162 (Fig. 1), IVPP V123524, and IVPP V119774,5 all follow this trend. IVPP V115531,4 runs contrary to this trend in having a straight premaxilla and curved rhamphotheca more similar to some tortoises30. Confuciusornithids display rhamphotheca traits similar to modern birds in some ways (caudal extent, shape in most) and completely contrary in others (rostral projection, shape in IVPP V11553). This supports the hypothesis that similar mutations across the Dinosauria triggered the initial formation of beaks (possibly mediated by BMP411) before differing minutiae in their development (e.g., differing activation of proliferation centres27) led to the diversity of forms seen in the dinosaur fossil record11,12.

In summary, modern imaging techniques have revealed heretofore unknown detail of the rhamphothecae of early fossil birds. Assumptions regarding the in vivo position and thickness of the rhamphotheca should be made with caution as disassociation of the rhamphotheca and beak is possible, though from our reconstruction two specimens4,5 appear to not have undergone disassociation. This potential for disassociation is tentatively suggested as an indicator of low in vivo jaw stress regimes and an impetus for further study of beak attachment in modern birds.

Fig. 2 Confuciusornis sanctus STM 13-162 compared to extant sally-striking and granivorous birds. The mandible as reconstructed in Fig. 1c is converted into a two-dimensional Finite Element model (a) and compared to models of a sally-striking bee-eater, Merops orientalis (b), an herbivorous pheasant, Chrysophalus pictus (c), an aquatic-gleaning kingfisher, Alcedo atthis (d), and a granivorous finch, Lonchura malacca (e). The mandible of C. sanctus reacts to loading like a sally-striker or herbivore, with areas of high stress (warm colors) more similar in size and extent to that of M. orientalis and C. pictus than A. atthis or L. malacca. Results of the intervals method for comparing finite element models47 (f) corroborate this interpretation. Bar height indicates the percent area of a model which experiences a given interval of stress, with higher interval numbers indicating higher Von Mises stress. The majority of the model area in L. malacca and A. atthis is under low amounts of stress. Model area of C. sanctus, M. orientalis, and C. pictus show similar trends in stress throughout, with a positively-skewed distribution peaking at intervals of moderate stress.
Finite element modeling based on the new reconstruction also suggests a weak jaw. Signs of differential development between confuciusornithids and later-diverging birds include the dis-connect between premaxillary foramina and rostral extent of the rhamphotheca as well as the potential mismatch between pre-maxilla and rhamphotheca curvature. Our characterization of the beak condition in confuciusornithids provides crucial new insights into beak structure and development outside of crown Aves. Future specimens will be particularly valuable in developing increasingly better-supported reconstructions of edentulous fossil species, potentially elucidating biomechanical implications of beak detachment, and deepening understanding of the pathways and mechanisms of rhamphotheca formation.

**Methods**

LSF imaging was performed according to an updated version of the methodology of Z. Hou et al. so only a brief description of the method is provided here. A 405 nm laser diode was used to fluoresce the specimen following standard laser safety protocol. Thirty second time exposed images were taken with a Nikon D810 DSLR camera and 425 nm blocking filter. Image post-processing (equalization, saturation, and color balance) was performed uniformly across the entire field of view in Photoshop CS6. Figure 1b was photostacked in this program to maximize the clarity of the skull and rhamphotheca in the final image.

Reconstruction of the skull and rhamphotheca were made in CorelDRAW X8. Reconstruction of the skull in Fig. 1c, d is based primarily on an overlay of an existing skull reconstruction from Fig. 4 of Elzanowski et al. onto an image (color balance) was performed uniformly across the entire skull and rhamphotheca in the Thirty second time exposed images were taken with a Nikon D810 DSLR camera of those of extant birds representing each of these feeding behaviors using

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