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The patterns and modes of the evolution of disparity in Mesozoic birds

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The origin of birds from non-avian theropod dinosaurs is one of the greatest transitions in evolution. Shortly after diverging from other theropods in the Late Jurassic, Mesozoic birds diversified into two major clades—the Enantiornithes and Ornithuromorpha—acquiring many features previously considered unique to the crown group along the way. Here, we present a comparative phylogenetic study of the patterns and modes of Mesozoic bird skeletal morphology and limb proportions. Our results show that the major Mesozoic avian groups are distinctive in discrete character space, but constrained in a morphospace defined by limb proportions. The Enantiornithines, despite being the most speciose group of Mesozoic birds, are much less morphologically disparate than their sister clade, the Ornithuromorpha—the clade that gave rise to living birds, showing disparity and diversity were decoupled in avian history. This relatively low disparity suggests that diversification of enantiornithines was characterized in exhausting fine morphologies, whereas ornithuromorphs continuously explored a broader array of morphologies and ecological opportunities. We suggest this clade-specific evolutionary versatility contributed to their sole survival of the end-Cretaceous mass extinction.

1. Introduction

Birds represent arguably one of the most morphologically and ecologically diverse clades of living tetrapods, with much of their evolutionary success pertaining to novel features [1]. Among them, powered flight expresses itself as one of the driving forces that resulted in convergent evolution of aerodynamically adaptive morphologies in the first half of avian evolution [2,3]. To comprehend to what degree and how an innovation as significant as flight shapes the bauplan across different lineages of Mesozoic birds as they diversified, macroecological methods that capture morphological changes and their adaptive significance within the context of phylogeny that spans the breadth of Mesozoic birds diversity are needed. Over the last three decades, fossil data have accumulated rapidly, with bird fossils collected from Mesozoic outcrops worldwide demonstrating a large scale of diversification and dragging back the appearances of many characteristic features of crown birds [2,4–6]. The wealth of this data now makes it feasible to investigate the tempo and mode of the diversity of Mesozoic birds, offering insight into critical questions in evolutionary biology. For instance, comparative studies show that Mesozoic birds evolved faster than non-avian theropods [7,8], and rates of morphological evolution can be shown to be heterogeneous among Early Cretaceous avian lineages [9,10]. However, these and other studies focused on either discrete morphological characters or continuous measurements independently, and with smaller taxonomic samples, which may not sufficiently capture macroevolutionary patterns. Continuous measurements (e.g. body mass, limb length) may be interpreted as changes in body proportions, whereas discrete characters attempt to encapsulate detailed anatomical change. These data types therefore provide different types of information and when integrated may provide greater insight

into the mechanism(s) of diversification. Here we show that, despite being more speciose, enantiornithines were less morphologically disparate than ornithuromorphs, and that the latter exhibited a greater exploration of novel morphologies during the Mesozoic.

2. Materials and methods

(a) Morphological and limb-length dataset

A character matrix targeting the phylogeny of Mesozoic birds was assembled, which is the latest version of the Mesozoic Avian Phylogeny (MAP) project, which is actively being maintained by the avian evolution research team of the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP). Compared to the most recent MAP dataset (electronic supplementary material), six recently described Mesozoic birds were added, their scorings based on direct observation and supplemented by published descriptions. The revised dataset, consisting of 280 discrete characters and 80 taxa (Mesozoic birds: $n = 77$; outgroup: Dromaeosauridae; extant taxa: *Gallus gallus*, *Anas platyrhynchos*), is currently the most comprehensive dataset of Mesozoic birds, including nearly all well-recognized taxa that are frequently evaluated in recent phylogenetic studies. The discrete characters cover anatomical traits from across the body (including both skeletal and integumentary characters), conveying an advantage over size measurements in capturing major morphological transitions and the appearance of novel features that underpin the macroevolution of Mesozoic birds. Given that these anatomical features focus on 'local' morphologies, it is useful to also investigate changes in 'global' morphology (e.g. body size, limb proportions) for comparison, to collectively capture the breadth of morphological evolution in early birds. Comparisons of the patterns recovered from these frameworks are valuable to key biological questions, such as why some clades exhibit greater disparity in 'local' features but not in body shape, or vice versa? To address this issue, we assembled a dataset of the length of six appendicular limb elements (humerus, ulna, carpometacarpus, femur, tibiotarsus and tarsometatarsus) from Mesozoic birds that are included in the character dataset (electronic supplementary material, data S1). These bones are generally better preserved and their lengths can be measured with greater accuracy. All included specimens were adults or subadults based on fusion of the compound elements and the well-ossified periosteal surfaces of preserved elements. Importantly, limb proportions are ecologically informative and are widely used in the ecological reconstruction of extinct vertebrates [11]. Due to the controversy regarding scaling relationships of limb size, only specimens that preserve complete length for all six limbs were included. The length dataset thus contains 54 taxa, but still covers all major Mesozoic avian clades and demonstrates diverse ranges of body sizes.

(b) Phylogenetic analyses and time-scaled phylogeny of Mesozoic birds

To reconstruct the phylogeny of Mesozoic birds, the character dataset was analysed under equally weighted parsimony using TNT v. 1.5 [12]. The 'New Technology search' method was applied to find the most parsimonious trees (MPTs) with sectorial search, ratchet, tree drift and tree fusion with default settings, and a minimum length tree was found in 10 replicates. The obtained MPTs were subjected to a final round of branch-swapping using the traditional tree-bisection-reconnection method to explore treespace more extensively. The branches of the MPTs were collapsed if the minimum length equalled zero. Bootstrap and Bremer values were calculated as the support

indices. The absolute bootstrap values were calculated via 1000 replicates using the same settings as the primary search, and the Bremer values were calculated using the Bremer script in TNT.

Phylogenetic inference produced 896 MPTs, with a length of 1393 steps (consistency index = 0.279, retention index = 0.663). The strict consensus tree (SCT) is well-resolved (figure 1), and the major relationships recovered are consistent with recent studies [13,14]. The SCT was time-scaled using tip dates bracketed by the first and last appearance datum of the geological stages where the fossils were collected [15]. Zero-length branches were avoided through two methods: with a fixed minimum duration (here, 1 million years), using the 'minimum branch length' (mbL) method [16]; alternatively, the branches were smoothed by allowing them an equal share of the duration of the preceding non-zero-length branch ('equal' method) [17]. We also performed Bayesian analyses using tip-dating methods as in [10], which co-estimate the topology and branch lengths (electronic supplementary material figure S1).

(c) Limb proportions phylomorphospace

To visualize the diversity of limb proportions among Mesozoic birds, the limb proportions phylomorphospace (liPhMoSp) was constructed using phylogenetically corrected ordination of the limb length data. The length measurements were \log_{10} -transformed to extract the non-allometric limb proportions before ordination. To account for size-dependent limb measurements, we calculated the residuals from a least-squares regression of the \log_{10} -transformed length data against body weight using the `phyl.resid` function of the R `phytools` package [18]. Circularity was avoided by estimating Mesozoic bird body masses using the empirical scaling relationships of the circumference of the femur with body weight derived from living bipedal tetrapods (electronic supplementary material, data S1) [19,20].

The time-scaled strict consensus tree was used as the phylogenetic backbone, and taxa that were not included in the length dataset were pruned from the tree. The size-corrected residuals of the six limb elements were subjected to phylogenetic principal components analysis (pPCA) to account for non-independence in phenotypes between species due to shared history [21,22]. pPCA was conducted using the `phyl.pca` function of the R package `phytools`, which estimated ancestral states using maximum likelihood under a Brownian motion model [18]; alternatively, the ancestral states were estimated using the lambda method. To visualize the morphospace, principal components (PC) scores of taxa and ancestral nodes were generated using the `phylomorphospace` function of the R package `phytools` (electronic supplementary material, data S2–S5) [18]. The resulting liPhMoSp allowed us to trace phylogeny and disparity simultaneously, displaying the direction and magnitude of morphological variations along different branches [23].

(d) Discrete morphological character morphospace

In order to explore the disparity of anatomical structures in Mesozoic birds, a morphospace was constructed by calculating a pairwise distance matrix from the discrete dataset, following a standard pipeline [24]. This discrete morphological character morphospace (dsMoSp) takes advantage of an ordination technique that results in far fewer axes than the number of input characters to summarize the morphological variance [7,23,25]. All the following analyses were performed using the R package `Claddis` [24]. First, a distance matrix was constructed by calculating a morphological distance between each pair of taxa. We chose the maximum observable rescaled distance (MORD) and the generalized Euclidean distance (GED) as our distance metrics, because they are more suitable to datasets containing fossils

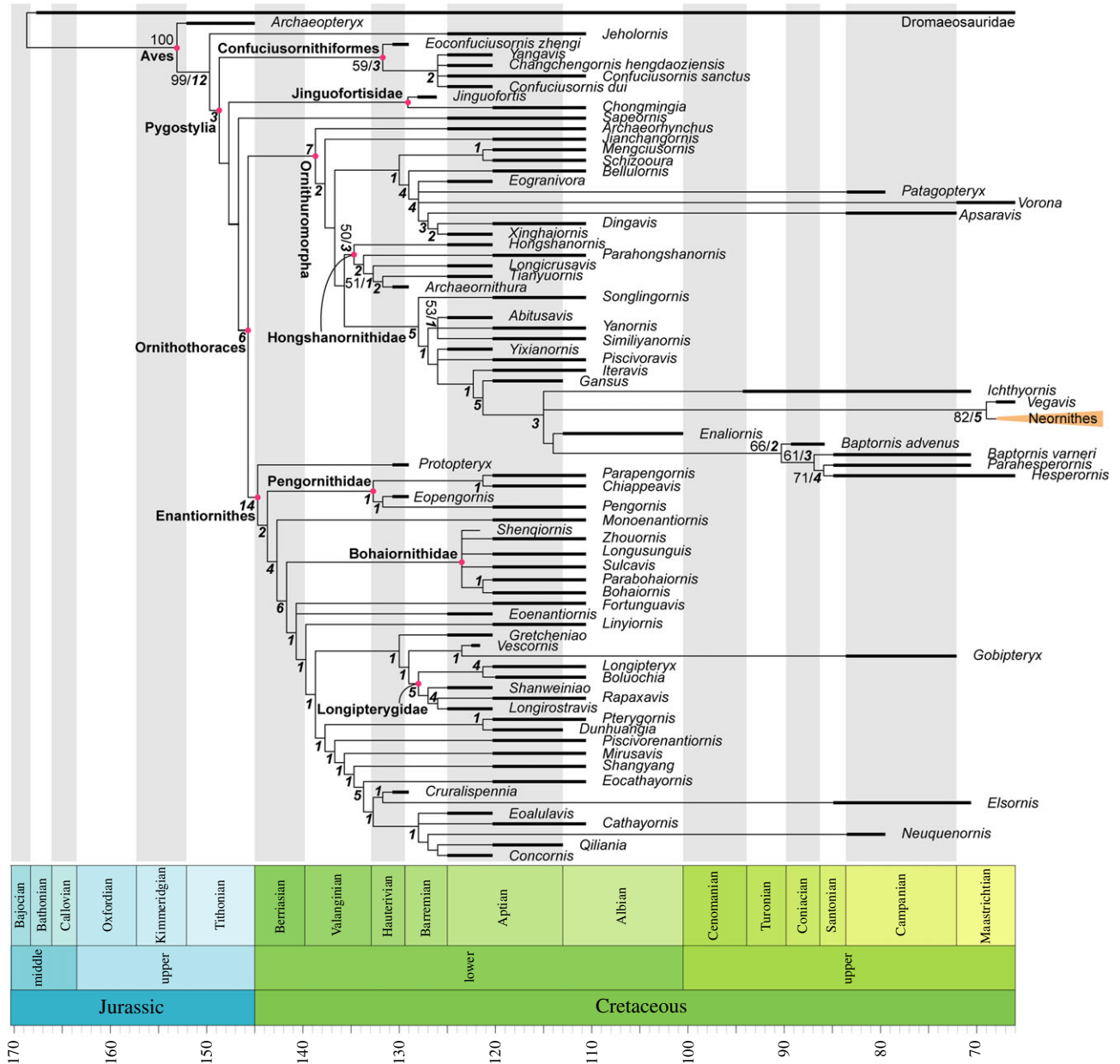


Figure 1. Time-scaled phylogeny of Mesozoic birds. The strict consensus tree is derived from 896 most parsimonious trees and is scaled using the ‘mbf’ method (see electronic supplementary material, figure S1 for result using the tip-dated phylogeny). Bootstrap and Bremer values are denoted in normal and *italic* formats, respectively. (Online version in colour.)

[24,26]. For comparison, distance metrics were also calculated using the ‘HSJ’ approach to better account for inapplicable characters implemented in Claddis [27]. Each pairwise distance matrix was then subjected to principal coordinates analysis (PCA). A non-phylogenetic morphospace was formed by projecting taxa based on their scores on principal coordinates axes (pcoas) and was used in disparity analysis (electronic supplementary material, data S6–S13).

We also constructed a discrete morphological character phylo-morphospace (dsMoPhSp) by taking phylogeny into account during PCA (electronic supplementary material, data S14–S21). States of ancestral nodes were estimated directly from the discrete characters using the likelihood methods and following the recommended settings [23]. The quantitative expression of disparity in discrete characters that is coupled with phylogeny in the dsPhMoSp enables us to trace disparity through time and compare patterns of morphological change between closely related groups (here, the sister clades Enantiornithes and Ornithuromorpha). We also test the effect of body mass on the discrete character distance matrix [28] (electronic supplementary material).

(e) Disparity and macroevolutionary models

To quantify and compare patterns of disparity through time and among groups, three commonly used disparity metrics were calculated, the sum of variances and ranges, and the median distance from centroids [29–31]. Variance describes the average dissimilarity among taxa and ranges captures the overall morphological dispersion [30]. The last metric denotes the median Euclidean distance of each taxon from the centroid of their own group in ordination space [29]. First, we investigated the disparity of limb proportions through time for all Mesozoic birds. Disparity metrics derived from PC scores were calculated and bootstrapped using 1000 replicates to give a confidence level using the *disPRity-through.time* function of the R package *disPRity* [32]. For intergroup comparisons, the phylogeny was divided into three contiguous groups, Enantiornithes, Ornithuromorpha and ‘non-Ornithothoraces’. The last group is paraphyletic and includes all sampled taxa outside Ornithothoraces (Enantiornithes + Ornithuromorpha), and consists of *Archaeopteryx*, *Jeholornis*, *Confuciusornithiformes*, *Jinguoformitidae* and *Sapeornis*, and is used here simply as a baseline comparison.

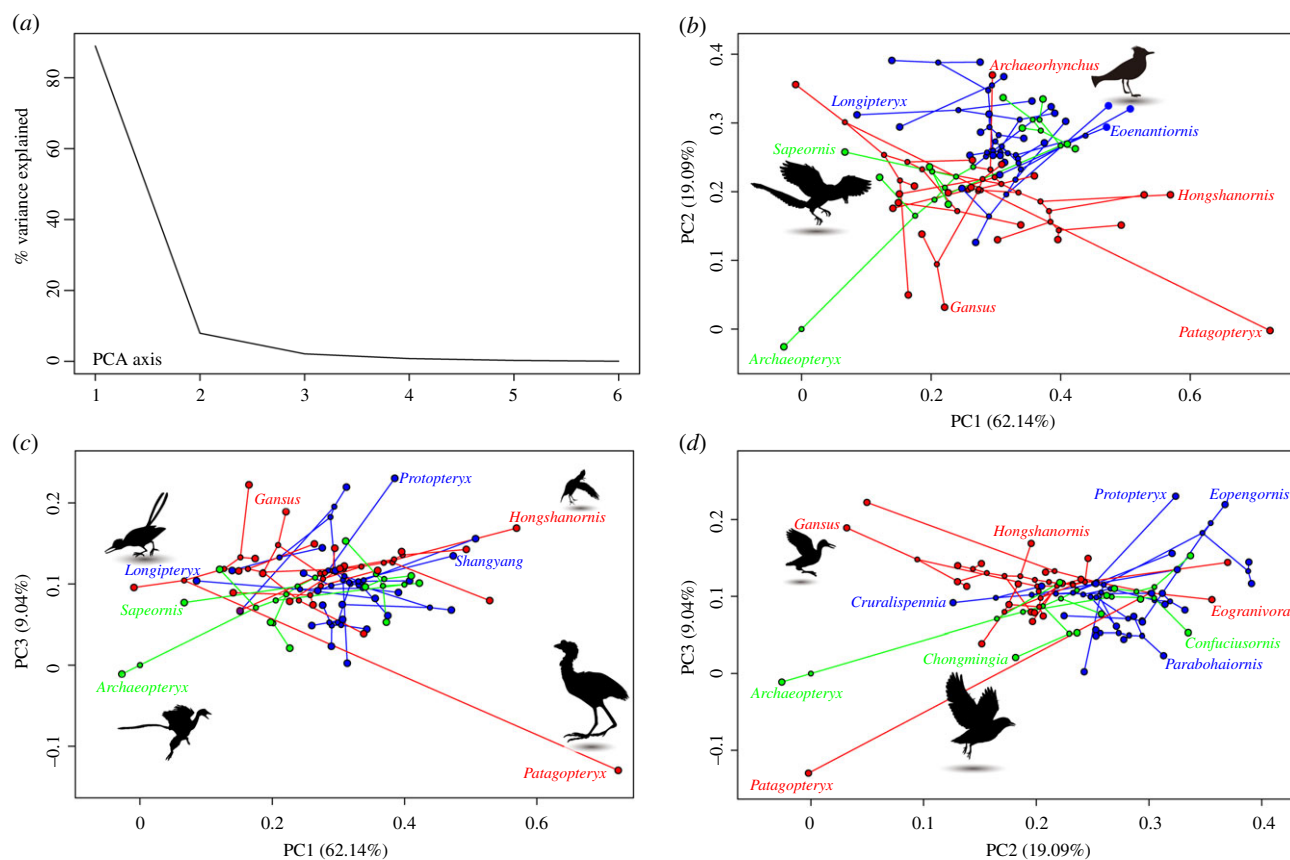


Figure 2. Limb proportions phylomorphospace of Mesozoic birds depicting appendicular limb length variations. The phylogenetic backbone is derived from the 'mbl'-scaled strict consensus tree. (a) Proportions of variances accounted by each principal component (PC) recovered from phylogenetic principal components analysis; (b–d) pairwise binary plots of PCs 1–3. 'Non-Ornithothoraces' are shown in green, Enantiornithes in blue and Ornithomorphs in red. Silhouettes are modified from [4,7], except *Patagopteryx* (from <http://www.phylopic.org>). See electronic supplementary material, figures S2, S5 and S6 for results using the lambda method, 'equal'-scaled and tip-dated phylogenies, respectively. (Online version in colour.)

The three disparity metrics were also calculated based on pcoas scores derived from the discrete dataset. Here, the outgroup Dromaeosauridae was assigned to 'non-Ornithothoraces' just to increase the sample size of this group. In order to test whether some groups are statistically unique in dsMoSp and dsMoPhSp occupation, we conducted the nonparametric multivariate analysis of variance (PERMANOVA) test using the *adonis* function of the R package *vegan* [33]. The Mahalanobis distance between a pair of groups was used as the dissimilarity index, and 999 permutations were performed to compute a significance value for each pairwise comparison. We also divided the discrete characters into six anatomical subregions (skull, pectoral girdle, vertebral column, forelimb, pelvis and hindlimb) [9], and repeated the aforementioned morphometric and disparity analyses to explore whether those subregions exhibit different patterns of morphological evolution.

Temporal disparity results may be biased by the fact that Mesozoic avian fossils are much better known from Early Cretaceous sites, potentially making Early Cretaceous birds appear more disparate than Late Cretaceous forms. To ameliorate this sampling bias, ancestral nodes were included in disparity analyses, which increased sample size from all-time bins as well as individual groups (in a fully resolved phylogeny, the number of ancestral nodes is one fewer than the number of terminal taxa). On the other hand, we also rarefy the data to account for uneven sampling by estimating the morphological changes for progressively reduced sample size [29], using the *boot.matrix* function of the R package *dispRity* [32]. These methods collectively provide robust estimates of disparity patterns [29,34,35].

Finally, we tested the fit of five commonly used models for disparity metrics of discrete characters using the *model.test* function of the R package *dispRity* [32]. The five models—stasis

(a null model describing time-invariant change in disparity), Brownian motion, trend, Ornstein–Uhlenbeck with one optimum, and the early burst models [36]—are compared using the sample-size-corrected Akaike information criterion (AICc) and Akaike weights [37].

3. Results

(a) Phylomorphospace and disparity of limb proportions

The pPCA using the appendicular limb length shows that Mesozoic avian groups, as well as their estimated ancestral nodes (using either BM or lambda methods), overlap substantially in phylomorphospace based on plots of the first three principal components (PCs 1–3: greater than 90% of limb proportions-related variance; figure 2a; electronic supplementary material figure S2), around which taxa with extreme limb proportions are scattered at the periphery (figure 2b–d). PC1 correlates negatively with all size-corrected residues (electronic supplementary material, table S1), suggesting that it refers to appendicular elongation and thus correlates with body size to some degree; the eigenvector coefficients of forelimb measurements are all greater than those of hindlimbs, indicating that PC1 describes whether the increase of limb length largely results from the elongation of the forelimb or the hindlimb. For instance, the Late Cretaceous ornithomorph *Patagopteryx*, a flightless taxon [38], is the largest taxon analysed here and has the proportionately shortest forelimb (less than half the hindlimb) and has the highest PC1 score

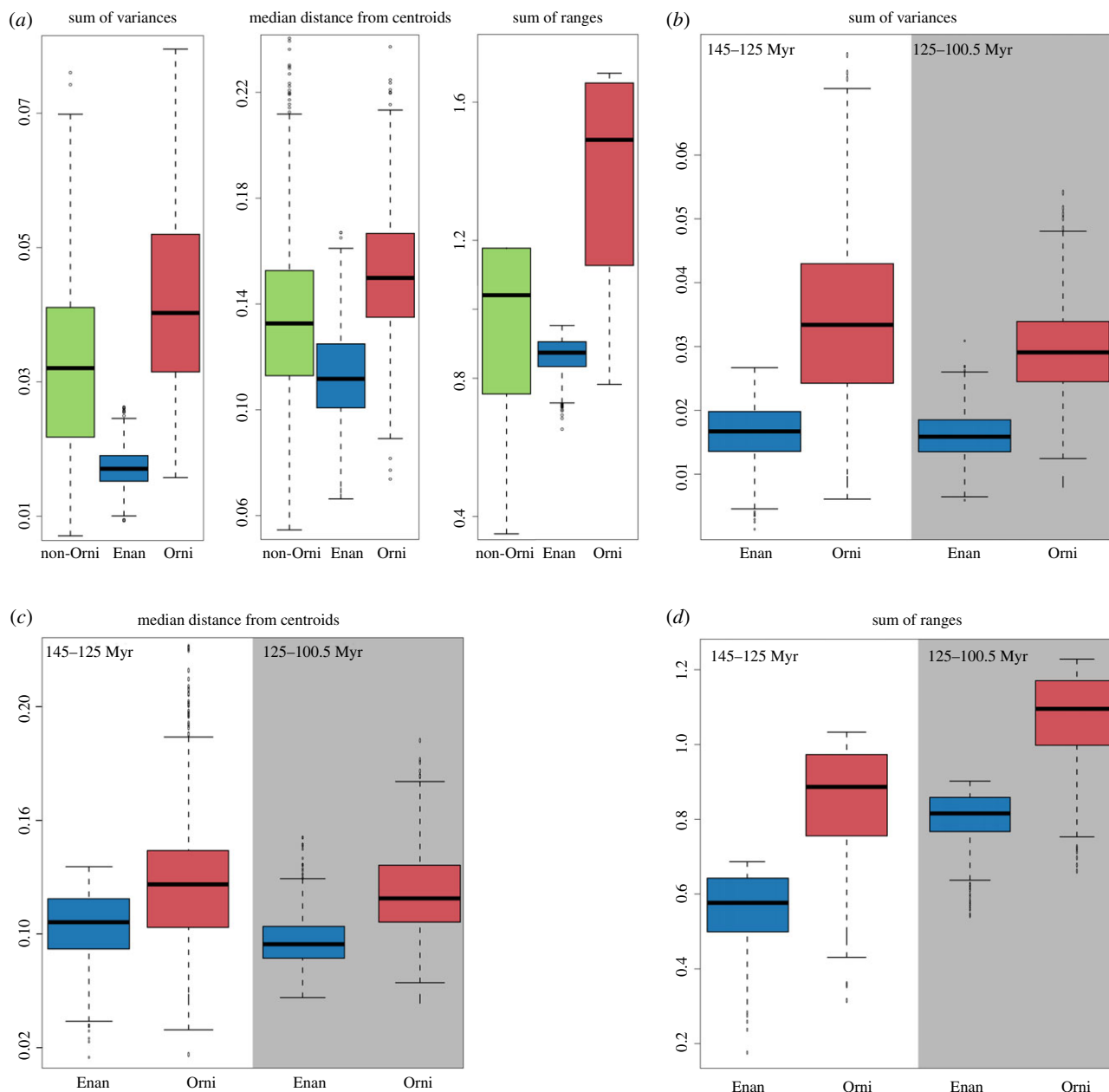


Figure 3. Comparison of limb proportion disparity among Mesozoic birds. The phylogenetic backbone is derived from the ‘mbI’-scaled strict consensus tree. Three disparity metrics are shown: sum of variances, median distance from centroids and sum of ranges. (a) Box plots showing disparity metrics among Mesozoic avian groups; (b–d) comparison of disparity metrics between Enantiornithes (Enan) and Ornithuromorpha (Orni) during the Early Cretaceous in two equal-length time bins (145–125, 125–100.5 Myr). Non-Orni, non-Ornithothoraces. See electronic supplementary material, figures S7 and S8 for results using the ‘equal’-scaled and tip-dated phylogenies. (Online version in colour.)

(data S2); by contrast, the basal pygostylian *Sapeornis*, although the second largest taxon in the dataset, is located far away from *Patagopteryx* with a PC1 score close to zero, because its large body size is mainly ascribed to its’ elongate forelimb (1.5 times hindlimb). The relatively high loading of tarsometatarsus length on PC2 suggests this primarily captures elongation of this element relative to other appendicular bones. PC3 correlates positively with both carpometacarpus and tarsometatarsus lengths, but negatively with other elements. The relatively high loadings of carpometacarpus and femur lengths, which are opposite in sign, indicate that PC3 describes the elongation of the former compared to the latter.

These results thus suggest that ‘non-Ornithothoraces’, Ornithuromorpha and Enantiornithes are convergent in morphospace defined by limb length, which is somewhat

counterintuitive given that these groups exhibit different ecological preferences and body shape (e.g. enantiornithines and ornithuromorphs are generally considered arboreal and terrestrial, respectively; *Archaeopteryx* and *Jeholornis* have long bony tails) [5,11].

Disparity analyses of limb proportions of all taxa as a whole reveal different patterns through time depending on the metrics used, and how the ancestral states were estimated. When ancestral states were estimated using the BM method, the sum of variances and the median distance from centroid change little through time, but the sum of ranges increases (figure 3a; electronic supplementary material, figure S3). The result suggests that the spread of limb proportion variations of Mesozoic birds expands in morphospace over time, but the average dissimilarity among species remains static (few taxa with aberrant limb proportions), supporting

the hypothesis that the limb proportions of early birds are evolutionarily conservative [8,39]. Intergroup comparison shows that ornithuromorphs are more disparate than other avian groups, regardless of metric choice (figure 3*a*). Enantiornithes, often considered the most taxonomically diverse clade of Mesozoic birds [2,40], are the least disparate, despite contributing more taxa to the length dataset ($n = 23$, compared with 21 ornithuromorphs, and 10 'non-ornithothoracines'; electronic supplementary material, data S1). This observation supports previous suggestions that enantiornithines are generally uniform in body shape [40]. The Early Cretaceous contains the majority of the evidence for the earliest diversification of Enantiornithes and Ornithuromorpha [5]. The greater disparity of the Ornithuromorpha is robust to data manipulation, such as removing the outlier such as *Patagopteryx* and subdividing the Early Cretaceous into two equal time bins (figure 3; electronic supplementary material figure S4). These time series show that overall morphological variations (sum of ranges) of both groups increases, but the average interspecies differences remain static (electronic supplementary material, figure S3*d-i*; results using the 'equal'-scaled and tip-dated methods are presented in electronic supplementary material, figures S5–S9 and tables S2–S4). Similar results were recovered when the ancestral states were estimated using the lambda method: ornithuromorphs are the most disparate in terms of the sum of ranges, but the 'non-ornithothoracines' appear to be more disparate in terms of the other two metrics (electronic supplementary material, figures S10–S12). However, this discrepancy is largely ascribed to a single taxa *Sapeornis* within the 'non-Ornithothoraces'. When *Sapeornis* was excluded, ornithuromorphs became the most disparate regardless of metric choice (electronic supplementary material, figure S10*b*).

(b) Discrete character morphospace and patterns of disparity

The first 40 and 30 pcoas are used for disparity analyses and constructing a phylomorphospace (dsMoPhSp) and non-phylogenetic morphospace (dsMoSp), respectively. The three groups of Mesozoic birds are statistically separated from one another in dsMoPhSp and dsMoSp based on PERMANOVA tests across all analyses ($p < 0.01$; electronic supplementary material, tables S5–S9) [31]. Particularly, in the binary plot of pcoas 1 and 2, the non-Ornithothoraces are grouped along the negative side of pcoa 2, whereas the Enantiornithes and Ornithuromorpha form two clusters that are separated along pcoa 1 (figure 4*a-c*). The distinctiveness of the three groups in discrete character morphospace is in stark contrast with the pattern from morphospace defined by limb proportions where they show clear overlap. Analyses using the different distance metrics and branch-scaling methods produce similar results (electronic supplementary material, figures S13–S16). The Mantel and multivariate phylogenetic least-squares tests recovered a significant correlation between body mass and pcoas (electronic supplementary material, figure S17; see electronic supplementary material for complete results). However, only pcoa 1 shows a correlation coefficient with body mass surpassing the two-tailed 95% confidence interval, and body mass only accounts for less than 9% of the variation of the morphological distance (electronic supplementary

material table S10). Therefore, our results are not strongly influenced by body mass.

Mesozoic birds as a whole show different patterns of disparity depending on the metric used. The sum of variances generally increases over time, but the lines for the sum of ranges and median distance from centroid drop sharply during the Early–Late Cretaceous transition (figure 5*a,b*; electronic supplementary material figure S18–S22). The latter pattern likely captures the generally poorer preservation of Late Cretaceous birds, alongside their smaller sample size. Hence, the variance metric probably more accurately describes the average dissimilarity among taxa, as it is relatively independent of sampling bias [25,29]. The increase in the sum of variances likely captures the novel morphology of Late Cretaceous taxa such as *Gobipteryx*, *Ichthyornis* and *Hesperornis*.

Intergroup comparisons show that the Ornithuromorpha are the most disparate in all quantitative metrics, especially sum of variances, indicating that Mesozoic ornithuromorphs continuously evolved new morphologies that contributed to overall morphological expansion (figure 5*c*; electronic supplementary material, figures S19*d*, S20*d*, S22*d*, S23*a-c*, and tables S11–S14). By contrast, the Enantiornithes, although having more taxa in the discrete dataset ($n = 34$, compared with 30 ornithuromorphs), are only slightly more disparate than 'non-Ornithothoraces' ($n = 11$) in terms of both sum of variances and median distance from the centroid, but do show larger disparity with respect to sum of ranges. The inclusion of ancestral nodes into each group theoretically averages the morphological difference among and within groups [23], because the ancestral states were estimated using those of terminal taxa which narrow the gap between groups. However, overall results are essentially the same, with ornithuromorphs clearly the most disparate group (electronic supplementary material, figures S21–S29). Rarefaction curves suggest that our results are not strongly affected by sampling bias (figure 5*h,i*; electronic supplementary material, figure S26). As the sister clades, the Enantiornithes and Ornithuromorpha show different patterns of disparity throughout the Cretaceous. Sum of variances and the median distance from centroids indicate increasing disparity of ornithuromorphs over time, peaking during the Late Cretaceous (figure 5*e*; electronic supplementary material, figures S18–S20, S24, S27–S29). By contrast, the same metrics applied to enantiornithines exhibit stasis during the Early Cretaceous followed by a slight decrease in the Late Cretaceous. In terms of sum of ranges, both groups show an inverse V-shaped curve: disparity increasing during the Early Cretaceous but decreasing towards the K-Pg boundary (figure 5*f,g*; electronic supplementary material, figures S20–S29). However, the sum of ranges for ornithuromorphs increases more rapidly and decreases more slowly than that of enantiornithines.

Comparisons of evolutionary models based on Akaike weights showed that a trend model best explains temporal changes in enantiornithine disparity regardless of metric used, or whether ancestral nodes are considered or not (electronic supplementary material, tables S15–S18). However, the sign of this trend varies. When ancestral nodes are excluded, the sum of ranges and median distance from centroids show a negative trend (decreasing disparity), whereas the sum of variances shows a marginal positive trend; when ancestral nodes are considered, the sum of ranges still shows a negative trend, but the other metrics are weakly positive (electronic

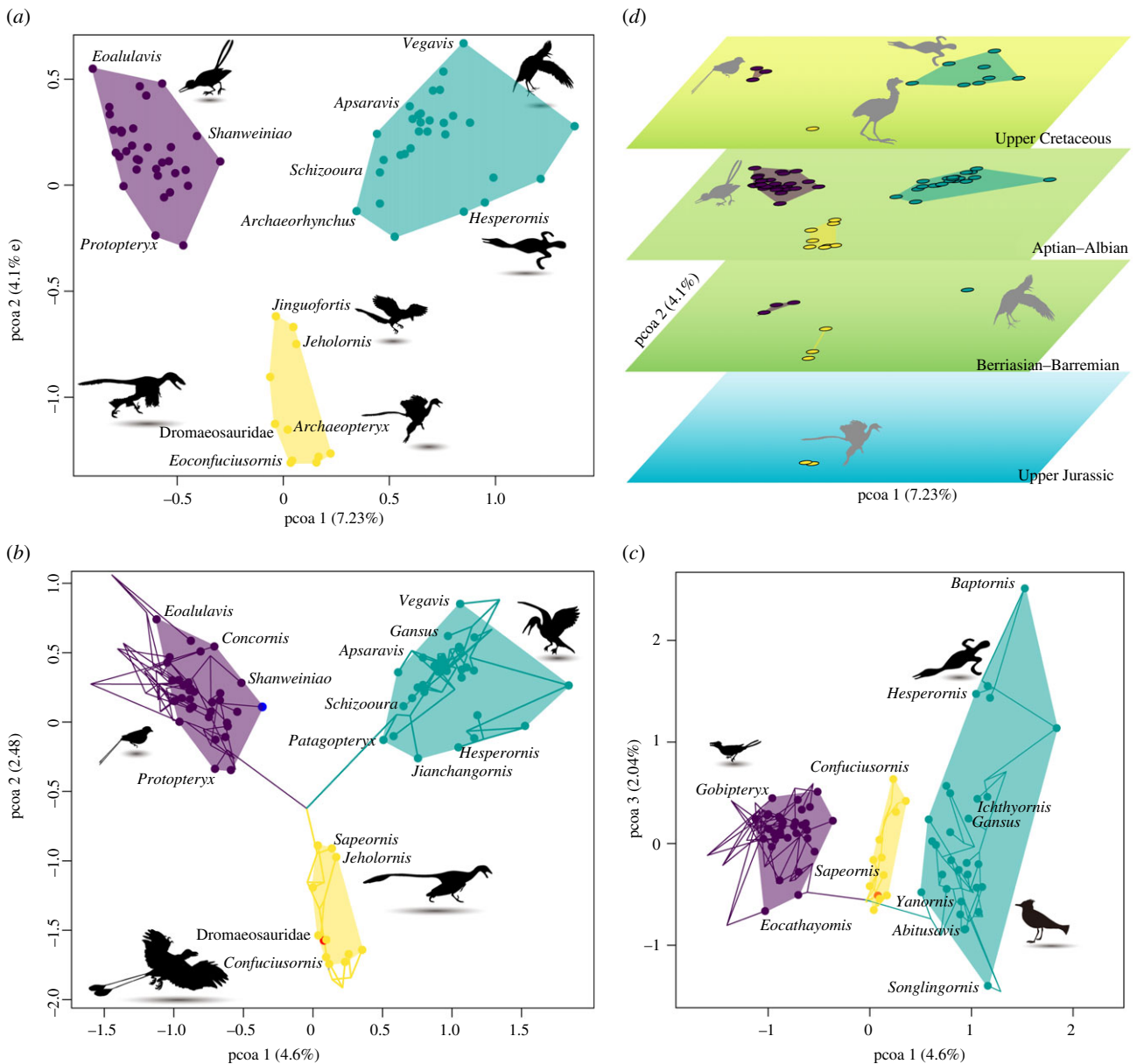


Figure 4. Discrete character morphospace of Mesozoic birds. The phylogenetic backbone is the ‘mb1’-scaled strict consensus tree, and maximum observable rescaled distance (MORD) was used as the distance metrics. (a) Binary plot of the first two principal coordinate axes (pcoas 1 and 2), and (b) stacked from the Late Jurassic to Cretaceous showing the changes in morphospace occupations (without ancestral nodes); (c,d) discrete character phylomorphospace (with ancestral nodes) derived from pairwise plots of pcoas 1–3. Yellow: non-Ornithoraces; green black: Ornithomorpha; purple: Enantiornithes. Silhouettes are modified from [4]. See electronic supplementary material, figures S13–S16 for results using the ‘equal’-scaled and tip-dated phylogenies, and the ‘HSJ’ and GED distance metrics. (Online version in colour.)

supplementary material, table S16). For Ornithomorpha (with and without ancestral nodes), a trend model again best describes their evolution regardless of metric choice, although the sum of ranges slightly favours the trend model over the single-optimum Ornstein–Uhlenbeck model (electronic supplementary material, table S18). The trend parameter is always positive and substantially larger than for enantiornithines. Overall, these results show that ornithuromorphs are morphologically more diverse than enantiornithines, despite the latter’s superior species richness.

Different disparity patterns were recovered from the six anatomical subregions. The three avian groups were statistically separated from one another in all dsMoSp of individual subregions (PERMANOVA test, $p < 0.01$; electronic supplementary material, tables S19). Ornithomorpha is the most disparate in terms of the skull, vertebral column, forelimb and pelvis morphology in all quantitative metrics. By contrast,

enantiornithines show a greater disparity in the hindlimb morphology, and no clear distinction was recovered for the pectoral girdle morphology (electronic supplementary material, figures S30–S39). Unlike the relatively static patterns recovered from the limb proportions of the Enantiornithes and Ornithomorpha through the Early Cretaceous, both groups show large changes in discrete characters of the forelimb and hindlimb across all metrics (electronic supplementary material, figures S37 and S38). Ornithomorpha show an increase in all metrics but the sum of ranges, whereas enantiornithines show an inverse V-shaped curve for all metrics but sum of variances.

4. Discussion

The Mesozoic represents a critical interval in avian evolution, with the appearances of novel morphologies, as well as

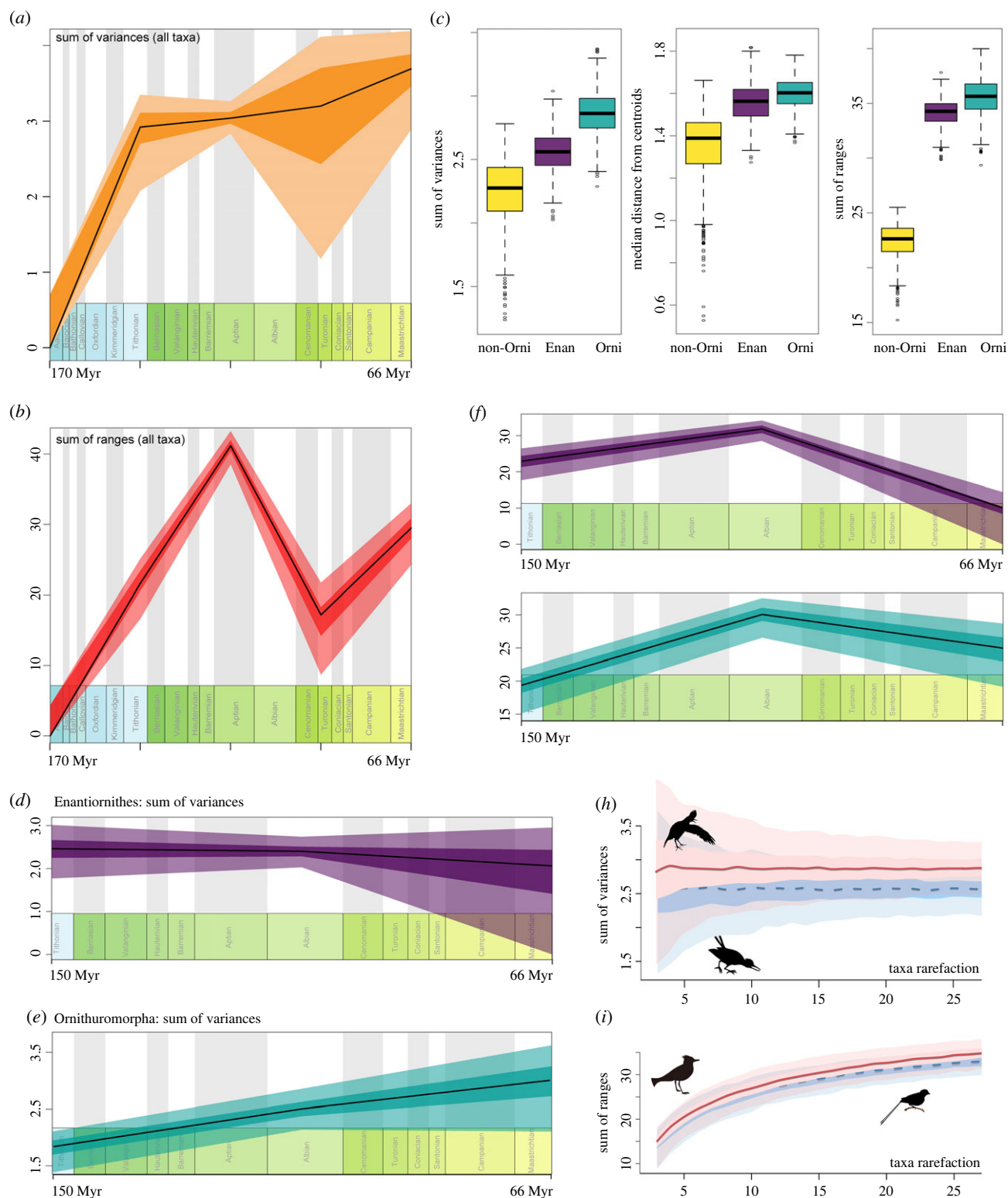


Figure 5. Morphological disparity of Mesozoic birds. The phylogenetic backbone is ‘mb1’-scaled, and MORD was used as the distance metrics. Two disparity metrics are shown: sum of variances and sum of ranges. (a,b) Disparity lines of Mesozoic birds as a whole across five time bins that roughly correspond to geological stages spanned by the scaled phylogeny: 170–145 Myr (Middle–Late Jurassic), 145–129.4 Myr (Berriasian–Hauterivian), 129.4–100.5 Myr (Barremian–Albian), 100.5–86.3 Myr (Cenomanian–Coniacian) and 86.3–66 Myr (Santonian–Maastrichtian); (c) box plots showing disparity metrics among Mesozoic avian groups; (d–g) comparison of disparity between Enantiornithes and Ornithuromorpha across three subequal-length time bins (150–125, 125–100.5, 100.5–66 Myr); (h,i) rarefaction of disparity curves of Ornithuromorpha (in red) and Enantiornithes (in blue) showing that the results are not strongly affected by sampling bias. The dark and light surfaces denote the 50% and 95% confidence intervals, respectively. Silhouettes are modified from [4]. See electronic supplementary material, figures S18–S29 for results derived from different branch-scaling methods, distance metrics and with/without ancestral nodes. (Online version in colour.)

recording the earliest phase of radiation [2,4,5]. Our disparity analyses reveal different modes and patterns of the evolution of disparity in Mesozoic birds demonstrated in limb measurements and discrete characters. In the limb proportions morphospace, the three avian groups overlap substantially.

Although exact determinations for the ecology of Mesozoic birds remain elusive, it is widely accepted that enantiornithines are arboreal and early ornithuromorphs are terrestrial [2,41]. The overlapping space defined by limb proportions of different groups provides evidence of

constrained evolution that explored the same set of functional solutions repeatedly. Limb proportions in crown groups vary greatly and are strongly correlated with niche adaptations [42,43]. However, conservativeness is still appreciable in some aspects such as the forelimb being longer than the hind limb and the ulna longer than the humerus in many taxa distributed across multiple habitats [44]. These shared features may imply the presence of developmental constraints and/or common functional requirements for locomotion. The powered flight could have imposed constraints on relative limb length, not only the wings but also the hindlimbs [45–47], and the extent to which these constraints act on different limb bones ratios requires future investigation.

Unlike limb proportions, Mesozoic birds occupy distinct areas of discrete character morphospace either measured as a whole or by individual anatomical regions, indicating that profound variation was profound evident in aspects of anatomical features other than limb proportions. This pattern to some degree echoes the radiation of the iconic Darwin's finches that is manifested largely by changes in beak form rather than limb proportions [48,49]. Differentiations in anatomical features could have facilitated access to new niches, circumventing the constraints imposed by limb proportions, illustrated by contrasting patterns of disparity demonstrated by limb proportions and discrete characters.

Enantiornithes and Ornithuromorpha represent the earliest avian lineages to undergo large-scale radiation [5,50], and thus offer important insights into how closely related clades travel along different trajectories to become evolutionarily successful. Surprisingly, ornithuromorphs are more disparate than enantiornithines in terms of both discrete characters and limb proportions, despite the latter being apparently more species rich. This comparative study adds to the tally of empirical evidence that species diversity and morphological disparity are decoupled [51,52]. The relatively lower degree of disparity implies that the diversification of enantiornithines is characterized by exhausting fine morphologies rather than exploiting new morphospace in terms of both limb proportions and anatomical features. This hypothesis is supported by our analyses of the exhaustion of character space during the evolution of enantiornithines and ornithuromorphs [53] (electronic supplementary material). The exhaustion curves show that enantiornithines realized fewer character states than ornithuromorphs in a given number of characters (electronic supplementary material, figure S40).

All the known enantiornithines are considered to be arboreal, including their earliest members [5,40,54]. This apparent habitat restriction may have promoted diversification through easing competition with other primarily terrestrial vertebrates such as non-avian dinosaurs as well as providing a separate food source (e.g. insect, fruits, small vertebrates). However, comparative studies—on lizard, snakes, and birds—show that arboreality can impose strong selective pressure and consequently constrain morphological variation [55,56]. In other words, the constraints of an arboreal mode of life in enantiornithines may have restricted the ability of enantiornithines to explore other niches, especially as transitions from arboreal to other ecological adaptations have rarely happened among crown birds [56]. By contrast, Mesozoic ornithuromorphs exhibit greater disparity in limb proportions and discrete characters. The continuous expansion in limb proportions and discrete morphospaces may have facilitated ornithuromorphs to invade new ecologies, attesting to the diverse habitats inferred for this clade (e.g. terrestrial, wading, swimming, diving and amphibious [11,50]). It has long been an area of significant interest that enantiornithines and ornithuromorphs suffered differentially in the end-Cretaceous mass extinction, with the former completely perishing [2]. A recent study suggested that the selective extinction of enantiornithines probably resulted from the global destruction of forests during this event [54]. The heavily devastated terrestrial ecosystem exerted great pressure for most vertebrates, particularly those highly adapted to certain habitats (here, Enantiornithes), while the more ecologically diverse ornithuromorphs survived. We posit that the different degree of evolutionary versatility of enantiornithines and ornithuromorphs quantified from limb proportion and discrete characters may forecast their different survivorship in this catastrophe.

Data accessibility. Electronic supplementary material is available online. All analyses were conducted in R v. 4.0.0; reproducible R scripts, MrBayes commands and all data files are available from the Dryad repository: <https://doi.org/10.5061/dryad.mcvdncjzv> [57] as well as on Github (<https://github.com/graemetlloyd/ProjectAncientBeak>).

Authors' contributions. M.W. designed the research project; M.W. and G.T.L. performed the phylogenetic and disparity analyses; C.Z. performed the tip-dating analyses; M.W., G.T.L., C.Z. and Z.Z. wrote the manuscript.

Competing interests. The authors declare no competing interest.

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