

SECTION 2. ANATOMICAL FRONTIERS

Chapter 6

Disparity and Macroevolutionary Transformation of the Maniraptoran Manus

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ABSTRACT

Multiple factors involved in the evolutionary transformation of the manus across the maniraptoran radiation, including its current morphology in modern birds, remain unexplored. Specifically, the morphological disparity of the manus has never been studied quantitatively, and there are no hypotheses about the possible mechanisms and constraints underlying its evolution. Morphological disparity is best studied with shape-analysis tools based on Procrustes methods, because they guarantee the independence of shape from size while depicting the results in expressive graphics. However, this methodology compares fixed configurations of coordinates, preventing their use in highly articulated and movable structures such as the maniraptoran manus. Here, we propose a new protocol, the one-dimensional Procrustes analysis (OPA), for transforming the chord lengths of these bones into unidimensional Cartesian coordinates, enabling treatment of the data under the operational advantages of the Procrustes methods. Our results applying this new method on a sample encompassing 174 maniraptoran dinosaurs manus, including 79 fossils (both avialan and nonavialan taxa) and 95 extant paleognathans and neognathans, document the morphological transition between early-diverging maniraptorans, nonavialan paravians, and birds over morphospace, highlighting an unexpectedly low disparity in the crown group when compared to early-diverging taxa. Within this transition, we show a common trend of proportional reduction and loss of distal elements, mostly in the minor and alular digits. Furthermore, our study reveals an allometric pattern characterizing manus morphological variation between early-diverging maniraptorans and enantiornithine avialans that disappears in crown birds and their closest early-diverging counterparts. This previously unnoticed allometric trend suggests a complex interplay of developmental, functional,

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and historical constraints in the evolution of the maniraptoran manus.

INTRODUCTION

Modern birds are the only living representatives of the maniraptoran theropod radiation (Gauthier, 1986; Padian and Chiappe, 1998; Sereno, 1999; Zhou, 2004; Norell and Xu, 2005). One of the most intriguing events of the macroevolutionary transition leading to anatomically modern birds is the transformation of the archetypal grasping forelimb of nonavian maniraptoran theropods into the forelimb of modern birds (fig. 1), which is largely enclosed in a soft-tissue airfoil and has limited grasping functions (Barta et al., 2018). This large morphological transition in proportion and size is often believed to have been fundamentally shaped by selective pressures related to the demanding biomechanical requirements of flight (Marden, 1994; Norberg, 1995). However, the great diversity of maniraptoran fossils found in recent decades is helping to mitigate such an exclusively adaptive view, given that aerial competence was probably acquired by taxa exhibiting non-modern forelimb morphologies (Xu et al., 2003, 2015; Barta et al., 2018; Wang et al., 2017, 2019; Pei et al., in press; Pittman et al., chapter 10; Dececchi et al., chapter 11; Larsson et al., chapter 12). Therefore, the history of the factors involved in shaping maniraptoran hand evolution is more complex than currently appreciated.

A miniaturization trend took place early in the lineage toward crown birds (Sereno, 1997; Carrano, 2006; Turner et al., 2007; Novas et al., 2012; Puttick et al., 2014; Lee et al., 2014). This trend was coupled with important quantitative changes in forelimb/hindlimb proportions (Dececchi and Larsson, 2013), yet the relative proportions of the skeletal elements of the forelimb (i.e., humerus, radius, ulna, carpals, metacarpals, and phalanges) changed very little (Middleton and Gatesy, 2000). However, it has been suggested that an increase in the morphological disparity of wing shapes occurred later

within the avialan radiation, after the origin of active flight, which in turn triggered the evolution of different flight strategies (Serrano and Chiappe, 2017; Serrano et al., 2018, chapter 13). Because the wing's remiges and patagia are anchored in the skeletal elements of the hand and forearm, their shapes, proportions, and sizes may have contributed to such an increase in wing disparity (Nudds et al., 2007), although this assumption has never been tested quantitatively. Addressing the evolution of the morphospace-occupancy of the maniraptoran hand skeleton (manus) is important, as it establishes a critical bridge between paleobiology and the biological disciplines that seek to identify mechanisms underpinning macroevolutionary trends (e.g., molecular genetics and Evo-Devo; Raff, 1996, 2000).

The study of morphospace construction and the analysis of disparity are eminently mathematically quantifiable (McGhee, 1999), and most studies in vertebrate paleobiology have used bone ratios as a proxy for anatomy and ternary diagrams to build morphospaces and visualize disparity. Although this procedure is practical and easy to apply (e.g., Marugán-Lobón and Bascalió, 2003), it is also analytically restrictive because ratios are highly limiting for statistical analyses (e.g., to study allometry; Rohlf and Marcus, 1993). In fact, geometric morphometrics (GM; Bookstein, 1991) is currently the optimal approach for understanding the evolution of morphological disparity, as it enables the separation of shape from size variation (Mitteroecker and Gunz, 2009; Marugán-Lobón et al., 2013a), which was impossible with "traditional morphometrics", namely, when working with absolute and/or relative lengths (Rohlf and Marcus, 1993). GM captures the geometry of forms as constellations of repeatable and corresponding points (often corresponding to primary homology positions), called landmarks. The Cartesian coordinates of these configurations are then compared in a multistep process, the generalized Procrustes analysis (GPA), which eliminates superfluous differences between the configurations due to position and orientation, together with the dif-

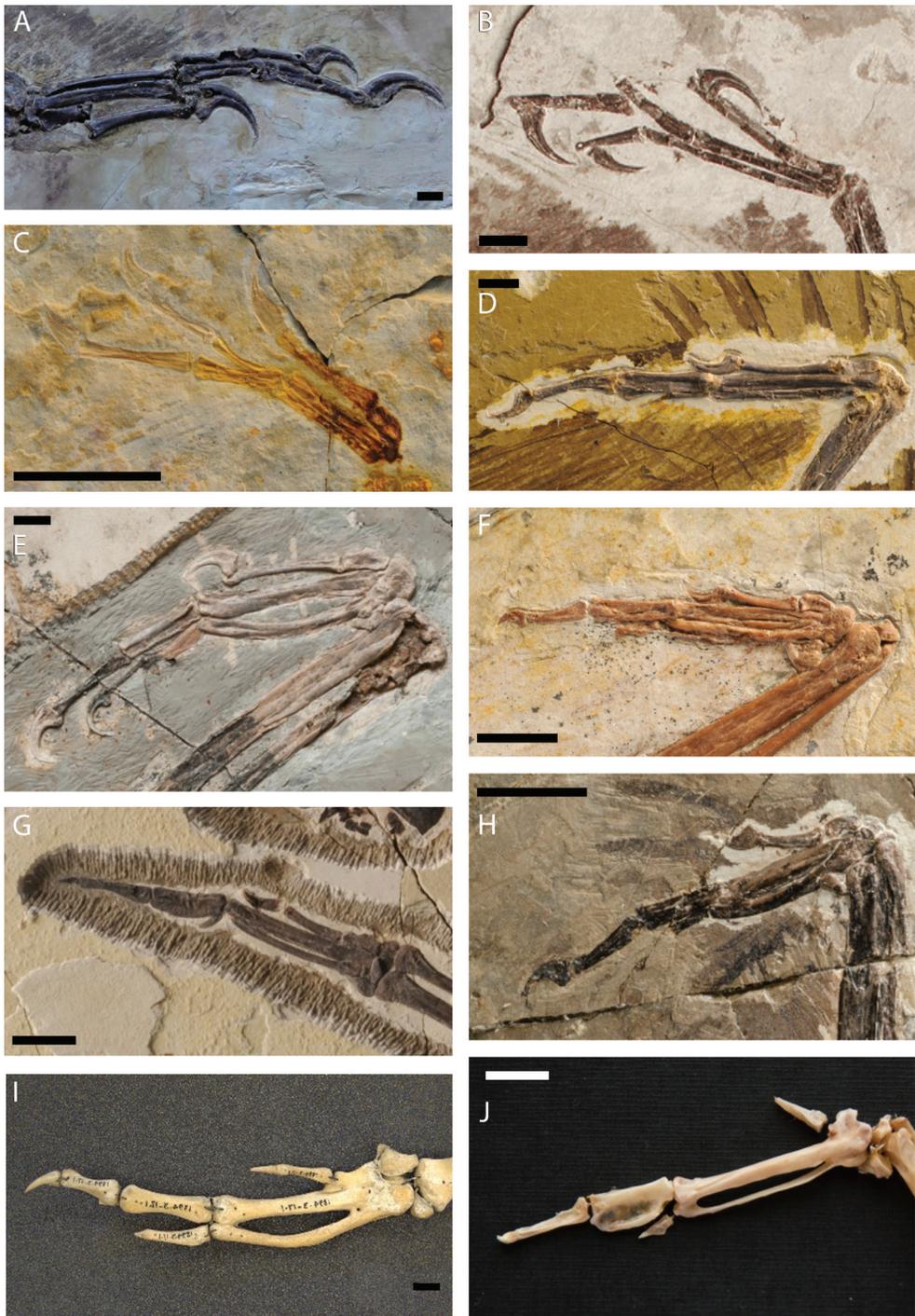


FIG. 1. Representative example of several maniraptoran hands, both fossil (A–H) and extant (I–J) specimens, illustrate the variability of shapes and sizes: A. *Zhenyuanlong*; B. *Anchiornis huxleyi*; C. *Zhongornis*; D. *Sapeornis chaoyangensis*; E. *Jeholornis*; F. *Zhouornis*; G. *Archaeorhynchus*; H. *Longipteryx*; I. *Struthio camelus*; J. *Falco tinnunculus*. Scale bars = 10 mm.

ferences due to isometric scaling between the forms (Gower, 1975). GPA calculates the centroid size for each configuration, a geometrically defined proxy for size that is calculated from the real distances between landmarks and rescales them to a standard called unit of centroid size. This key algebraic procedure of standardization was a breakthrough in morphometrics, as it makes size and shape orthogonal, securing the statistical independence between shape and size, and guaranteeing that any difference in shape between configurations associated with size (i.e., allometry) will never be spurious (Rohlf and Marcus, 1993; Mitteroecker and Gunz, 2009; Klingenberg, 2016).

Although the advantages of GM are widely recognized (e.g., Adams et al., 2013), there are certain limitations when applied to articulated skeletal structures. Specifically, the use of GM entails comparing configurations by superimposition to detect minimal differences between corresponding landmarks and, as a result, it requires working on integral structures that have no moveable or articulable parts. The reason is that with the Procrustes alignment of landmark configurations in articulated structures such as the manus, mobility and the ability to flex the digits implies that there are multiple possible spatial configurations between the elements (as many as the joints allow). Therefore, there will be as many configurations of landmarks of the same manus as there are possible positions between the digits, and this will drastically confuse interpretations derived from the method. Although criteria have been established to standardize the placement of moveable structures in one single position (e.g., Adams, 1999; Vidal-García et al., 2018), most choices are either arbitrary or may imply biologically inconsistent assumptions (Marugán-Lobón et al., 2013b). Moreover, the case of the maniraptoran manus is even more intricate given that early-diverging maniraptorans are known only from fossils and the manus elements

are likely to be disarticulated or to be preserved in unnatural poses.

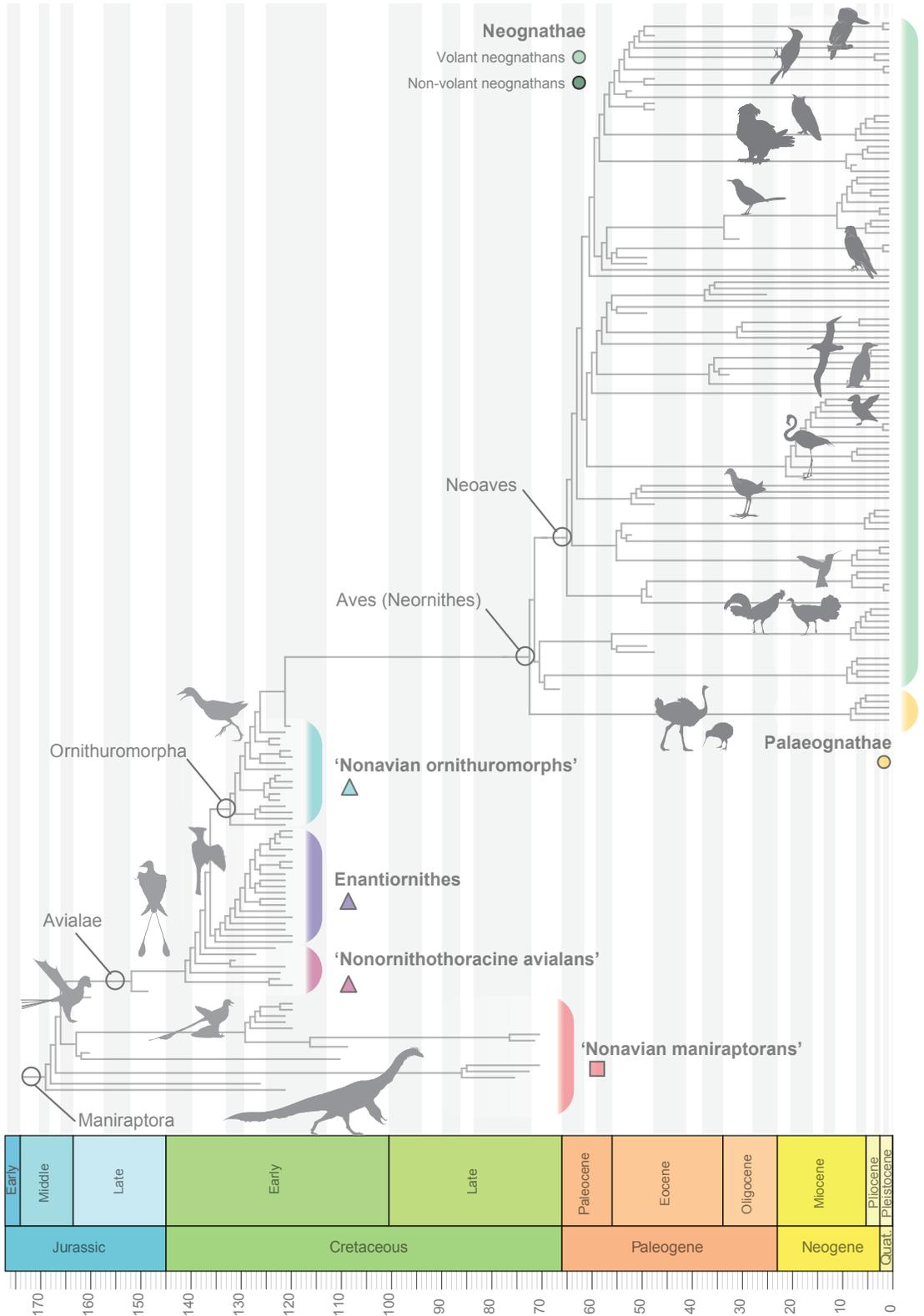
Here, we propose a simple morphometric protocol that allows exploiting the accessibility of longitudinal measurements from skeletons and fossils to obtain Procrustes data. Our method, from now on called one-dimensional Procrustes analysis (OPA), involves the transformation of longitudinal measurements of the elements of the manus into one-dimensional Cartesian coordinates, which can be aligned using the Procrustes criterion and analyzed using GM's standard multivariate tools (Dryden and Mardia, 1998), such as multivariate regression to assess allometry (Monteiro, 1999; Marugán-Lobón et al., 2013b). We applied this protocol to a large sample of maniraptoran dinosaur manus ranging from the Jurassic to the present. The objective of the present study was to analyze the morphological disparity of the maniraptoran manus in a macroevolutionary context, from the earliest-diverging taxa to the crown group (Aves), the present-day representatives of the clade. Within this context, the objective was to study the pattern of transformation of its shape understood as a set of proportional lengths of the conforming elements of the manus and its relationship with size (evolutionary allometry).

MATERIALS AND METHODS

MATERIALS

In order to accurately capture the morphological transformation of the maniraptoran manus (fig. 1), we gathered data from a large sample of fossil and extant specimens (from early-diverging maniraptoran taxa to crown birds; fig. 2) with a complete autopod. The sampling of crown birds (Aves, equivalent to Neornithes here) was designed to cover the maximum morphological disparity in the distal region of the forelimb. We measured 174 speci-

FIG. 2. Random phylogenetic tree of the maniraptoran lineage out of the 100 trees used in this work, showing the topology of each member of the sample ($N = 174$), branch length, and time scale. Colour coding and schematic representations consistent in all figures. See supplemental figure for a large version of this figure that includes taxon names and a more detailed geological time scale (<https://doi.org/10.5531/sd.sp.44>).



mens from the Natural History Museum of Los Angeles County (LACM) and Natural History Museum (NHMUK, Tring), as well as from high-definition photographs provided by one of the authors (L.M.C.) and the literature (pictures and published measurements). The total sample includes 16 specimens of nonavian maniraptorans (Therizinosauria, Oviraptorosauria, Deinonychosauria, and Scansoriopterygidae), 44 early-diverging birds (nonornithothoracine taxa, Enantiornithes, and nonavian ornithuromorphs), and 114 crown birds (Palaeognathae and Neognathae, which includes Galloanserae and Neoaves, with fossil and extant taxa). All measured specimens are adults, except a juvenile enantiornithine (NIGP 130723; Chiappe et al., 2007) and the nonornithothoracine of uncertain affinities *Zhongornis* (DNHM D2456; Gao et al., 2008; Rashid et al., 2018; but see O'Connor & Sullivan 2014 about possible non-avian theropod identity).

MANUS ANATOMY AND DATA ACQUISITION

The structure of the manus has changed dramatically during the transition from early-diverging maniraptorans to modern birds, with the reduction and loss of elements, fusions, and alterations in proportions (fig. 1). Therefore, the establishment of a homology framework for the identity of the digits of present-day birds with respect to their dinosaurian ancestors has been challenging (Wagner and Gauthier, 1999; Vargas and Wagner, 2009; Xu et al., 2009, 2011; Bever et al., 2011; Young et al., 2011; Xu and Mackem, 2013). Traditionally in paleontology and zoology the sequence of digits I-II-III has been considered to correspond to the alular, major and minor digits, in modern birds. This hypothesis has been supported mainly by fossil and morphological evidence and is supported by the embryological “frame shift” hypothesis (Wagner and Gauthier, 1999; Bever et al., 2011; Young et al., 2011). However, other embryological studies have proposed that the identity of the manual digits of modern birds is equivalent to the II-III-IV positions of the ancestral pentadactyl hand

(i.e., the “lateral change” hypothesis; Xu et al., 2011). Although the debate continues, both hypotheses coincide in the homology of digit identities between the early-diverging maniraptoran and avian manus, thus allowing comparison. Furthermore, these hypotheses are congruent in that the morphogenesis (or condensation) of the digits and their identity is decoupled, thus allowing independent evolutionary changes (Wagner and Gauthier, 1999). Here, we followed the identity of avian digits as I-II-III, the ancestral condition in Theropoda in which digits IV and V are highly reduced (Gauthier, 1986; Xu et al., 2009). At the base of the maniraptoran clade (Gauthier, 1986) the autopod is elongated with a characteristic phalangeal formula of 2-3-4 (digits I-II-III, respectively). Digit II is normally the longest and digit I the shortest, and the wrist is characterized by the presence of a semilunate carpal that contacts the proximal portions of metacarpals (mc) I and II. In contrast, the autopod of crown birds (Aves), while still tridactyl, bears elements that have become fused and have changed their relative lengths as a result of reductions and/or losses of some of their phalanges. In modern groups, the general phalangeal formula is typically 1-2-1, with some exceptions with the formula 2-2-1 or 2-3-1 in forms that retain claws, such as some fowls (Galloanserae) or the ostrich (Struthionidae), respectively (Mayr, 2017). Here, the length of semilunate carpal in nonavian maniraptorans was included in the measurement of mc-II to aid direct comparisons with avian forms in which these elements are fused (Middleton and Gatesy, 2000). We measured chord lengths of the hand bones of modern birds using calipers (by the authors S.M.N. and T.S.) and using the tpsDig2 software (Rohlf, 2017, v. 2.30) on digital images of the fossils forms (by first author, S.M.N.). The measurements represent the maximum length between the central points of the proximal and distal articulations between bones (chord lengths). For the unguis phalanges, we took the straight length from the central point of the articulation region to the bone tip (fig. 3).

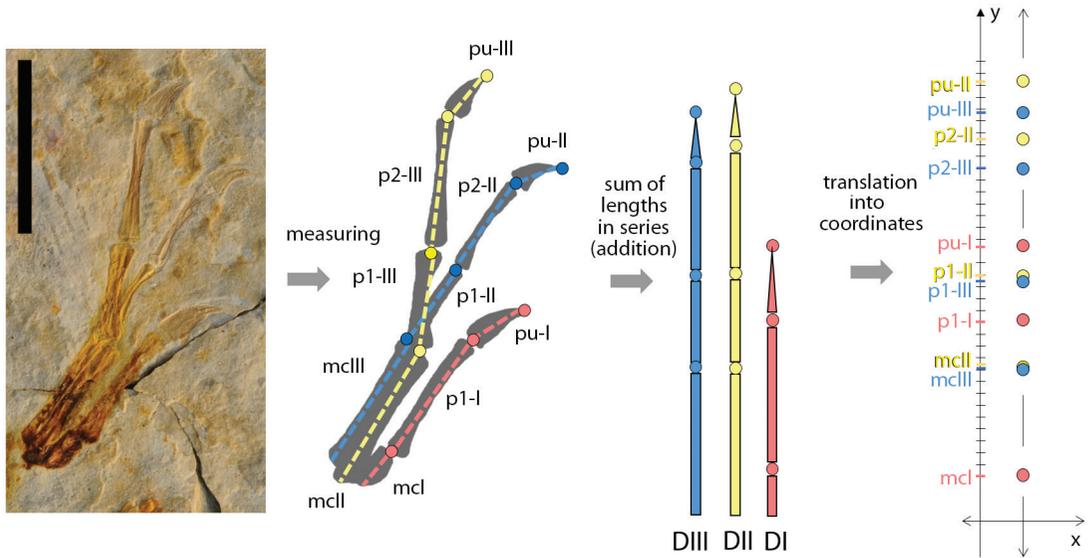


FIG. 3. Schematic representation of the one-dimensional Procrustes analysis (OPA) method carried out in the data processing, from lengthwise. Exempling manus belongs to the juvenile early-diverging avialan *Zhongornis* (DNHM D2456; Gao et al., 2008; Rashid et al., 2018).

TRANSFORMATION TO PROCRUSTES COORDINATE DATA

OPA, the protocol to transform the linear measurements into Cartesian coordinates (fig. 3), starts from a baseline on a zeroed x -axis. Then, the length of metacarpal I yields the first y -coordinate value (fig. 3, red elements). Subsequently, the length of the first phalanx of digit I is added to the latter y -value, always with x set to 0. This yields a second y -coordinate that, in turn, corresponds to the sum of the lengths of metacarpal I plus the length of its first phalanx. The coordinate of the second phalanx is similarly attained by adding its chord length to the latter sum of lengths, yielding a third y -coordinate, thus giving the coordinates of the entire digit I. This procedure is repeated for digit II and III (fig. 3, yellow and blue elements respectively). Importantly, the phalangeal formula might be different given that there are evolutionary phalange losses (e.g., most modern forms lack unguals). This mismatch is solved by adding a repeated last coordinate (summing a length of zero could not be carried out because the software does not run with two equal

coordinates, hence we added a negligible 0.01). This is equivalent to having two identical coordinates on top of each other at the tip of the affected digit. This adding of repeated landmarks allows performing the Procrustes superimposition with missing landmarks, without adding any extra information or variance to the data (Klingenberg, 2008).

The entire protocol (OPA) is unidimensional because there is only one coordinate axis with data. To separate size from shape and compare configurations we used a generalized Procrustes analysis (GPA; Gower, 1975), which uses the least-squares criterion to minimize, in a rigid way, the distance between corresponding landmarks among different configurations. This alignment uses the mean configuration as a reference for correcting the position and orientation of the configurations through superimposition and, in addition, rescales them to units of centroid size (CS). CS is an independent variable representing the scale or size of the configuration (it represents the real distances between the coordinates) and is calculated as the square root of the sum of the squared distances of all the coordinates (Book-

stein, 1991). It is important to emphasize that working with coordinates that present values only on one axis implies that neither the translation nor the rotation steps are relevant for this study. The standardization of size of the coordinate data is particularly relevant, since it guarantees that the isometry (differences only in scale) are eliminated from the data (Gower, 1975), and the residuals of this analysis, the Procrustes coordinates, represent the different proportions of manus elements per specimen without size information. This “shape data” is used in downstream analyses, and we refer to it hereafter as manus shape.

PHYLOGENETIC HYPOTHESIS AND MULTIVARIATE STATISTICS

We built an informal supertree (fig. 2) using Mesquite v. 3.40 (Maddison and Maddison, 2011) and based on the comprehensive phylogeny of Prum et al. (2015) for the relationships of birds within the crown group, Turner et al. (2012) for the relationships between early-diverging maniraptorans and Paraves, and O'Connor and Zhou (2012) as well as Wang and Lloyd (2016) for Mesozoic birds. The topology of the terminal branches not included in the previous studies was resolved with information on the specific groups. We calibrated the resulting tree using the R package Paleotree (Bapst, 2012), scaling the branches of taxa based on their stratigraphic range obtained from the Paleobiology Database (www.paleodb.org) and from the literature. To avoid zero-length branches we set a minimum branch length of 1 million years. We generated 100 trees using this method, which randomly assesses the first appearance dates (FADs) and last appearance dates (LADs) of each species inside the stratigraphic interval where they are assigned, generating 100 different tree distributions and solving randomly the polytomies that could not be resolved with the literature. See supplemental figure for a larger version of figure 2 that includes taxon names and a more detailed geological timescale (<https://doi.org/10.5531/sd.sp.44>). All of the following analyses were carried out in consideration of the phy-

logenetic relationships between all specimens in our sample.

We applied two of the main types of analysis from the custom statistical toolkit in GM (Bookstein, 1991), namely, principal components analysis (PCA), which allows us to reduce the dimensionality of the data and summarizes the differences (shape variance), and multivariate regression, a multivariate tool to test the relationship between shape variation (dependent variable) with the variation of any type of independent variable (Monteiro, 1999). This method is the most reliable for studying allometry in the context of shape analysis using size as the predictor or independent variable (e.g., Marugán-Lobón et al., 2013b; Mitteroecker et al., 2013). PCA generates an ordination that is generally interpreted as an empirical morphospace (Marugán-Lobón and Buscalioni, 2004) on which phylogeny can be mapped, including the reconstruction of the ancestral shapes represented by the nodes. This graph, called phylo-morphospace, represents a reconstruction of the phylogenetic history of changes in shape, reflecting how different clades have evolved. We mapped a randomly selected tree from the population of the 100 trees we generated by means of weighted (i.e., including branch length information) squared-change parsimony (Maddison, 1991) into the PC1-3 morphospace using MorphoJ v. 1.06d (Klingenberg, 2011).

We also quantified mean manus shape disparity (Procrustes variance) and tested for statistically significant differences between groups using the function *morphol.disparity* in the R package *geomorph* v.3.0.7 (Adams and Otárola-Castillo, 2013). Specifically, we tested for differences between: (1) the stem and crown lineages, (2) all the main maniraptoran grades, and (3a) palaeognathans, all neognathans, (3b) only volant neognathans and non-volant neognathans to each other and to all the other main maniraptoran grades. The latter comparisons was triggered by the visual observation that flightless palaeognathans and neognathans seem to exhibit the most disparate manus morphologies among crown

group birds. Because Procrustes variance provides information only about mean shape disparity within each group, we also calculated the convex hull volumes encapsulated by the manus morphologies of each of the groups in the tangent shape space defined by the first three principal components of manus shape. Convex hulls were computed as the smallest three-dimensional volume encompassing all the specimens within each group. Convex hull volumes were calculated using the `convhull.volume` function in the R package `disparity` (Guillerme, 2018).

Multivariate linear regression was performed between the manus shape variables (Procrustes data, as a dependent variable) and the log-transformed CS (log-CS) of the specimens (independent variable or predictor). In principle, the data cannot be considered independent because lineages are phylogenetically related (i.e., phylogenetic inertia). We therefore used phylogenetically informed regressions (phylogenetic generalized least squares, PGLS) to test the relationship between manus shape and manus size in all maniraptorans using the `procD.pgls` function in the R package `geomorph` v.3.0.7. All PGLS linear models included group category as an additional independent variable both to test for shape and size differences between clades and to test for group-specific allometries. We specifically tested for allometric differences between: (1) the stem and crown lineages, and (2) all the “grades” outlined in figure 2 (i.e., crown birds, early-diverging ornithuromorphs, enantiornithines, nonornithothoracine avialans, nonavian maniraptorans). To test for group-specific allometries we conducted pairwise comparisons of slope vector angles and slope vector lengths between all the groups. PGLS regressions were repeated using all the corresponding 100 trees, and median and range values for all the main statistics (R^2 , F , Z , P) were recorded, to address how differences in branch lengths affect our results. Furthermore, because log-CS and clade categories covary (table 1) we used type II (conditional) sums of squares to assess the statistical significance of all PGLS linear models (Adams and

Collyer, 2018). Because our variables are unevenly dispersed across our phylogeny (e.g., different lineages exhibit different disparities of shape and size), which can severely reduce statistical power of linear models (Adams and Collyer, 2018), we used randomizing residuals in a permutation procedure (10,000 iterations implemented in `geomorph` v.3.0.6, Adams et al., 2018) to assess statistical significance for all PGLS regressions, as this has been shown to be more robust to group-clade aggregations (Adams and Collyer, 2018).

RESULTS

The first three main components of the PCA capture 92.62% of the total shape variance (i.e., proportional differences between the elements of the studied manus), which is graphically summarized in a 3D phylomorphospace (fig. 4). PC1 is clearly the dominant dimension, capturing as much as 63.38% of the total variance, while PC2 and PC3 capture 23.95% and 5.29%, respectively. PC1 describes the most notable morphological differences in the nonavian-to-avian transition, accounting for the differences in proportional lengths of the proximal elements of the manus with respect to the distal ones, namely, encompassing the proportional reduction and loss of phalanges in the lineage, toward crown birds. Along the PC1 axis, maniraptorans with proportionally shorter phalanges are grouped in the negative region, while those with autopods with proportionally more elongated phalanges are in the positive region (fig. 4). This distribution thus clearly separates some early-diverging avialans, nonavian paravians and early-diverging maniraptorans, distributed exclusively in the positive region, from later-diverging nonavian avialans (Enantiornithes, early-diverging ornithuromorphs), ultimately polarizing the situation of Aves (Neornithes) in the negative region of PC1 due to their usual lack of distal phalanges. PC2 accounts for large changes in digit III, with only minor changes in digits I and II. Specifically, this axis describes the variation of the length of

TABLE 1

Differences in manus shape disparity between maniraptoran theropod groups

Pairwise comparisons of shape disparity (Procrustes variance) between **A.** crown and stem maniraptoran lineages; **B.** major maniraptoran grades. The comparison between major maniraptoran grades was also performed by dividing **C.** Aves between neognathans and palaeognathans and with **D.** neognathan birds further split in volant and non-volant taxa. Statistically significant values ($P < 0.05$) are highlighted in bold.

Numbered table columns correspond to the numbered clades in the table rows.

A	Crown					
Stem	<0.0001					
B	1	2	3	4		
1. Nonornithothoracine avialans						
2. Enantiornithes	0.016					
3. Nonavian maniraptorans	0.096	0.198				
4. Aves (Neornithes)	0.009	0.681	0.047			
5. Nonavian ornithuromorphs	0.012	0.721	0.137	0.975		
C	1	2	3	4	5	
1. Nonornithothoracine avialans						
2. Enantiornithes	0.016					
3. Nonavian maniraptorans	0.096	0.197				
4. Neognathae	0.006	0.329	0.024			
5. Nonavian ornithuromorphs	0.013	0.718	0.135	0.69		
6. Palaeognathae	0.035	6E-04	0.004	2E-04	3E-04	
D	1	2	3	4	5	6
1. Nonornithothoracine avialans						
2. Enantiornithes	0.015					
3. Nonavian maniraptorans	0.094	0.192				
4. Volant neognathans	0.004	0.226	0.017			
5. Nonvolant neognathans	0.438	0.104	0.358	0.04		
6. Nonavian ornithuromorphs	0.013	0.713	0.134	0.568	0.089	
7. Palaeognathae	0.036	4E-04	0.004	2E-04	0.034	3E-04

digit III with respect to the other two digits; such variation is more pronounced in the more distal elements of digit III and separates taxa with digits I and II longer with respect to III in the positive region of this axis, from those with a digit III proportionally similar (or even longer) to the other two digits (I and II). Finally, PC3 describes

changes in the proportional length of metacarpal I as compared with the length of the phalanx 1 of digit III, that is, when one increases in length the other decreases and vice versa. Manus with an elongated metacarpal I and a shorter phalanx 1 of digit III are located in the positive region, while those with a reduced metacarpal I and an

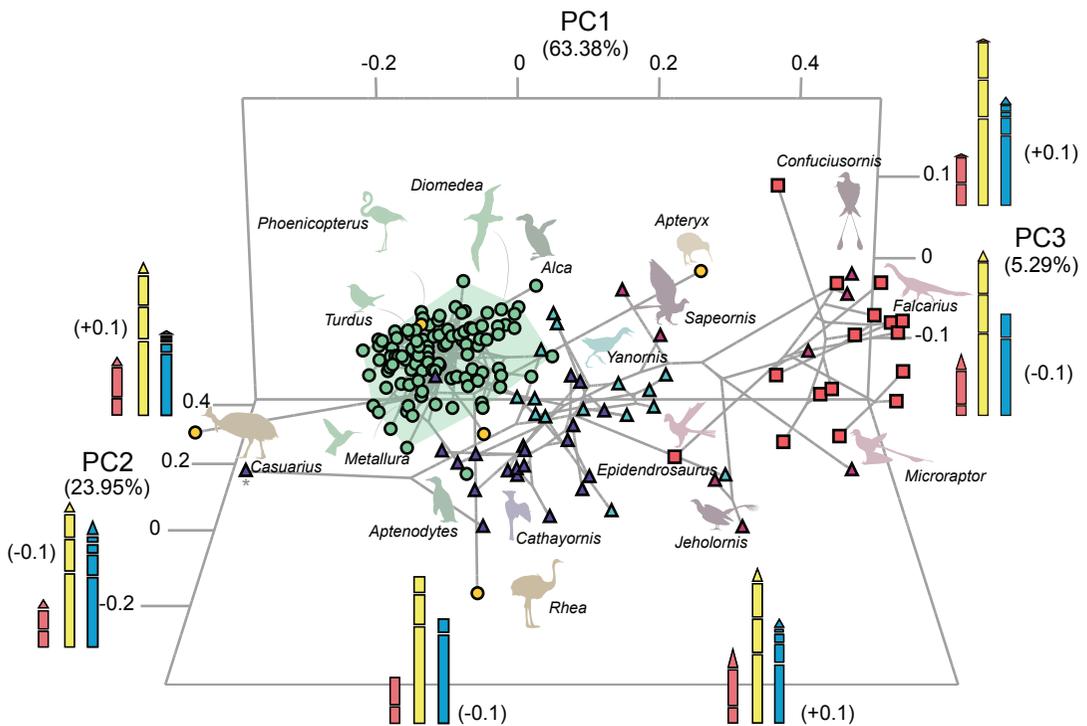


FIG. 4. Phylomorphospace of hand evolution in Maniraptora. Phylogeny mapped over the scores of the first three principal components of shape. Color coding is consistent with figure 2. Asterisk indicates juvenile enantiornithine NIGP 130723 (Chiappe et al. 2007).

elongated phalanx 1 of digit III are grouped in the negative region. Those that have similar relative lengths (such as the bulk of taxa within the crown group of birds) are distributed in the central region (fig. 4).

In the PC1-3 phylomorphospace, crown birds together with enantiornithes and early-diverging ornithuromorphs occupy a more central area of morphospace, clearly separated from the rest of the phylogenetically earlier-diverging taxa (non-ornithothoracine birds, scansoriopterygids, deinonychosaurs, and early-diverging maniraptorans), which occupy more peripheral positions (fig. 4). The latter are generally characterized by presenting more disparate manus morphologies, with distal elements proportionally more elongated with respect to the proximal (i.e., longer fingers). The greatest disparity lies mainly in the different relative lengths of digit III with respect to the other two digits. Likewise, there are certain taxa within these

groups with very divergent manus from their parent clades, like the early-diverging birds *Jinguofortis perplexus* and *Sapeornis chaoyangensis*, whose manus display a reduced digit III more similar to that of ornithuromorphs, the paravian *Balaur bondoc* with a highly reduced digit III, or the non-ornithothoracine *Epidendrosaurus* (Scansoriopterygidae) and *Jeholornis curvipes* in which digit III is relatively long.

The rest of the dinosaurs studied cluster around a group consisting of Aves and its closest Mesozoic relatives (early-diverging ornithuromorphs and Enantiornithes) situated in the left region of morphospace. That these taxa are clustered separately from the rest of maniraptorans underscores their greater degree of morphological similarity (fig. 4). Enantiornithes and early-diverging ornithuromorphs have, however, more elongated digits than crown birds and differ between them in the relative length of digit III,

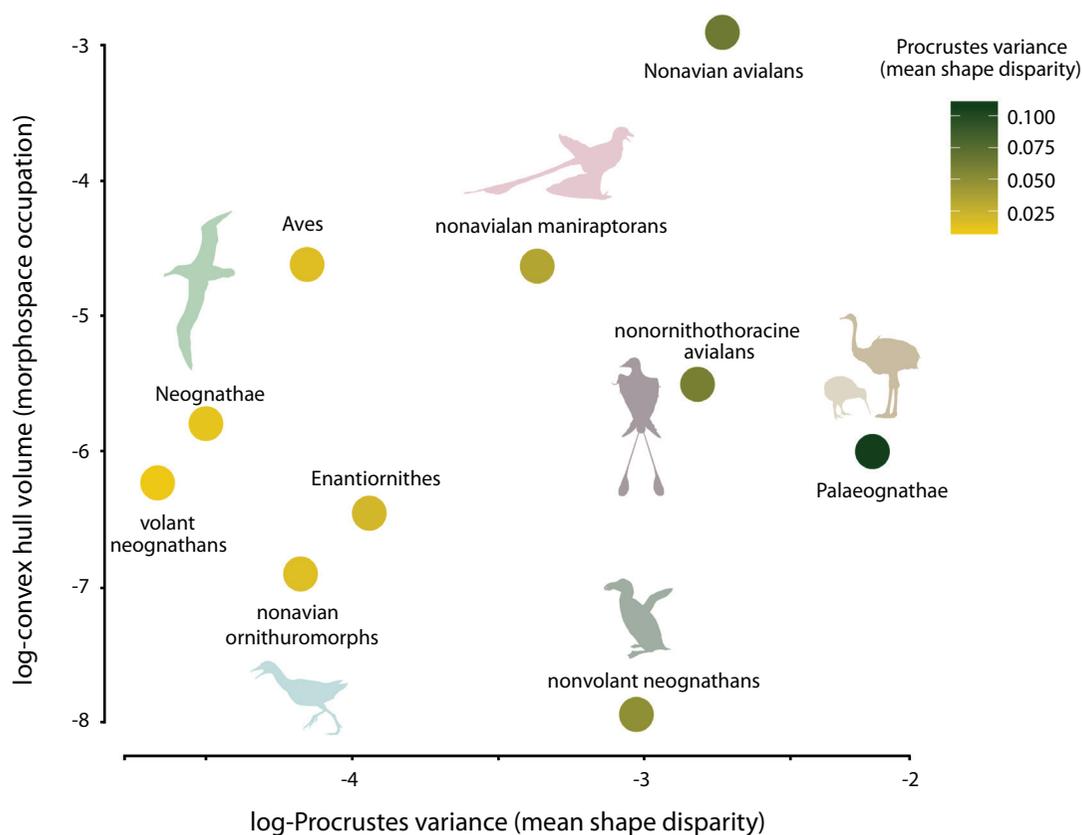


FIG. 5. Hand shape disparity (i.e., Procrustes variance) and morphospace occupation (i.e., convex hull volume) of the grades of Maniraptora.

which is generally longer in Enantiornithes (fig. 4). It is worth mentioning that the juvenile enantiornithine NIGP 130723 displays an incompletely ossified manus, thus leading to unusual proportions in which the distal elements are extremely reduced with respect to the proximal ones (even more than in crown birds); these proportions lead to significant differences between this taxon and the rest of the sample.

Interestingly, crown birds display very low manus disparity (fig. 5), all clustering in a very localized and reduced region of the morphospace, corresponding with manus morphologies with phalanges significantly shorter than those of their Mesozoic relatives. Palaeognathan taxa are an exception among the crown group in which the manus contains the greatest disparity (fig. 5),

diverging substantially from one another and from the rest of the sample. These birds are nearly fingerless (e.g., *Casuarus bennetti*, situated in the lower left region of morphospace), with extremely small manus lacking digit III (e.g., *Apteryx australis*, situated in the upper right region of morphospace), or displaying large manus with slightly elongated, clawed digits, similar to those of Enantiornithes (e.g., *Struthio camelus* or *Rhea americana* in a more central region). Among the neognathans, Aequorlornithes (seabirds, shorebirds, herons, cormorants and relatives) have some flightless forms diverging from their relatives, as in the case of the two penguins (*Spheniscus humboldti* and *Aptenodytes forsteri*), the Galapagos' cormorant (*Phalacrocorax harrisi*), and the great auk (*Alca impennis*) included in the sample.

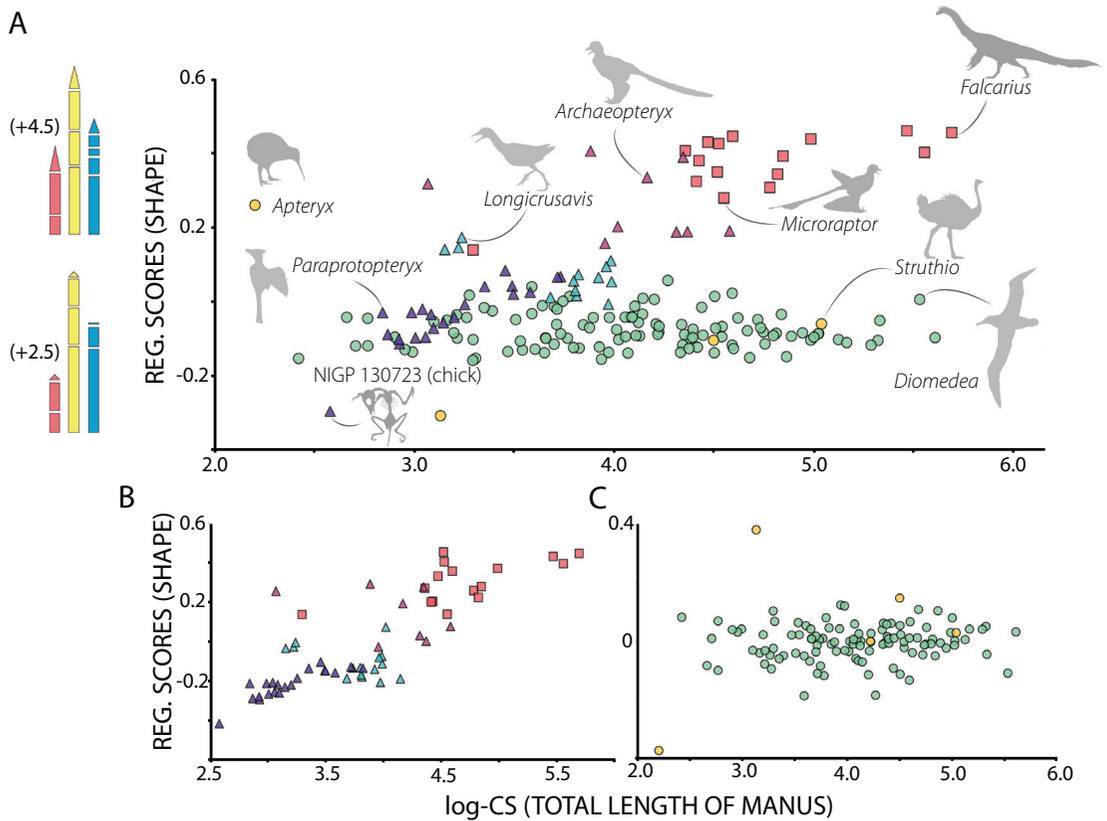


FIG. 6. Correlation between size and shape based on a multivariate regression. A significant correlation results in the existence of an allometric factor in the change of hand shape, significant in nonavian maniraptorans. Among neognathans (in green tones) there is no allometry (i.e., shape changes occur regardless of size variation). Notice as well that variation is strikingly low). Below the main graph (A) dispersions B and C show the allometric and non-allometric trends, respectively.

Although differences in disparity are visible over morphospace, the pairwise statistical comparisons between the Procrustes disparities further corroborates significant differences in mean shape disparity between the crown and stem lineages, while revealing a drastic decrease in manus-shape morphological diversity in maniraptorans after the K-Pg (fig. 5, table 1). Non-ornithothoracine avialans (i.e., long tailed birds and early-diverging pygostylians) are significantly more disparate than all the other lineages of birds, excluding flightless crown birds, and exhibit higher manus mean disparity (although lower range of morphologies) than nonavian maniraptorans. Furthermore, palaeognathans are more

disparate than all the other lineages of avialan and nonavian maniraptorans. Nonavian maniraptorans, although more disparate than all the ornithothoracine lineages including crown birds, exhibit significant differences only in mean shape disparity with crown birds (but not with flightless taxa alone). Early-diverging ornithothoracine lineages, namely, enantiornithines and early-diverging ornithuromorphs, exhibit intermediate values of mean shape disparity, but these differences are not statistically significant. Furthermore, crown birds explore a larger range of morphologies than enantiornithines and early-diverging ornithuromorphs (lower values of convex hull volumes), although this observation is influenced by large

TABLE 2

Allometric differences between stem and crown lineages and between the four main grades of maniraptorans

Summary of statistics from the PGLS regression of hand shape as a function of log-CS and group A. stem/crown and B. maniraptoran grades. Median and range values (maximum and minimum) are summarized. Statistically significant values ($P < 0.05$) are highlighted in bold. R^2 is the observed proportion of shape (dependent variable) explained by differences in size and group (independent variables) in each of the PGLS regressions; F-values are a measure of the variance explained by each of the variables as compared with the error term (unexplained variance); Z are the standard deviates from the F-values obtained in the 1,000 sampling permutations and give a measure of the intensity of the effects of size, group, and interactions between both, on shape.

A. Between stem and crown				
	R^2	F	Z	P
Log-CS	0.003 (0.002, 0.006)	0.463 (0.297, 1.020)	-0.406 (-0.852, 0.461)	0.653 (0.334, 0.799)
Group (stem/crown)	0.001 (0.001, 0.002)	0.218 (0.160, 0.322)	-1.129 (-1.455, -0.733)	0.86745 (0.764, 0.927)
Log-CS:group	0.04 (0.02, 0.06)	6.561 (3.939, 10.760)	2.484 (1.932, 2.986)	0.004 (<0.001, 0.023)
B. Between the main four grades of maniraptoran				
	R^2	F	Z	P
Log-CS	0.004 (0.002, 0.006)	0.763 (0.362, 1.256)	0.273 (-0.554, 0.824)	0.401 (0.212, 0.71)
Group (grades)	0.119 (0.101, 0.150)	6.381 (5.172, 8.542)	3.673 (3.211, 4.078)	>0.001 (>0.001, 0.001)
Log-CS:group	0.108 (0.084, 0.150)	5.783 (4.305, 8.416)	3.287 (2.750, 3.747)	>0.001 (>0.001, 0.008)

differences in sample size. Similarly, flightless neognathans (penguins, flightless cormorant, and giant auk) exhibit high mean shape disparity.

A PGLS regression between manus shape (Procrustes coordinates) and size (log-CS) for the total sample is statistically significant but only captures 2.67% of variance (fig. 6, table 2), which represents a very low percentage of shape change explained by size (evolutionary allometry). However, from a visual inspection of the plot (fig. 6) it is evident that the allometric scaling between nonavians and avians is completely different, and such difference is confirmed by the statically significant differences between allometric vectors (table 2). In fact, when analyzed separately by groups, PGLS regression in early-diverging bird taxa explains up to 42.3% of the total variance ($p < 0.0001$), whereas in Aves, the explained variance drops down to a negligible 0.5041% ($p < 0.0001$). These striking results imply that maniraptoran manus shape differences are essentially non-allometric only in crown birds.

The general allometric trend within the stem lineage equates small sized manus with relatively shorter digits (short phalanges with respect to metacarpals), very small claws, and a digit III that has lost the most distal elements (i.e., third phalanx and claw), in contrast with larger manus with relatively elongated digits (longer phalanges with respect to metacarpals), large ungual phalanges and a complete digit III (fig. 6). Few cases fall outside the general allometric trend with manus shapes that do not correspond to those expected by their size condition. In general, these outliers coincide with those observed in the PCA, such as the paleognathan *Apteryx australis* and *Casuarinus bennetti*, and the early-diverging bird *Zhongornis*.

The range of values of the statistics from the PGLS regressions did not vary much due to differences in estimated branch lengths in our 100 trees (table 2). We thus picked only one tree for pairwise allometric comparisons between the five maniraptoran grades. The results largely

TABLE 3

Pairwise differences in pattern and strength of allometry between all the grades of maniraptorans studied
Significant p-values (<0.05) indicate differences in the A. strength (slope lengths)
or pattern (slope angles) of allometry.

A. Strength of allometry					
	Avialae	Enantiornithes	Maniraptora	Aves (Neornithes)	Ornithuromorpha
Avialae	1	0.056	0.4376	0.4209	0.9351
Enantiornithes	0.056	1	0.0556	0.0026	0.021
Maniraptora	0.4376	0.0556	1	0.0083	0.2431
Aves (Neornithes)	0.4209	0.0026	0.0083	1	0.2649
Ornithuromorpha	0.9351	0.021	0.2431	0.2649	1

P values of slope lengths = differences in the amount of shape change per unit of size change

B. Pattern of allometry					
	Avialae	Enantiornithes	Maniraptora	Aves (Neornithes)	Ornithuromorpha
Avialae	1	0.0327	0.056	0.9936	0.9781
Enantiornithes	0.0327	1	0.9739	0.0045	0.0178
Maniraptora	0.056	0.9739	1	0.008	0.013
Aves (Neornithes)	0.9936	0.0045	0.008	1	0.9679
Ornithuromorpha	0.9781	0.0178	0.013	0.9679	1

P values of slope angles = differences in the pattern of the relationship between shape and size

confirm our observations by revealing only significant differences in the pattern and the strength of manus allometry between ornithuromorphs (both crown and stem) and the rest of the nonornithuromorph lineages, but nonsignificant differences between the crown (Aves) and early-diverging ornithuromorphs, therefore substantiating that both groups share a common manus allometric pattern (table 3).

DISCUSSION

Morphological evolution needs to be studied quantitatively and at high taxonomical levels if we aim to map the variational limits of disparity and to determine which main factors underlie this disparity at macroevolutionary scales (Raff, 1996). However, this requires using approaches that allow preserving both the physical integrity of the structure and the information that pertains to the organization of the whole. Geometric morpho-

metrics is one of those analytical methodologies, yet given the osteological complexity of certain anatomical structures, such as the maniraptoran manus, it is impossible to apply the method in its standard manner. In order to bridge this methodological gap, we have devised a simple protocol that translates traditional longitudinal measurements of hand bones into linear Cartesian coordinates, making them directly treatable under the scope of shape analysis based on Procrustes methods. Using OPA we have visually expressed the transition between early-diverging forms (e.g., therizinosaurians, oviraptorosaurians, and early-diverging paravians, including earliest-diverging birds), toward crown group birds, and how these compare with their closest relatives (early-diverging ornithuromorphs and enantiornithines). Given that this new way of treating data allows mapping evolutionary bone loss, we have not only shown that bone reduction and loss of phalanges are key morphological transformations, but also

that the alular (I) and minor (III) digits are the most modified elements involved in the evolutionary transition of the manus from early-diverging maniraptorans to modern birds. Uniquely, using this method we have also unveiled two previously unreported aspects of the evolution of the manus in these dinosaurs. First, we have shown that there is a marked decrease of manus disparity stemming from changes in skeletal proportions along the maniraptoran lineage, which, unexpectedly, becomes minimal in neognathan birds. Secondly, we have revealed that in most early-diverging maniraptorans, morphological variation largely scales according to an equivalent allometric pattern. In fact, in crown birds and their early-diverging ornithuromorph relatives, the low structural variation of the manus is almost completely non-allometric, entailing an important evolutionary shift in the construction of manus variation.

The distribution of the main clades over morphospace shows a minimal overlap and effectively documents the stepwise acquisition of the modern avian manus across maniraptoran evolution. This ordination altogether epitomizes the view about the evolution of the avian manus along the dinosaur-bird transition, from a more typically “dinosaurian” grasping organization of early-diverging nonavian maniraptorans (therizinosaurians and oviraptorosaurians), nonavian paravians (deinonychosaurs and scansoriopterygids), and the earliest-diverging birds, to that of a more “avian” one visible in enantiornithines, early-diverging ornithuromorphs and crown birds. Effectively, the first dimension of variation accounts for the largest morphological change, namely, the proportional distal elongation to distal reduction of the digits’ elements, showing even the loss of phalanges and the different phalangeal formulas in the lineage. This trend is one of the most relevant in the differential distribution of taxa across the phylo-morphospace. The second and third dimensions show the two digits triggering this pattern (I and III). Interestingly, our results suggest that much of the evolution of the manus across the dinosaur-bird transition has occurred in an inte-

grated way within a macroevolutionary trend of distal element reduction and loss, which is congruent with the fact that during the early stages of digit formation most proportional changes in phalanges are regulated as a system rather than individually (Kavanagh et al., 2013).

Previous studies proposed that the reduction and loss of the most distal elements of a winged manus may have been functionally adaptive, given that the mass and moment of inertia of the hand is reduced, making it more effective during active flight (Berg and Rayner, 1995; Bakker et al., 2013). Moreover, it has been proposed that the wide variety of manus morphologies of different early-diverging paravian taxa, such as the divergent scansoriopterygids, the first avialans (e.g., *Archaeopteryx*), and *Anchiornis* or *Microraptor*, could have triggered substantial flight experimentation prior to the acquisition of the modern avian manus (Xu et al., 2015; Brusatte, 2017; Wang et al., 2017, 2019). However, important morphological differences exist between crown and stem birds for which similar flight types have been inferred (e.g., *Sapeornis chaoyangensis* as a soaring bird aerodynamically similar to a screamer: Serrano and Chiappe (2017) and Serrano et al. (chapter 13); the enantiornithines *Concornis lacustris* and *Eoalulavis hoyasi*, functionally similar to small passerines or woodpeckers: Serrano et al., 2018; see Pittman et al., on early flight study, chapter 10). Our results show that the morphology of the manus in crown birds, compared with the wide range of morphologies in nonavian forms, exhibits very low disparity in spite of its uniformity in species displaying different flight styles. In light of these results, it is likely that drivers other than flight were also at play during the morphological transformation of the manus from nonavian maniraptorans to modern birds, despite highly diverse morphologies existing in the early stages of paravian evolution possibly related to experimentation of aerodynamic capabilities (as well as with a complex trade-off between aspects such as grasping, folding, and tegument support). Rather, it seems as if many different skeletal morphologies might have been functionally equiva-

lent, entailing that other morphological traits, such as aspects of the manus shape not taken into account in this study, the proportions of the forelimb elements (Serrano et al., 2017), and/or the evolution of integumentary structures such as feathers or patagia (Navalón et al., 2015; Wang et al., 2017, 2019), are probably more relevant to understand the evolution of avian flight.

It is surprising that in spite of being the most speciose group of terrestrial vertebrates (more than 10,000 species) and displaying an enormous size range (from 5 cm and 1.8 g of the hummingbird bee, *Mellisuga helenae*, up to 3 meters and 180 kg in the ostrich, *Struthio camelus*) as well as a huge ecological and functional diversity, modern birds show such a strikingly low disparity in the proportions of their hand skeleton. From a paradigmatic standpoint, this low disparity could be interpreted as a result of the filtering effect of the K-Pg mass extinction that constrained the morphological variation available for the subsequent radiation during the Cenozoic. From a paleobiological point of view, this scenario is also congruent with the type of “bottom-up” trends whereby clades accumulate high morphological disparity during their early radiation and slow down as they diversify later (Foote, 1997). Interestingly, bottom-up trends are also explained as an outcome of early experimentation of development programs through geological time (Raff, 2000). However, at lower macroevolutionary scales, a remarkable exception in such trends is the high disparity of hand skeletal proportions and phalangeal formulas of paleognathan birds. Ratites are paraphyletic due to the position of the tinamous (e.g., Prum et al., 2015), the only modern palaeognathan with flying capabilities. The manus of these avians sometimes bears claws (*Struthio camelus* and *Rhea americana*), lacks phalanges in digit III (*Apteryx australis*), entirely lacks fingers (*Casuarius bennetti*), and can even have a morphology similar to that of flying neognathan (as the case of the tinamou, *Tinamus solitarius*). Members of these lineages have different ontogenetic trajectories (Faux and Field, 2017), possibly translating into their different manual morphologies and the absence of

flight-related selective pressures eliminated a vital constraint, therefore favoring greater variability in forelimb elements. For instance, the emu (*Dromaius novaehollandie*) has greater variability in wing bones than any flying bird (Maxwell and Larsson, 2007). Among neognathans, specialized underwater divers such as the Galapagos cormorant (*Phalacrocorax harrisi*), the great auk (*Alca impennis*), and the penguins (*Spheniscus humboldti* and *Aptenodytes forsteri*) are also examples of higher disparity in different functional selective regimes.

Our results also showed that an important and common allometric trend explains a large portion of manus disparity in Mesozoic taxa, including Enantiornithes and the earlier-diverging maniraptoran lineages spectrum. This allometric trend is unambiguously truncated in early-diverging ornithuromorphs and crown birds, in which manus size and shape variation are completely decoupled (i.e., evolutionary allometry disappears). A similar pattern of allometric decoupling had been reported for the forelimb in the dinosaur-bird transition (Dececchi and Larsson, 2013), probably implying that the morphology of the manus first evolved as strongly integrated with the changes in body size that took place in the avialan stem lineage (Turner et al., 2007; Puttick et al., 2014; Lee et al., 2014). More strikingly, in light of the observed allometric pattern (fig. 6), modern avians have almost completely decoupled their manus proportions and size variation, a condition that appears to have started in early-diverging ornithuromorphs. Interestingly, the adult manus of the neognathans retains a skeletal morphology that is more similar to that of enantiornithines at early ontogenetic stages (see the manus of juvenile enantiornithine NIGP 130723 as example; Chiappe et al., 2007), hinting that their low disparity and variation, apart from the filtering effect of the K-Pg, may have also resulted from an heterochronic truncation of the primitive allometric pattern, similar to what happened with their skull (Bhullar et al., 2012). Thus, heterochrony may be a systemic process underlying many main features of the modern avian bauplan, including the manus.

CONCLUSIONS

We have proposed a new method (and named it one-dimensional Procrustes analysis, OPA), that allows transforming traditional morphometrics into unidimensional configurations of Cartesian coordinates that can be submitted to a Procrustes transformation and thereafter analyzed using the multivariate kit of geometric morphometrics. We applied the method to a large sample of maniraptoran dinosaurs, fossil and modern, and the obtained results are highly consistent with qualitative descriptions of the maniraptoran manus, thus further substantiating the applicability of the proposed morphometric protocol. Moreover, using this new protocol we have discovered that the elements evolving in the maniraptoran manus are mostly digits I and III, digit II (the central digit) being the least modified, and that much of the early stages of this evolutionary pattern are highly allometric. In contrast, we show that the transformation of the ancestral manus into that of crown group birds is not allometric (it is size independent) and that it encompasses a significant decrease of disparity, which is likely linked to heterochrony. Our morphometric proxy could be extrapolated to other articulated anatomical structures such as, but not restricted to, the manus of other dinosaurs, and other archosaurs and tetrapods in general, as well as for studies aiming at understanding other aspects of morphological evolution, such as the integration and modularity in articulated structures, which require reliable morphometric data for their statistics.

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