

Evolution of tooth crown shape in Mesozoic birds, and its adaptive significance with respect to diet

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Abstract

Both the evolution of tooth morphology and the relationship between dental features and diet in toothed birds have long been studied. Here we quantify variation in tooth crown shape in 28 key Mesozoic bird species, and examine differences in dental morphology among birds belonging to different taxonomic groupings and inferred to have had different diets. Using geometric morphometric methods (GMM) and phylogenetic comparative methods (PCM), we found few clear differences in tooth crown shape between different taxonomic and ecological categories, and our analysis provides little support for many dietary inferences drawn in previous studies. However, the Solnhofen *Archaeopteryx*, *Jeholornis*, *Protopteryx*, *Pengornis*, *Longipteryx*, *Tianyuornis*, *Mengciusornis*, *Ichthyornis* and *Hesperornis* all were found to possess relatively specialized tooth crown shapes, perhaps reflecting specialized diets such as insectivory, granivory, piscivory and consumption of soft-shelled arthropods. Similarity in tooth crown shape across many Mesozoic birds may indicate the lack of dietary specialization, and the association between tooth form and diet may have been weakened in any case by ‘functional replacement’ of the dentition by a horny beak and, in many cases, gastroliths.

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1. Introduction

Although extant birds are toothless (Gill, 1995), the majority of their Mesozoic precursors retained teeth. Various studies have examined the evolutionary process of tooth loss in birds from the perspectives of adaptive significance, changes in dental morphology, molecular mechanisms, and evolutionary developmental mechanism (Dilger, 1957; Proctor and Lynch, 1993; Feduccia, 1999;

Zhou et al., 2010; Louchart and Viriot, 2011; Meredith et al., 2014; Wang et al., 2017, 2019). Before the eventual disappearance of teeth in the avian lineage, a considerable diversity of tooth forms existed both interspecifically (Louchart and Viriot, 2011; O’Connor and Chiappe, 2011; O’Connor, 2019) and intraspecifically (Rauhut et al., 2018), a situation which has been hypothesized to reflect adaptation to diverse diets.

A clear relationship between tooth shape and diet is typically present in mammals (Williams and Kay, 2001; Schwenk and Rubega, 2005; Muhlbachler and Solounias, 2006; Ungar, 2010), and has also been demonstrated in some teleost fishes (Rüber et al., 1999; Streelman et al.,

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2003), Mesozoic reptiles (Massare, 1987; Jones, 2009), and living amphibians (Gregory et al., 2016). It is probable that tooth shape was also correlated with diet in Mesozoic birds (Louchart and Viriot, 2011), but very few Mesozoic avian specimens provide direct evidence of diet such as preserved stomach contents, making it difficult to link tooth crown shape to specific dietary preferences. Accordingly, functional evaluation of the morphology of the skeleton, guided by mechanical principles and living analogs, has been widely regarded as the most reliable method of inferring feeding behavior that can be applied to most fossil birds. Dental morphology, however, has provided the primary basis for many such dietary inferences (O'Connor and Chiappe, 2011; O'Connor et al., 2013; O'Connor, 2019), including insectivory for *Archaeopteryx* (Elzanowski, 2002; Wellnhofer and Haase, 2009), durophagy for *Shenqiornis* and *Sulcavis* (O'Connor et al., 2013), and specialization on soft-shelled arthropods for *Pengornis* (O'Connor and Chiappe, 2011). Broader analyses combining information from the teeth, the rostrum and other portions of the skeleton, have been used to identify *Longirostravis* as a mud-prober (Hou et al., 2004) and *Shanweinia* (O'Connor et al., 2009) and *Longirostravis* (O'Connor, 2019) as insectivorous bark-probers.

Nevertheless, the dentition of most known toothed Mesozoic birds has been described and discussed in sufficient detail to support the interpretation that Mesozoic avian taxa were diverse in their diets (Louchart and Viriot, 2011; O'Connor and Chiappe, 2011; O'Connor, 2019). Within the genus *Archaeopteryx*, variation in crown shape and other aspects of dental morphology among different individuals has been variously explained as a product of: 1) sexual dimorphism (Howgate, 1984); 2) ontogenetic variation (Howgate, 1984); 3) intraspecific polymorphism (Howgate, 1984); 4) interspecific variation (Howgate, 1984); 5) adaptation to different diets and/or feeding behaviors (Howgate, 1984; Rauhut et al., 2018). The same set of explanations could also be applied to the diversity in tooth crown shape in other Mesozoic birds. However, most of the various dietary interpretations that have been put forward for Mesozoic avian taxa have never been subjected to any form of rigorous quantitative testing.

Here we investigate tooth crown shape variation in a range of Mesozoic birds, including representatives of key avian clades and individuals for which direct evidence of diet is available in the form of stomach contents, using a more quantitative approach than has been adopted in previous studies (O'Connor and Chiappe, 2011; O'Connor, 2019). The increasing number of Mesozoic birds whose tooth crown shapes have been well-studied made it possible for us to use the information from published works for this study. Geometric morphometric methods (GMM; Zelditch et al., 2012) and phylogenetic comparative methods (PCM; Garamszegi, 2014) were used to collect and analyze 2D tooth crown shape data. Our primary goal was to shed light on the functional morphology and evolution of the dentition in Mesozoic birds by evaluating variation in

crown shape across taxa, and by exploring the relationship between crown shape and diet.

2. Materials and methods

The 2D curvatures of the anterior and posterior edges of 103 well-preserved teeth from 28 species of 21 genera of toothed Mesozoic birds were measured using stereo camera reconstruction, implemented with the R package STEREO-MORPH (version 1.6.2, Olsen and Westneat, 2015). Given the lack of taxonomic clarity within *Archaeopteryx*, each individual of this genus was treated for purposes of the analysis as a representative of a different species, and the phylogenetic relationships among these notional *Archaeopteryx* species were resolved as a random dichotomous pattern (Wellnhofer and Haase, 2009; Kundrát et al., 2018; Rauhut et al., 2018). Data of *Archaeopteryx*, *Mengciusornis*, *Tianyuornis*, *Ichthyornis* and *Hesperornis* are measured from high-quality photos in the literature (Martin and Stewart, 1977; Wellnhofer and Haase, 2009; Zheng et al., 2014; Field et al., 2018; Kundrát et al., 2018; Rauhut et al., 2018; Wang et al., 2019), while data for other taxa were collected from specimen photos taken for this study.

We used 28 evenly spaced points (semilandmarks, Fig. 1) to capture the curvature of each edge (anterior and posterior) of each tooth included in the analysis. Semilandmarks from all teeth representing a given species were aligned by Procrustes superimposition, which scaled the tooth outlines to the same centroid size and aligned them as closely as possible by translating and rotating them in

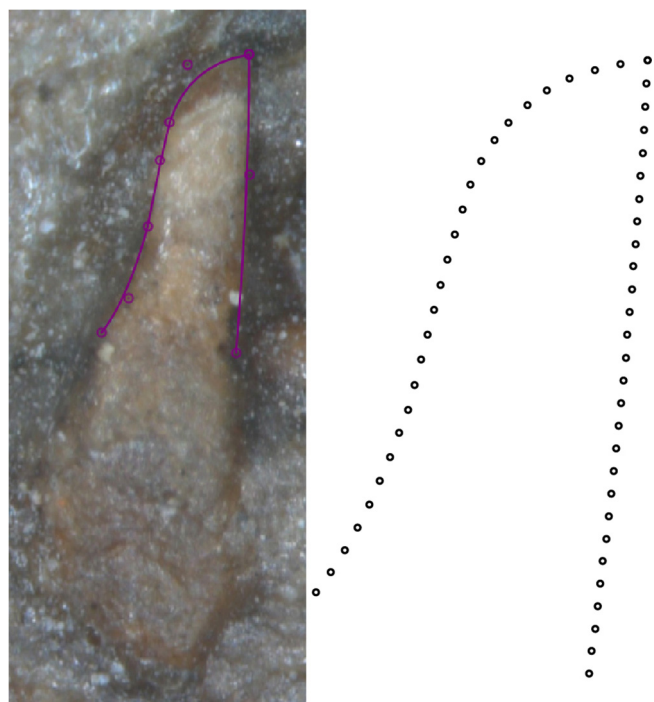


Fig. 1. Semilandmarks defining the margins of a tooth crown of Mesozoic birds.

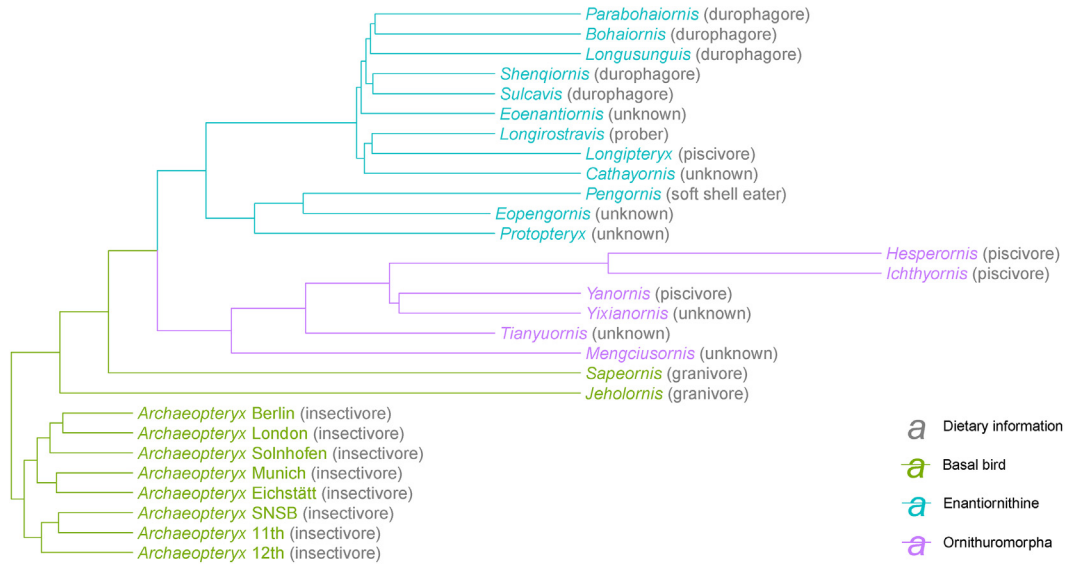


Fig. 2. Time-scaled phylogeny of Mesozoic birds included in this study, annotated with the diets inferred for various taxa in previous studies.

2D. The outlines were then averaged to generate a single mean semilandmark set for each species. All species mean sets were then aligned by a second Procrustes superimposition to collect Procrustes coordinates. Procrustes superimposition was done using the ‘gpagen’ function in the R package GEOMORPH (version 3.1.2; Adams et al., 2020).

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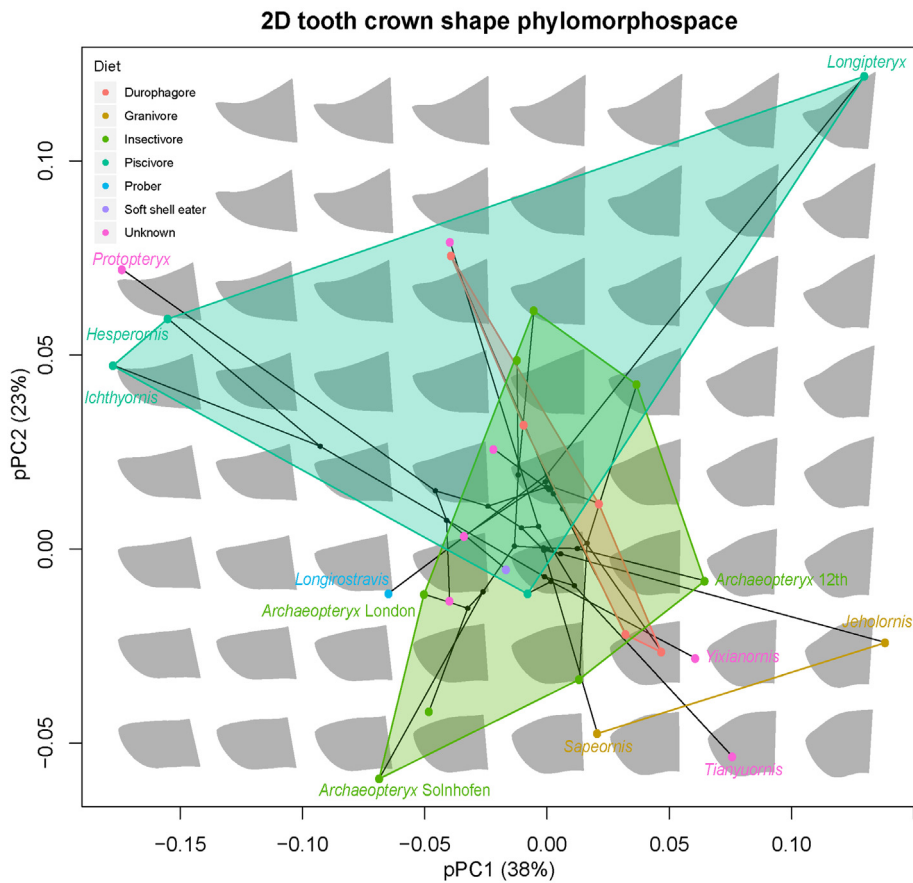


Fig. 3. 2D tooth crown shape phylomorphospace (PC1 vs PC2) for 28 species. Points, lines and polygons of different colors indicate different previously inferred diets. The tips of crown pointing left, the anterior edge of crown facing down.

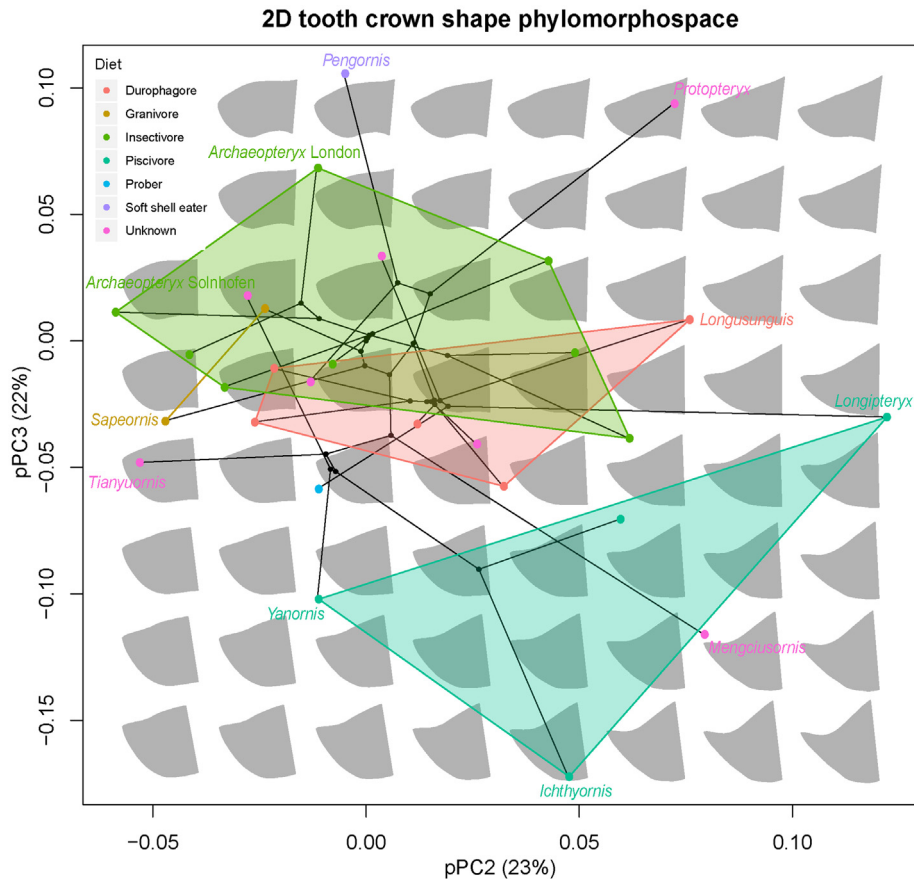


Fig. 4. 2D tooth crown shape phylomorphospace (PC2 vs PC3) for 28 species. Points, lines and polygons of different colors indicate different previously inferred diets. The tips of crown pointing left, the anterior edge of crown facing down.

Phylogenetic time trees were taken from publications (Wang, M. et al., 2015; Wang et al., 2019), simplified by removal of all taxa not included in the analysis, and combined with a randomly resolved trees for *Archaeopteryx* (Fig. 2). Phylogenetic signal in tooth shape (K_{mult} , Blomberg et al., 2003; Adams, 2014) was quantified using the GEOMORPH function ‘physignal’ (Blomberg et al., 2003).

Because tooth shape showed significant phylogenetic signal (K_{mult} : 0.668, $P > 0.001$), we performed a phylogenetic principal component analysis (pPCA) on the Procrustes coordinates in order to explore multivariate morphological variation in the data set, using the ‘phyl.pca’ function in the R package PHYTOOLS (version 0.6-60, (Revell, 2012)). To better visualize this variation, we used open source code (Olsen and Gremillet, 2017) to generate the backtransformations (MacLeod, 2009) of the principal components. This method took advantage of the fact that, in PCA, the original input matrix can be recovered by multiplying the PC score matrix by the inverse of the eigenvector matrix.

Most of the variation of tooth crown shapes is explained by the first five PC axes ($> 95\%$), so we used an adapted version of this procedure (Olsen and Gremillet, 2017) to visualize shape change along each of the first five PC axes generated in our analysis. These backtransform shapes rep-

resent the theoretical shape corresponding to a particular PC score or a pair of PC scores in multivariate space (Olsen and Gremillet, 2017).

2.1. Evolutionary allometric relationship tests

Evolutionary allometry is covariation of size and shape across a phylogeny (Cheverud, 1982). When the taxa included in a given study vary in both size and shape, some degree of covariance is usually present. In our analysis, crown size measures were used to test for an allometric relationship between crown shape (the Procrustes coordinates) and crown size (the log centroid size). Both non-phylogenetic and phylogenetic versions of this test were performed, using the function ‘procD.lm’ and ‘procD.pgls’ in the R package GEOMORPH.

2.2. Interpretation of dietary and taxonomic differences in tooth crown shapes

To visualize the results of our pPCA, we generated bivariate plots for each pair of successive PCs, from PC1 to PC5, with backtransformed tooth shapes to show theoretical shape variation across the bivariate phylomorphospace. Two sets of polygons, one representing

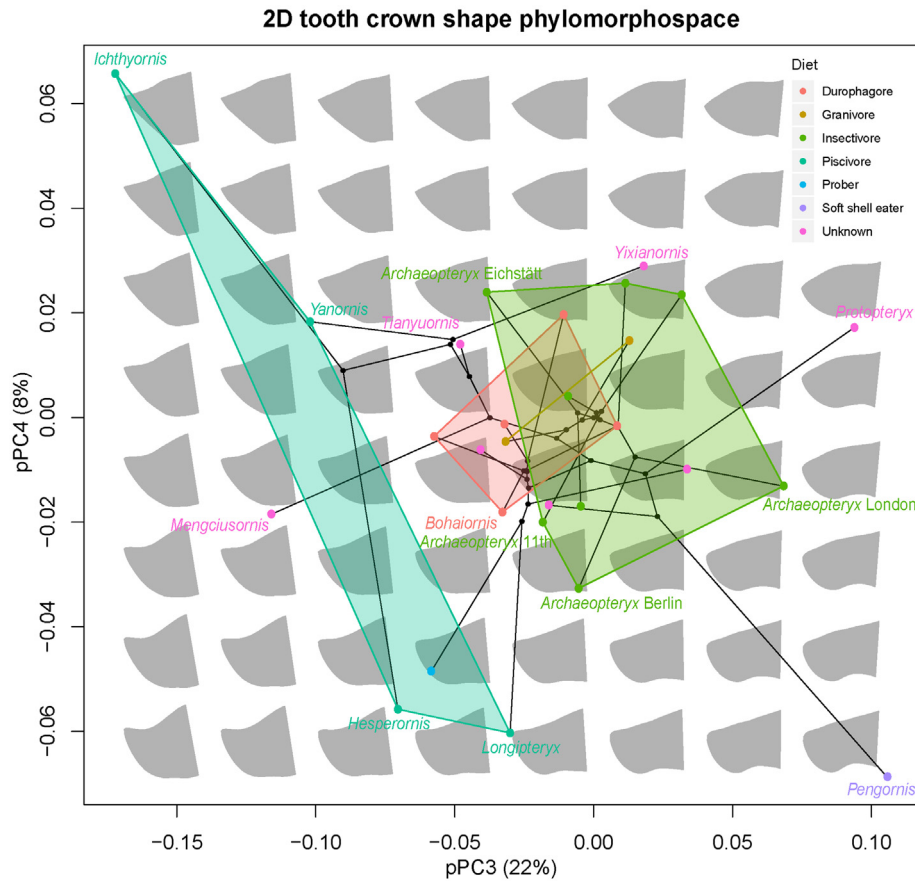


Fig. 5. 2D tooth crown shape phylomorphospace (PC3 vs PC4) for 28 species. Points, lines and polygons of different colors indicate different previously inferred diets. The tips of crown pointing left, the anterior edge of crown facing down.

taxonomic groupings and the other dietary categories, were overlaid (on separate versions of each plot) on the points representing the taxa in our analysis. Each polygon was drawn to encompass all of the birds in the taxonomic or dietary category in question. Three taxonomic polygons were used, corresponding to ‘basal birds’, enantiornithines and ornithuromorphs. The positions on the plots of *Jeholornis*, *Sapeornis* and *Yanornis* are particularly noteworthy (Fig. 10), as specimens of these birds preserve direct evidence of piscivory in the case of *Yanornis* and of granivory in the case of the other two taxa.

A quantitative method was used to test for significant differences in tooth shape between each possible pair of dietary categories, and each possible pair of taxonomic groupings. Specifically, a Euclidean NPMANOVA was performed on the 5 pPC scores using open source code (Martinez Arbizu, 2017) and function ‘adonis’ in the R package VEGAN (version 2.5-4, (Oksanen et al., 2019)).

3. Results

3.1. Tooth crown shape variation

Most of the variation in 2D curvature of tooth crown in Mesozoic birds (96%) is explained by 5 PC axes (pPC1-

pPC5: 36%, 25%, 23%, 9%, 3%, respectively; Figs. 3–10. A higher pPC1 value indicates a proportionally higher crown, more ‘*Ichthyornis*-like’ and less ‘*Jeholornis*-like’. A higher pPC2 value indicates a relatively straight anterior edge, more basally constricted crown, and more sloping posterior edge. A higher pPC3 value indicates a more anteriorly directed crown tip and less ‘hook-like’ crown, a higher pPC4 value indicates a more convex apical part of the anterior edge of the crown, and a higher pPC5 corresponds to a blunter crown tip and more robust crown base. Taken together, the remaining pPCs explain < 5% of the crown shape variation in the data set.

The principal components analysis of 2D tooth crown shape failed to clearly separate birds belonging to different dietary or taxonomic groupings into different regions of tooth crown phylomorphospace, (Figs. 3–10; note that the apices of the tooth crowns point left, and their anterior edges face downward). Separation of polygons is seen only in two of the phylomorphospaces, and only then with respect to diet (PC2 vs PC3, and PC3 vs PC4; Figs. 4, 5, 8, 9). The polygon representing piscivores is displaced in these phylomorphospaces from all other polygons shown on the plot, although the position of *Mengciusornis* ensures that a polygon plotted for taxa scored as having “unknown” diets would overlap that plotted for piscivores.

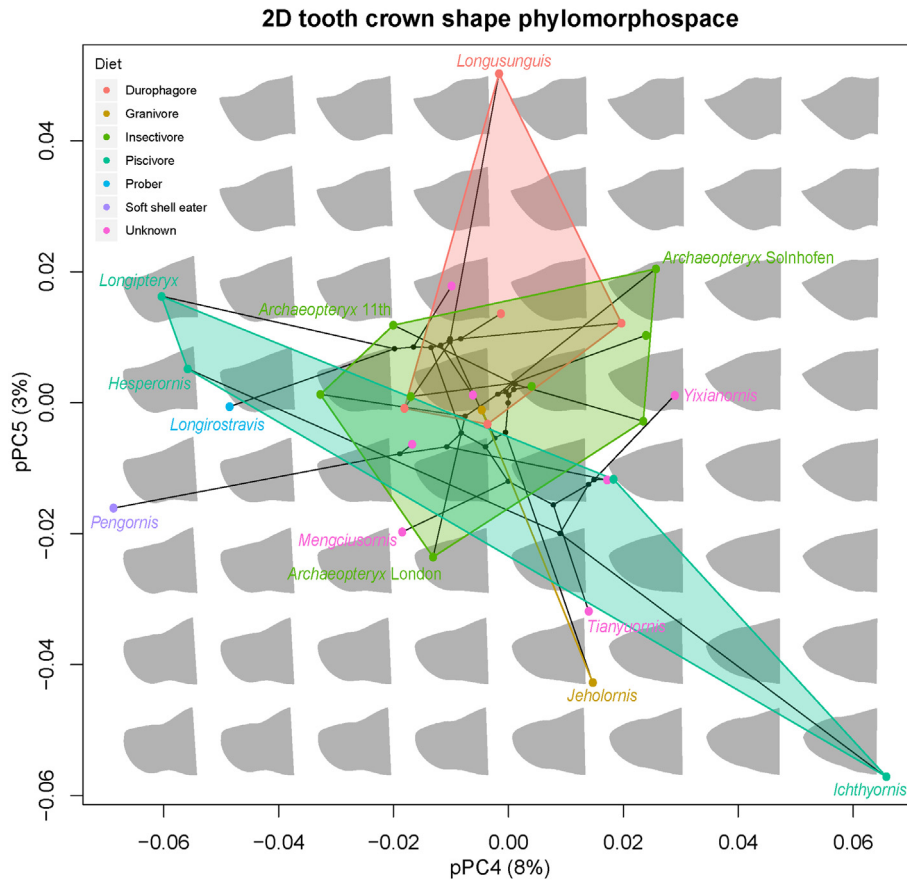


Fig. 6. 2D tooth crown shape phylomorphospace (PC4 vs PC5) for 28 species. Points, lines and polygons of different colors indicate different previously inferred diets. The tips of crown pointing left, the anterior edge of crown facing down.

Most of the polygons plotted for dietary or taxonomic groupings overlap with at least one other polygon in every phylomorphospace, although the single prober (*Longirostravis*) and single eater of soft-shelled arthropods (*Pengornis*) included in the analysis consistently plot outside the various polygons.

3.2. Evolutionary allometric relationship tests

Phylogenetic and non-phylogenetic tests both failed to demonstrate any statistically significant effect of crown size (log centroid size of crown) on crown shape (Table 1).

3.3. Taxonomic and dietary pairwise test

The NPMANOVA test failed to find statistically significant differences in tooth shape among the various dietary and taxonomic groupings (Fig. 1; Table 2). Furthermore, significant differences could not be identified even when the Bonferroni correction was not applied to the recovered *P*-values. Therefore, quantitative pairwise tests rejected the hypothesis that taxonomic and dietary differences in tooth crown shape exist among the Mesozoic birds included in our study.

4. Discussion

4.1. Influence of taxonomic difference on tooth crown shapes

Consistent with the non-significant results of the pairwise tests, and the significant phylogenetic signal present in our tooth shape data, the taxonomic and ecological polygons plotted in our phylomorphospaces showed substantial overlap (Figs. 3–10). Such results suggest considerable similarity in tooth crown shape across most Mesozoic birds.

The teeth of birds are less complex than those of many other theropods (Louchart and Pouech, 2017), particularly in lacking serrations on both the anterior and the posterior edges of the crown. Small, vertically oriented enamel ridges or grooves have been reported in *Longipteryx* (Wang, X. et al., 2015) and *Sulcavis* (O'Connor et al., 2013), but most Mesozoic bird teeth lack these specialized structures and can be referred as “peg-like” or “subtriangular”. Our results suggest narrowly limited diversity in tooth crown shape among basal birds, enantiornithines and ornithuromorphs. For this reason, identifying isolated teeth that are suspected to belong to Mesozoic birds may prove difficult, although considering the size and provenance of the

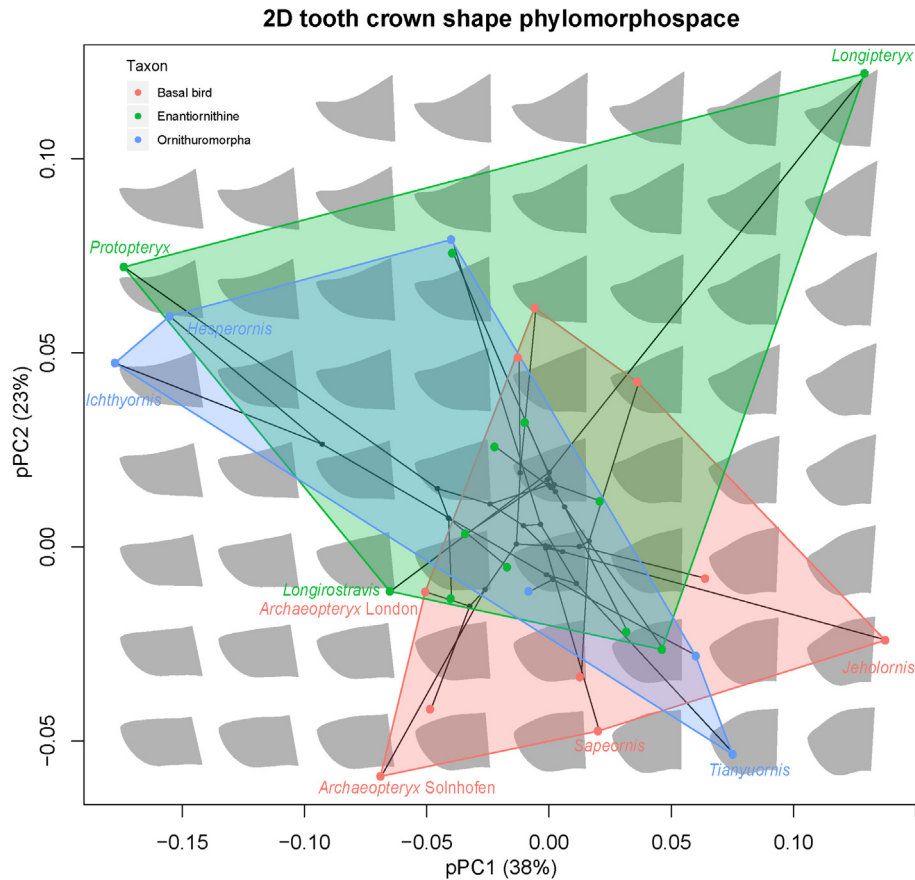


Fig. 7. 2D tooth crown shape phylomorphospace (PC1 vs PC2) for 28 species. Polygons of different colors represent different taxa. The tips of crown pointing left, the anterior edge of crown facing down.

specimens in conjunction with their shape is a potentially fruitful strategy (Louchart and Pouech, 2017).

4.2. Dietary interpretations, and adaptive significance of tooth crown shape

Our results show that tooth crown shape is too uniform across Mesozoic birds to provide strong support for most dietary interpretations that have been proposed in the literature. However, some such interpretations have been based not only on tooth crown shape, but also on other factors including tooth number, skull shape (*Longipteryx*, Zhang et al., 2001; Longipterygidae, O'Connor et al., 2011), forelimb shape and its locomotor implications (*Longipteryx*, Zhang et al., 2001) and even association of other fossil taxa (Wellnhofer and Haase, 2009). To address the question of why tooth shape is so uniform, we explore 3 possible explanations based on assumptions (Fig. 11).

4.2.1. First possible explanation: insufficient data

If we assume that 1) current dietary interpretations for Mesozoic birds are mostly correct, and 2) tooth crown shape has substantial adaptive importance and is subject to relatively strong selective pressure based on diet, then

it is surprising that our study differed from many previous ones (Bright et al., 2016; Pineda-Munoz et al., 2016) in failing to recover positive results such as a significant NPMA-NOVA P -value. Our negative results could then be interpreted as based on insufficient data, reflecting the limited nature of the currently available information on diet and tooth crown shape in Mesozoic birds. Some dietary categories in this study included few taxa (prober, $n = 1$; soft shell (soft-shelled arthropods) eater, $n = 1$; granivore, $n = 2$), and these small sample sizes may have a considerable influence on the results of our pairwise tests.

Consolidating the original seven dietary categories into four (invertebrate = insectivore + soft shell eater + prober + durophage; vertebrate = piscivore; unknown = unknown; seed = granivore). With all invertebrate-eating birds in a single enlarged category (Fig. 10), NPMA-NOVA produced lower P -values and more significant pairwise comparisons than were recovered in the seven-category version of the analysis (Table 3).

These pPCA results suggest that invertebrate-eating birds differ significantly in tooth shape from both granivorous and vertebrate-eating ones, diet group by pPCA and increasing sample sizes could improve the results of NPMA-NOVA. However, the total sample size ($n = 28$)

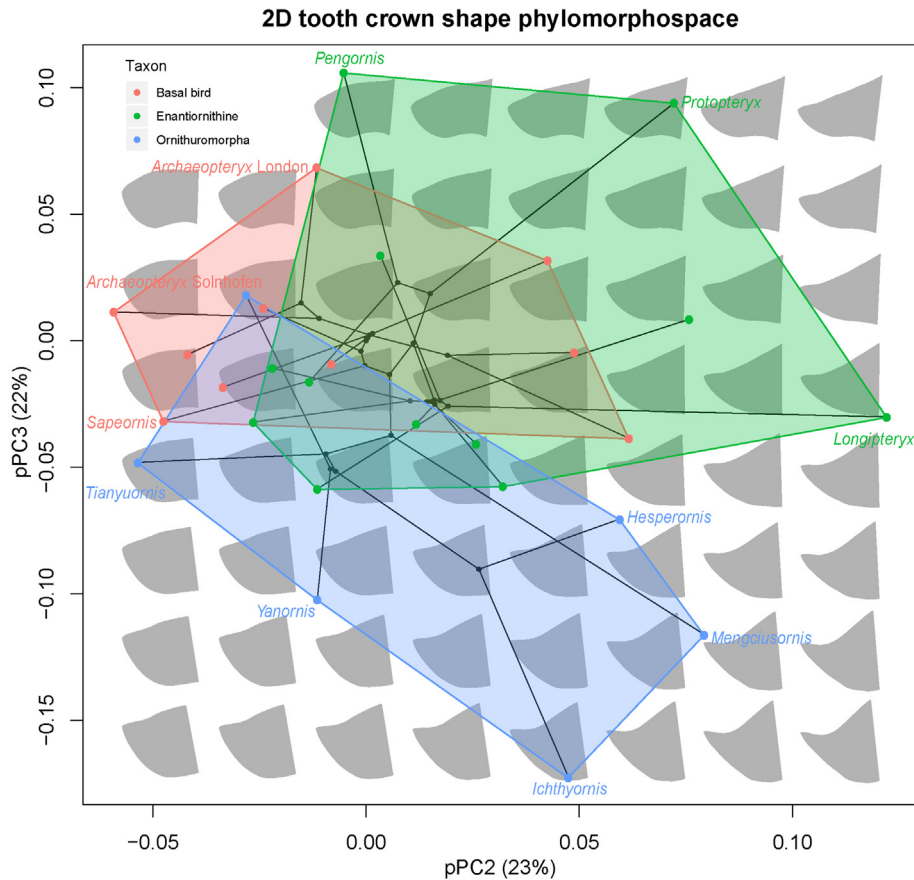


Fig. 8. 2D tooth crown shape phylomorphospace (PC2 vs PC3) for 28 species. Polygons of different colors represent different taxa. The tips of crown pointing left, the anterior edge of crown facing down.

used in our analysis is likely too small for clear differences in tooth shape to be detectable among members of all seven original dietary categories, even if such differences exist. Therefore, building a larger sample by collecting data from a greater number of well-preserved fossils, as well as refining interpretations of diet using more advanced methods (e.g., stable isotope analysis), could be essential for improving the results obtained in the present analysis and more accurately inferring diet from tooth crown shape data in Mesozoic birds.

4.2.2. Second possible explanation: a considerable number of Mesozoic birds are omnivores, and functional trade-offs strongly influenced tooth crown shape

Aside from the small size of our data set, another possible explanation for the failure of our results to corroborate many previous dietary interpretations may be that many of the birds in the study were to some extent generalist rather than specialist feeders. Their tooth crown shapes would then be strongly influenced by functional trade-offs involving dental adaptation to different food types (Shoval et al., 2012). This would imply that the generalist feeders should form a cluster in phylomorphospace, while taxa with morphologically specialized teeth should plot far from this main cluster and can be interpreted as likely to have been specialized in dietary terms as well.

In each 2D phylomorphospace considered in this study, we plotted a confidence ellipse surrounding the region that contains 60% of all taxa drawn from the Gaussian (normal) distribution of 4 pairs of PC scores (PC1 vs PC2; PC2 vs PC3; PC3 vs PC4; PC4 vs PC5; Fig. 12). We interpret the taxa within the confidence ellipses as generalist feeders, and those outside the ellipses as dietary specialists.

Following this logic, the tooth crown shapes of those taxa outside the ellipses (Solnhofen specimen of *Archaeopteryx*, insectivore; *Jeholornis*, granivore; *Protopteryx*, unknown diet; *Pengornis*, soft-shelled arthropod eater; *Longipteryx*, piscivore; *Tianyuornis*, unknown diet; *Mengciusornis*, unknown diet; *Ichthyornis*, piscivore; and *Hesperornis*, piscivore) reflect evolutionary adaptation to specialized diets. The tooth crown shapes of these taxa are substantially different from those of most Mesozoic birds in at least some phylomorphospaces, which makes the hypothesis that they were specialist feeders of various kinds more plausible.

4.2.3. Third possible explanation: teeth were decreasing in importance as they were functionally replaced a rhamphothecal and a muscular gizzard, often containing gastroliths

This explanation is essentially based on the hypothesis that Mesozoic birds underwent a trend towards functional

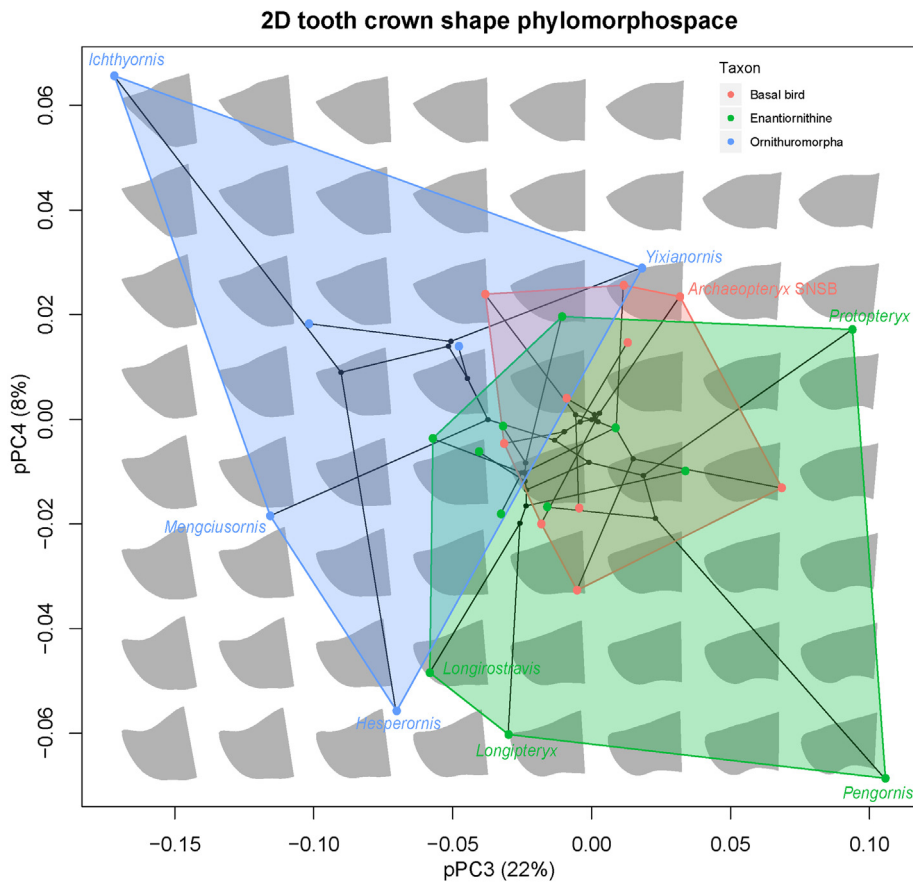


Fig. 9. 2D tooth crown shape phylomorphospace (PC3 vs PC4) for 28 species. Polygons of different colors represent different taxa. The tips of crown pointing left, the anterior edge of crown facing down.

‘replacement’ of the teeth by the combination of a rhamphothecal beak and a muscular gizzard (Davit-Béal et al., 2009; Louchart and Viriot, 2011), a process that may have accelerated reduction and eventual loss of the dentition. Although rarely preserved (Hou et al., 1999; Falk et al., 2019), the beaks of Mesozoic birds may have exhibited considerable diversity based on adaptation to different diets, as demonstrated in many living birds (Schluter and Grant, 1984; Gosler, 1987; Benkman, 1988; Price, 1991; Peterson, 1993; Gill, 1995; Barbosa and Moreno, 1999; Bardwell et al., 2001; Grant and Grant, 2006; Olsen and Gremillet, 2017).

If such diet-driven adaptation of the beak indeed occurred in toothed early avians, the functional importance and adaptive significance of tooth crown shape would have diminished accordingly. Gastroliths have also been found in some Mesozoic birds, representing another food-processing adaptation that could have partially usurped the role of the dentition.

The relatively high phylogenetic signal (Table 4) (Olsen and Gremillet, 2017; Felice and Goswami, 2018; Bright et al., 2019) recovered in our analysis for tooth crown shape under a Brownian motion model (Felsenstein, 1985) together with the negative results of the pairwise tests for dietary associations, offer at least circumstantial sup-

port for the hypothesis that the functional significance of the dentition had been transferred to the beak and/or muscular gizzard. Other anatomical features could also have played an important role in adaptation of different diets. Some dietary interpretations of Mesozoic birds have been based not only on dental features, but also on claw shape (Wang et al., 2014), rostrum shape (O’Connor and Chiappe, 2011; O’Connor, 2019), body size (O’Connor, 2019) and dozens of anatomical characters (Zanno and Makovicky, 2011).

More broadly, the adaptive importance of tooth crown shape must have diminished on the line to crown-group birds as additional feeding-related features evolved. These novel components of the feeding system, such as the rhamphotheca, may provide a stronger basis for dietary interpretations than the tooth crown shapes or other dentitional features.

If the function of the dentition was indeed being replaced to a substantial degree by other parts of the digestive system in Mesozoic birds, then the power of our analysis of tooth crown shape to generate robust dietary inferences is necessarily limited. Unfortunately, soft parts of the digestive system (oesophagus, crop, proventriculus (glandular stomach), ventriculus (muscular stomach or gizzard), the intestines, and ceca) are rarely preserved in these

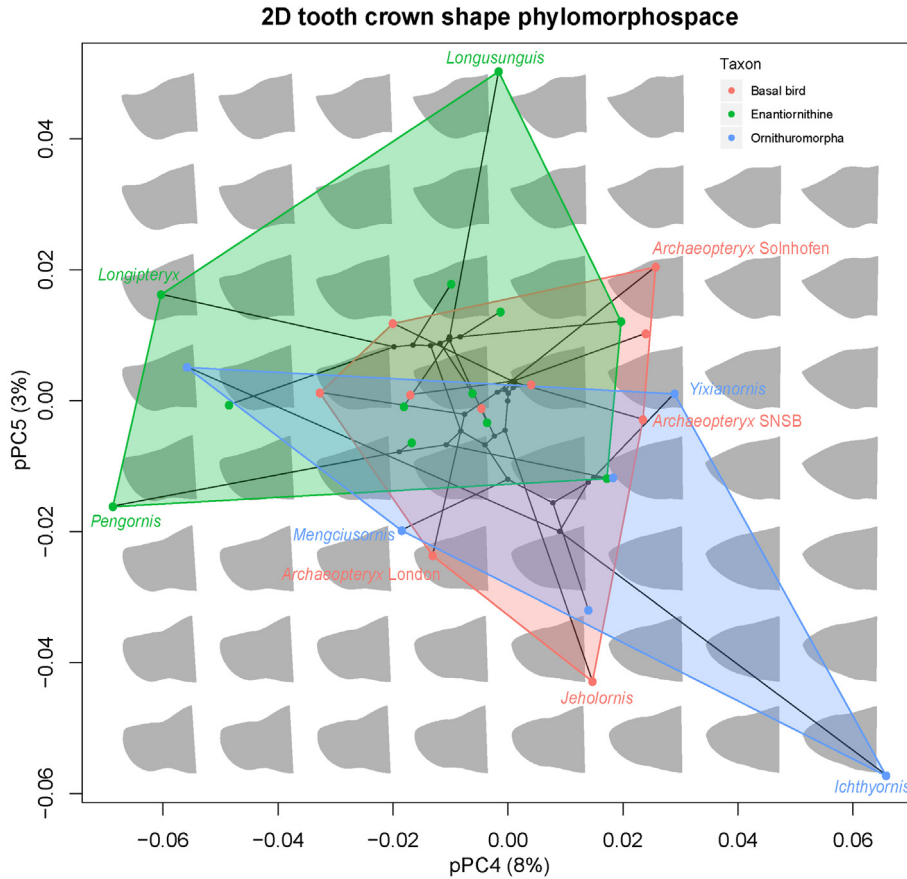


Fig. 10. 2D tooth crown shape phylomorphospace (PC4 vs PC5) for 28 species. Polygons of different colors represent different taxa. The tips of crown pointing left, the anterior edge of crown facing down.

Table 1
Tests of the evolutionary allometric relationship between tooth crown shape and centroid size.

	R ²	F	Z	P	Function
Shape-logCS	0.04943	1.3519	0.7299	0.239	procD.lm
Shape-logCS	0.03133	0.841	0.055968	0.5088	procD.pgls

taxa, and this is also largely true of gastroliths and ingested food items. Furthermore, gastroliths are found in living birds species which feed on a variety of different hard food items (Gionfriddo and Best, 1996), so that the presence of gastroliths in a fossil taxon would be consistent with an inference of frugivory, granivory, insectivory or omnivory.

Table 2
Pairwise tests for significant differences in tooth crown shape between various taxonomic and dietary categories based on pPC scores.

Method	NPMANOVA (5 pPCs scores)
Basal birds vs Enantiornithines	0.824
Basal birds vs Ornithuromorphs	0.116
Enantiornithines vs Ornithuromorphs	0.199
Insectivore vs piscivore	0.172
Insectivore vs durophagore	1
Piscivore vs durophagore	0.738

	AS strong	AS weak
DI mostly accepted	First possible explanation	Third possible explanation
DI mostly rejected	Second possible explanation	explanation

Fig. 11. Assumptions underpinning the three different possible explanations for the failure of our analysis to clearly support previous dietary inferences given in the discussion. AS: adaptive significance; DI: Dietary interpretation.

Table 3

Pairwise tests for significant differences in tooth crown shape between various taxonomic and dietary categories based on pPC scores, adjusted to test first possible explanation (see text). * indicates a significant difference ($P < 0.05$).

Method	NPMANOVA (5 pPCs score)	
	P	corrected P
Invertebrate vs seed	0.005 *	0.015 *
Invertebrate vs vertebrate	0.014 *	0.042 *
Seed vs vertebrate	0.267	0.801

Beak shapes is commonly seen as the most informative dietary indicator in birds, but its reliability has been called into question by studies in living birds of dietary adaption (Olsen and Gremillet, 2017) and correlated evolution between the beak and braincase (Bright et al., 2016, 2019), employing similar methods to those used in the present study.

Encouragingly, a recent study successfully revealed important relationships between form and ecological

function in living birds (Pigot et al., 2020). This suggests that more comprehensive data on the morphology of Mesozoic birds, including information on skull shape and limb proportions as well as on the dentition, may hold the key to drawing clearer inferences about these and other aspects of their ecology in the future.

5. Conclusion

Our analysis of tooth crown shape in Mesozoic birds fails to provide clear support for dietary interpretations presented in many previous studies, but shows that morphologically specialized tooth crown shapes occur in several Mesozoic birds including the Solnhofen *Archaeopteryx*, *Jeholornis*, *Protopteryx*, *Pengornis*, *Longipteryx*, *Tianyuornis*, *Mengciusornis*, *Ichthyornis* and *Hesperornis*. Based on the previously inferred diets of these taxa, insectivory, granivory, piscivory and consumption of soft-shelled arthropods were the feeding modes related to specialized tooth crown morphologies. Functional

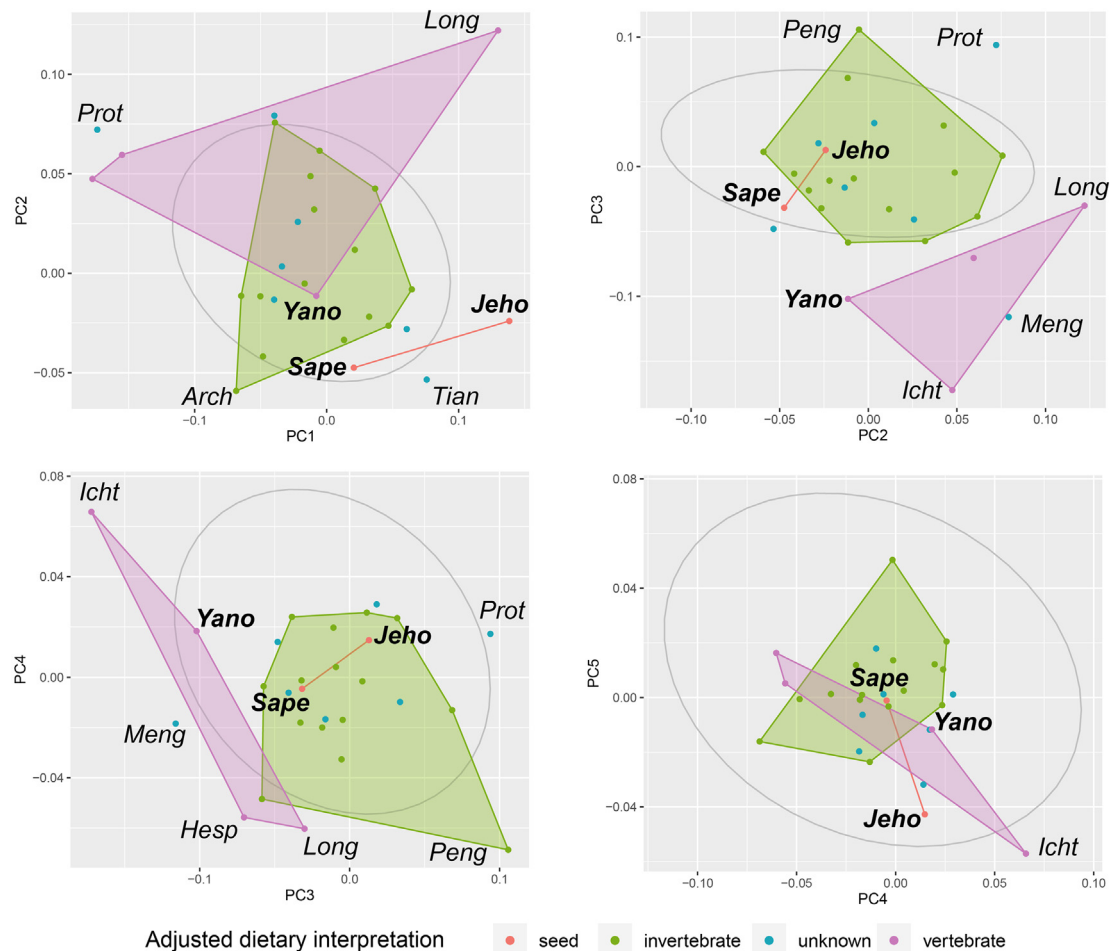


Fig. 12. Modified plots reflecting the possibility that tooth crown shape exhibits strong adaptive significance (first and second possible explanations). Polygons and lines in different colors represent adjusted dietary interpretations (first possible explanation); 60% confidence ellipses (gray) contain the 60% of total species which may be omnivores (second possible explanation). Abbreviated names of taxa for which direct evidence of diet is available are given in bold (Jeho and Sape: seed; Yano: fish; Arch, Solnhofen *Archaeopteryx*; Hesp, *Hesperornis*; Icht, *Ichthyornis*; Jeho, *Jeholornis*; Meng, *Mengciusornis*; Peng, *Pengornis*; Prot, *Protopteryx*; Sape, *Sapeornis*; Tian, *Tianyuornis*; Yano, *Yanornis*).

Table 4

Measures of phylogenetic signal (K_{mult}) for different phenotypic features in various groups of birds.

k	P	Taxon	Phenotype	Sample size	Data type	Reference
0.668	$P < 0.001$	Mesozoic birds	tooth crown shapes	28	shape coordinates	this study
0.661	$P < 0.001$	Mesozoic birds	tooth crown shapes	28	PC scores (95%)	this study
0.66	$P < 0.001$	living birds	pterygoid-quadrates	352	PC scores (95%)	(Felice and Goswami, 2018)
0.65	$P < 0.001$	living birds	palate	352	PC scores (95%)	(Felice and Goswami, 2018)
0.59	$P < 0.001$	living birds	occiput	352	PC scores (95%)	(Felice and Goswami, 2018)
0.59	$P < 0.001$	living birds	whole skull	352	PC scores (95%)	(Felice and Goswami, 2018)
0.58	$P < 0.001$	living birds	rostrum	352	PC scores (95%)	(Felice and Goswami, 2018)
0.56	$P < 0.001$	living birds	basisphenoid	352	PC scores (95%)	(Felice and Goswami, 2018)
0.56	$P < 0.001$	waterfowl	beak shapes	42	shapes coordinate	(Olsen and Gremillet, 2017)
0.55	$P < 0.001$	living birds	vault	352	PC scores (95%)	(Felice and Goswami, 2018)
0.48	$P < 0.001$	living birds	naris	352	PC scores (95%)	(Felice and Goswami, 2018)
0.344	$P = 0.001$	parrots and cockatoos	beak and braincase	170	shape coordinates	(Bright et al., 2019)

replacement of the dentition by a horny beak, and in some cases also by gastroliths, undermined the adaptive significance of tooth crown shape in other Mesozoic birds, among which the tooth crowns were quite similar in form.

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Supplementary data

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References

- Adams, D.C., 2014. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Systematic Biology* 63, 685–697.
- Adams, D.C., Collyer, M., Kaliontzopoulou, A., Sherratt, E., 2020. Geomorph: Software for geometric morphometric analyses. R package version 3.2.1. <https://cran.r-project.org/web/packages/geomorph/index.html>.
- Barbosa, A., Moreno, E., 1999. Evolution of foraging strategies in shorebirds: an ecomorphological approach. *The Auk* 116, 712–725.
- Bardwell, E., Benkman, C.W., Gould, W.R., 2001. Adaptive geographic variation in western scrub-jays. *Ecology* 82, 2617–2627.
- Benkman, C.W., 1988. Seed handling ability, bill structure, and the cost of specialization for crossbills. *The Auk* 105, 715–719.
- Blomberg, S.P., Garland Jr., T., Ives, A.R., 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57, 717–745.
- Bright, J.A., Marugán-Lobón, J., Cobb, S.N., Rayfield, E.J., 2016. The shapes of bird beaks are highly controlled by nondietary factors. *Proceedings of the National Academy of Sciences of the United States of America* 113, 5352–5357.
- Bright, J.A., Marugán-Lobón, J., Rayfield, E.J., Cobb, S.N., 2019. The multifactorial nature of beak and skull shape evolution in parrots and cockatoos (Psittaciformes). *BMC Evolutionary Biology* 19, 104.
- Cheverud, J.M., 1982. Relationships among ontogenetic, static, and evolutionary allometry. *American Journal of Physical Anthropology* 59, 139–149.
- Davit-Béal, T., Tucker, A.S., Sire, J.-Y., 2009. Loss of teeth and enamel in tetrapods: fossil record, genetic data and morphological adaptations. *Journal of Anatomy* 214, 477–501.
- Dilger, W.C., 1957. The loss of teeth in birds. *The Auk* 74, 103–104.
- Elzanowski, A., 2002. Archaeopterygidae (Upper Jurassic of Germany). In: Chiappe, M., Witmer, L. (Eds.), *Mesozoic Birds: Above the Heads of Dinosaurs*. University of California Press, Berkeley, Los Angeles, London, pp. 129–159.
- Falk, A., O'Connor, J.K., Wang, M., Zhou, Z., 2019. On the preservation of the beak in *Confuciusornis* (Aves: Pygostylia). *Diversity* 11, 212.
- Feduccia, A., 1999. *The Origin and Evolution of Birds*. Yale University Press, New Haven, 466 pp.
- Felice, R.N., Goswami, A., 2018. Developmental origins of mosaic evolution in the avian cranium. *Proceedings of the National Academy of Sciences of the United States of America* 115, 555–560.
- Felsenstein, J., 1985. Phylogenies and the comparative method. *The American Naturalist* 125, 1–15.
- Field, D.J., Hanson, M., Burnham, D., Wilson, L.E., Super, K., Ehret, D., Ebersole, J.A., Bhullar, B.S., 2018. Complete *Ichthyornis* skull illuminates mosaic assembly of the avian head. *Nature* 557, 96–100.
- Garamszegi, L.Z., 2014. *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology: Concepts and Practice*. Springer, Heidelberg, New York, Dordrecht, London, 552 pp.
- Gill, F.B., 1995. *Ornithology*. W.H. Freeman, New York, 763 pp.
- Gionfriddo, J.P., Best, L.B., 1996. Grit-use patterns in North American birds: the influence of diet, body size, and gender. *The Wilson Bulletin* 108, 685–696.
- Gosler, A.G., 1987. Pattern and process in the bill morphology of the Great Tit *Parus major*. *Ibis* 129, 451–476.
- Grant, P.R., Grant, B.R., 2006. Evolution of character displacement in Darwin's finches. *Science* 313, 224–226.
- Gregory, A.L., Sears, B.R., Wooten, J.A., Camp, C.D., Falk, A., O'Quin, K., Pauley, T.K., 2016. Evolution of dentition in salamanders: relative roles of phylogeny and diet. *Biological Journal of the Linnean Society* 119, 960–973.
- Hou, L., Martin, L.D., Zhou, Z., Feduccia, A., Zhang, F., 1999. A diapsid skull in a new species of the primitive bird *Confuciusornis*. *Nature* 399, 679–682.
- Hou, L., Chiappe, L.M., Zhang, F., Chuong, C.M., 2004. New Early Cretaceous fossil from China documents a novel trophic specialization for Mesozoic birds. *Naturwissenschaften* 91, 22–25.
- Howgate, M.E., 1984. The teeth of *Archaeopteryx* and a reinterpretation of the Eichstätt specimen. *Zoological Journal of the Linnean Society* 82, 159–175.
- Jones, M., 2009. Dentary tooth shape in Sphenodon and its fossil relatives (Diapsida: Lepidosauria: Rhynchocephalia). In: Koppe, T., Meyer, G.,

- Alt (Eds.), Comparative Dental Morphology. Karger Publisher, Basel, pp. 9–15.
- Kundrát, M., Nudds, J., Kear, B.P., Lü, J., Ahlberg, P., 2018. The first specimen of *Archaeopteryx* from the Upper Jurassic Mörnsheim Formation of Germany. *Historical Biology* 31, 3–63.
- Louchart, A., Pouech, J., 2017. A tooth of Archaeopterygidae (Aves) from the Lower Cretaceous of France extends the spatial and temporal occurrence of the earliest birds. *Cretaceous Research* 73, 40–46.
- Louchart, A., Viriot, L., 2011. From snout to beak: the loss of teeth in birds. *Trends in Ecology & Evolution* 26, 663–673.
- MacLeod, N., 2009. Form & shape models. *Palaeontological Association Newsletter* 72, 14–27.
- Martin, L.D., Stewart, J.D., 1977. Teeth in *Ichthyornis* (Class: Aves). *Science* 195, 1331–1332.
- Martinez Arbizu, P., 2017. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.0 1. <https://github.com/pmartinezarbizu/pairwiseAdonis>.
- Massare, J.A., 1987. Tooth morphology and prey preference of Mesozoic marine reptiles. *Journal of Vertebrate Paleontology* 7, 121–137.
- Meredith, R.W., Zhang, G., Gilbert, M.T.P., Jarvis, E.D., Springer, M.S., 2014. Evidence for a single loss of mineralized teeth in the common avian ancestor. *Science* 346 (6215), 1254390.
- Mihlbachler, M.C., Solounias, N., 2006. Coevolution of tooth crown height and diet in oreodonts (Merycoidodontidae, Artiodactyla) examined with phylogenetically independent contrasts. *Journal of Mammalian Evolution* 13, 11–36.
- O'Connor, J.K., 2019. The trophic habits of early birds. *Palaeogeography, Palaeoclimatology, Palaeoecology* 513, 178–195.
- O'Connor, J.K., Chiappe, L.M., 2011. A revision of enantiornithine (Aves: Ornithothoraces) skull morphology. *Journal of Systematic Palaeontology* 9, 135–157.
- O'Connor, J.K., Wang, X.R., Chiappe, L.M., Gao, C.L., Meng, Q.J., Cheng, X.D., Liu, J.Y., 2009. Phylogenetic support for a specialized clade of Cretaceous enantiornithine birds with information from a new species. *Journal of Vertebrate Paleontology* 29, 188–204.
- O'Connor, J.K., Chiappe, L.M., Gao, C.L., Zhao, B., 2011. Anatomy of the Early Cretaceous Enantiornithine bird *Rapaxavis pani*. *Acta Palaeontologica Polonica* 56, 463–475.
- O'Connor, J.K., Zhang, Y.G., Chiappe, L.M., Meng, Q.J., Li, Q.G., Di, L., 2013. A new enantiornithine from the Yixian Formation with the first recognized avian enamel specialization. *Journal of Vertebrate Paleontology* 33, 1–12.
- Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGinn, D., 2019. vegan: Community ecology package. R package version 2.5-4. R Foundation for Statistical Computing. <https://cran.r-project.org/web/packages/vegan/index.html>.
- Olsen, A.M., Gremillet, D., 2017. Feeding ecology is the primary driver of beak shape diversification in waterfowl. *Functional Ecology* 31, 1985–1995.
- Olsen, A.M., Westneat, M.W., 2015. StereoMorph: an R package for the collection of 3D landmarks and curves using a stereo camera set-up. *Methods in Ecology and Evolution* 6, 351–356.
- Peterson, A.T., 1993. Adaptive geographical variation in bill shape of scrub jays (*Aphelocoma coerulescens*). *The American Naturalist* 142, 508–527.
- Pigot, A.L., Sheard, C., Miller, E.T., Bregman, T.P., Freeman, B.G., Roll, U., Seddon, N., Trisos, C.H., Weeks, B.C., Tobias, J.A., 2020. Macroevolutionary convergence connects morphological form to ecological function in birds. *Nature Ecology & Evolution* 4, 1–10.
- Pineda-Munoz, S., Lazagabaster, I.A., Alroy, J., Evans, A.R., Cooper, N., 2016. Inferring diet from dental morphology in terrestrial mammals. *Methods in Ecology and Evolution* 8, 481–491.
- Price, T., 1991. Morphology and ecology of breeding warblers along an altitudinal gradient in Kashmir, India. *The Journal of Animal Ecology* 60, 643–644.
- Proctor, N.S., Lynch, P.J., 1993. *Manual of Ornithology: Avian Structure & Function*. Yale University Press, New Haven, 340 pp.
- Rauhut, O.W.M., Foth, C., Tischlinger, H., 2018. The oldest *Archaeopteryx* (Theropoda: Avialiae): a new specimen from the Kimmeridgian/Tithonian boundary of Schamhaupten, Bavaria. *PeerJ* 6, e4191. <https://doi.org/10.7717/peerj.4191>.
- Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things): phytools: R package. *Methods in Ecology and Evolution* 3, 217–223.
- Rüber, L., Verheyen, E., Meyer, A., 1999. Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proceedings of the National Academy of Sciences of the United States of America* 96, 10230–10235.
- Schluter, D., Grant, P.R., 1984. Determinants of morphological patterns in communities of Darwin's finches. *The American Naturalist* 123, 175–196.
- Schwenk, K., Rubega, M., 2005. Diversity of vertebrate feeding systems. In: Sarck, M.J., Wang, T. (Eds.), *Physiological and Ecological Adaptations to Feeding in Vertebrates*. Science Publishers, Enfield, pp. 1–41.
- Shoval, O., Sheftel, H., Shinar, G., Hart, Y., Ramote, O., Mayo, A., Dekel, E., Kavanagh, K., Alon, U., 2012. Evolutionary trade-offs, Pareto optimality, and the geometry of phenotype space. *Science* 336, 1157–1160.
- Streelman, J., Webb, J., Albertson, R., Kocher, T., 2003. The cusp of evolution and development: a model of cichlid tooth shape diversity. *Evolution & Development* 5, 600–608.
- Ungar, P.S., 2010. *Mammal Teeth: Origin, Evolution, and Diversity*. Johns Hopkins University Press, Baltimore, 304 pp.
- Wang, M., Zhou, Z.H., O'Connor, J.K., Zelenkov, N.V., 2014. A new diverse enantiornithine family (Bohaiornithidae fam. nov.) from the Lower Cretaceous of China with information from two new species. *Vertebrata Palasiatica* 52, 31–76.
- Wang, M., Zheng, X., O'Connor, J.K., Lloyd, G.T., Wang, X., Wang, Y., Zhang, X., Zhou, Z., 2015. The oldest record of Ornithuromorpha from the Early Cretaceous of China. *Nature Communications* 6, Article number 6987, doi: 10.1038/ncomms7987.
- Wang, M., O'Connor, J.K., Zhou, S., Zhou, Z., 2019. New toothed Early Cretaceous ornithuromorph bird reveals intraclade diversity in pattern of tooth loss. *Journal of Systematic Palaeontology* 18, 1–15.
- Wang, S., Stiegler, J., Wu, P., Chuong, C.M., Hu, D.Y., Balanoff, A., Zhou, Y.C., Xu, X., 2017. Heterochronic truncation of odontogenesis in theropod dinosaurs provides insight into the macroevolution of avian beaks. *Proceedings of the National Academy of Sciences of the United States of America* 114, 10930–10935.
- Wang, X., Shen, C., Liu, S., Gao, C., Cheng, X., Zhang, F., 2015. New material of *Longipteryx* (Aves: Enantiornithes) from the Lower Cretaceous Yixian Formation of China with the first recognized avian tooth crenulations. *Zootaxa* 3941, 565.
- Wellnhofer, P., Haase, F., 2009. *Archaeopteryx: The Icon of Evolution*. Verlag Dr. Friedrich Pfeil, München, 208 pp.
- Williams, S.H., Kay, R.F., 2001. A comparative test of adaptive explanations for hypsodonty in ungulates and rodents. *Journal of Mammalian Evolution* 8, 207–229.
- Zanno, L.E., Makovicky, P.J., 2011. Herbivorous ecomorphology and specialization patterns in theropod dinosaur evolution. *Proceedings of the National Academy of Sciences of the United States of America* 108, 232–237.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D., 2012. *Geometric Morphometrics for Biologists: A Primer*. Academic Press, California, 444 pp.
- Zhang, F., Zhou, Z., Hou, L., Gu, G., 2001. Early diversification of birds: evidence from a new opposite bird. *Chinese Science Bulletin* 46, 945–949.
- Zheng, X.T., O'Connor, J.K., Wang, X.L., Zhang, X.M., Wang, Y., 2014. New information on Hongshanornithidae (Aves: Ornithuromorpha) from a new subadult specimen. *Vertebrata Palasiatica* 52, 217–232.
- Zhou, Z., Zhang, F., Li, Z., 2010. A new Lower Cretaceous bird from China and tooth reduction in early avian evolution. *Proceedings of the Royal Society B: Biological Sciences* 277, 219–227.