

Phylogeny and foraging behaviour shape modular morphological variation in bat humeri

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Abstract

Bats show a remarkable ecological diversity that is reflected both in dietary and foraging guilds (FGs). Cranial ecomorphological adaptations linked to diet have been widely studied in bats, using a variety of anatomical, computational and mathematical approaches. However, foraging-related ecomorphological adaptations and the concordance between cranial and postcranial morphological adaptations remain unexamined in bats and limited to the interpretation of traditional aerodynamic properties of the wing (e.g. wing loading [WL] and aspect ratio [AR]). For this reason, the postcranial ecomorphological diversity in bats and its drivers remain understudied. Using 3D virtual modelling and geometric morphometrics (GMM), we explored the phylogenetic, ecological and biological drivers of humeral morphology in bats, evaluating the presence and magnitude of modularity and integration. To explore decoupled patterns of variation across the bone, we analysed whole-bone shape, diaphyseal and epiphyseal shape. We also tested whether traditional aerodynamic wing traits correlate with humeral shape. By studying 37 species from 20 families (covering all FGs and 85% of dietary guilds), we found similar patterns of variation in whole-bone and diaphyseal shape and unique variation patterns in epiphyseal shape. Phylogeny, diet and FG significantly correlated with shape variation at all levels, whereas size only had a significant effect on epiphyseal morphology. We found a significant phylogenetic signal in all levels of humeral shape. Epiphyseal shape significantly correlated with wing AR. Statistical support for a diaphyseal-epiphyseal modular partition of the humerus suggests a functional partition of shape variability. Our study is the first to show within-structure modular morphological variation in the appendicular skeleton of any living tetrapod. Our results suggest that diaphyseal shape correlates more with phylogeny, whereas epiphyseal shape correlates with diet and FG.

KEYWORDS

Chiroptera, foraging ecology, functional morphology, geometric morphometrics, humerus, modularity

1 | INTRODUCTION

Animal locomotion is a key component of the ecological interactions that shape ecosystem functioning (da Silva et al., 2014; Denzinger &

Schnitzler, 2013). Locomotory strategy has an important role in the evolvability and adaptability of taxa (Dececchi et al., 2016; Witton, 2015) by shaping biological traits both at a micro- and macroscale (Luo et al., 2019; Martin-Serra et al., 2014; Medina et al., 2018; Patel

et al., 2013; Verde Arregoitia et al., 2017). Locomotion has also been a major evolutionary driver in vertebrates, enabling occupation of novel ecological niches in some cases (Sallan & Friedman, 2012; Simmons et al., 2008) and an evolutionary constraint limiting adaptability in other cases (Gutarra et al., 2019; McInroe et al., 2016). Phenotypic specialisations (e.g. increased bone density and higher metabolic rates) have evolved in flying vertebrates (i.e. pterosaurs, birds and bats), fulfilling the functional demands associated with locomotory strategies (Carter & Adams, 2016; Dececchi et al., 2016; Voigt et al., 2012). Moreover, phenotypic adaptations for locomotion also have a phylogenetic component, reflecting evolutionary relationships among taxa (Fabre et al., 2015; Hand et al., 2009). The link between phylogeny, ecology and morphology in studies of bat locomotion has proven to be variable and sometimes inconsistent with predictions based on various ecomorphological hypotheses (Diogo, 2017).

Bats are remarkably diverse from both a taxonomic (second most speciose mammal group after rodents) and ecological standpoint (widest dietary range among mammals; Hedrick et al., 2019), as well as being the only mammals capable of self-powered flight (Rayner, 1988). Despite possessing a hyper-specialised postcranium adapted for flight (e.g. elongated metacarpals, increased bone density and keeled sternum; Panyutina et al., 2015), bats show a remarkable repertoire of locomotory and foraging behaviours, including hovering flight, terrestrial locomotion, water-surface trawling and long-distance migration (Hand et al., 2009; Norberg & Rayner, 1987; Weller et al., 2016). Understanding the form-to-function interdependence in bat postcranial morphology is crucial for contextualising how the group has evolved and diversified (Ferry-Graham et al., 2002). Locomotory and foraging strategies have allowed for the incursion and colonisation of novel niches that are unique to bats among mammals, for example, terrestrial locomotion coevolved with sanguivory in vampires and hovering flight with nectar feeding (Amador, Almeida, et al., 2019). However, most studies on functional morphology in bats have focused on cranial morphology and adaptations that can be linked directly to diet and echolocation (Arbour et al., 2019; Monteiro & Nogueira, 2011; Rossoni et al., 2017; Rossoni et al., 2019). Both diet and echolocation have linked cranial phenotypic diversification and evolutionary adaptive radiations in bats (Arbour et al., 2019; Hedrick et al., 2019; Rossoni et al., 2017; Rossoni et al., 2019; Santana & Cheung, 2016; Santana et al., 2012), shedding light on the macroevolutionary trajectories that shaped modern bat diversity (Dumont et al., 2012; 2014). Tooth row complexity, cranial shape and size, nose-leaf morphology and biomechanical performance have all been linked to the colonisation of dietary and echolocating niches during major diversification events (Arbour et al., 2019; Brokaw & Smotherman, 2020; Monteiro & Nogueira, 2011; Rossoni et al., 2019; Santana et al., 2011).

Studies on postcranial functional morphology in bats, on the other hand, have mostly focused on the evolution of flight (Amador et al., 2018; Norberg & Rayner, 1987; Simmons et al., 2008; Stanchak et al., 2019). Evolutionary trajectories of the bat wing have been reconstructed using traditional metrics of aerodynamic properties and

descriptive anatomy of the wing of living and a few complete fossils (Amador, Almeida, et al., 2019; Amador, Simmons, et al., 2019; Norberg & Rayner, 1987), providing support for an arboreal ancestor for bats (e.g. Bishop, 2008; Rayner, 1988; Simmons et al., 2008; Smith, 1977). Studies reconstructing the aerofoil of *Onychonycteris finneyi* (one of the most complete bat fossils ever found) concluded that it had a primitive locomotor apparatus and was capable of self-powered flight (Amador, Simmons, et al., 2019). That study suggested the armwing was a key innovation during early bat flight evolution, whereas the subsequent evolution of the handwing enhanced flight manoeuvrability and was linked to the origin of many modern families (Amador, Simmons, et al., 2019). Another study gathered data on the aerodynamic properties of the wing of a wide variety of modern bats to reconstruct the evolutionary trajectory of bat wing aerodynamics (Amador, Almeida, et al., 2019). It hypothesised an Oligo-Miocene aerial diversification in bats that was associated with dietary specialisations, loss of echolocation in one lineage and optimal adaptation to environmental change (Amador, Almeida, et al., 2019). Body size evolution has also been linked to the evolution of flight in bats, flight acting as a selective constraint in limiting maximum body size in lineages of larger modern bats (e.g. pteropodids; Moyers Arévalo et al., 2018). Overall, there is increasing evidence that the bat postcranium evolved in association with other traits beyond the acquisition of self-powered flight.

Bat foraging guilds (FGs) have been described based on morphological traits, interpreting differences in the aerodynamic properties of the wing (Amador, Almeida, et al., 2019; Bullen & McKenzie, 2001; Denzinger & Schnitzler, 2013; Norberg & Rayner, 1987), depicting foraging differences across a bivariate gradient of aspect ratio (AR) and wing loading (WL). AR is a wingspan to wing area ratio that is correlated with the energetic cost and speed of flight, with higher AR values being interpreted as decreased energetic costs (Rayner, 1988). WL is a body weight to wing area ratio that can be used to assess manoeuvrability and mass-carrying ability, with higher WL values corresponding to increased manoeuvrability (Norberg & Rayner, 1987). As a result, wing morphology has been associated with different foraging strategies ranging in flight speed and manoeuvrability (Norberg & Rayner, 1987). Phyllostomid frugivores show below-average AR and average WL, reflecting that frugivory does not require fast and agile flight (Norberg & Rayner, 1987). On the other hand, pteropodid frugivores show some of the highest WL values among bats, possibly correlated to increased mass-carrying capabilities resulting from their larger sizes (Norberg & Rayner, 1987). Fast hawkers (aerial pursuit of prey at high speeds) usually show high values of WL, whereas slow hawkers (understory and slow aerial pursuit of prey) show low WL (Norberg & Rayner, 1987). Carnivorous bats usually experience higher demands for take-off and considerable prey load-carrying within clutter, and they tend to show low WL and AR (Norberg & Rayner, 1987). AR and WL have also been traditionally used to study wing shape and flight evolution in birds and pterosaurs (Bell et al., 2011; Habib & Ruff, 2008; McGowan & Dyke, 2007). How AR and WL correlate with individual bone

morphology along the wing remains unclear, leaving a gap in our understanding of wing ecomorphology.

Recent studies have challenged a mosaic evolution of the postcranium, where postcranial and cranial evolution are partitioned (Hedrick et al., 2020; Morales et al., 2019). Traditionally, cranial and postcranial evolution have been interpreted to be driven by different adaptive regimes, postcranial evolution associated only with locomotion and cranial evolution with diet respectively (Hedrick et al., 2020; Morales et al., 2019). Studies in flying vertebrates have also suggested that adaptations for flight are more multi-faceted than previously assumed, indicating that traditional metrics used to describe wing morphological specialisations (e.g. AR and WL) can limit our understanding of vertebrate flight ecology (Baliga et al., 2019; Chin et al., 2017). Moreover, the need to test traditional interpretations of morphological adaptations to flight at a single structure or trait level has also become evident (Amador et al., 2018; Baliga et al., 2019; Stanchak et al., 2019). Computational and mathematical approaches have allowed for the application of biomechanical and kinematic principles to the study of flight ecology and evolution (Baliga et al., 2019; Chin et al., 2017; Dececchi et al., 2016). The advent of geometric morphometrics (GMM) and phylogenetic comparative methods has also enabled the development of theoretical frameworks from which to interpret patterns of phenotypic diversification (Adams & Collyer, 2018a; Klingenberg, 2014). The interaction between morphological disparity, integration (i.e. high covariation between traits) and modularity (i.e. modules of highly correlated traits within a structure) is a major recent development in evolutionary theory (Felice et al., 2018; Gerber, 2013; Klingenberg, 2013). Integration and modularity have been shown to either increase or constrain phenotypic variation, shaping evolutionary patterns and ecological adaptations (Felice et al., 2018; López-Aguirre et al., 2019b; Zelditch et al., 2016).

GMM and phylogenetic comparative methods have also been applied to study the bat postcranium, revising our understanding of bat postcranial morphology (Louzada et al., 2019), development (López-Aguirre et al., 2019a, 2019b) and evolution (López-Aguirre et al., 2019; Stanchak et al., 2019). Prenatal development of the postcranium indicates a positive interaction between integration and disparity across development, while also revealing differences in allometric trajectories between bat suborders (López-Aguirre et al., 2019a, 2019b). Calcar development and histology in bats have also indicated a kinematic role in flight performance, highlighting the importance of previously underestimated morphological traits during the evolution of mammalian flight (Stanchak et al., 2019). Bat hindlimb morphology has also been found to reflect taxonomic- and locomotory-related differences, shedding new light on form-to-function interplay in bats (Louzada et al., 2019).

Here, we investigate patterns of phenotypic disparity in the bat wing by studying humeral shape and the possible concordance between cranial and postcranial morphological adaptations. The humerus is a bone uniquely specialised in bats to perform under multiple functional demands: withstand high mechanical loading

(Swartz et al., 1992; Watts et al., 2001), increase muscle insertion area for muscles associated with wingbeat (Tokita et al., 2012) and control manoeuvrability of the wing by rotation of the shoulder and elbow joints (Boerma, Breuer, et al., 2019). Diaphyseal and epiphyseal morphology respond to different functional needs (i.e. resistance to torsion and bending in the diaphysis and joint range of motion in the epiphyses; Cooper & Tabin, 2008; Cooper et al., 2012). We aim to test whether humeral epiphyseal and diaphyseal morphological disparities vary independently, responding to different functional constraints. Using GMM to study 3D virtual models of the humerus, we tested the relationship between phenotypic disparity and phylogeny (evolutionary relatedness), ecology (diet and foraging strategy) and biology (body size). In order to test decoupled patterns of morphological variation across functionally dissimilar sections of the humerus, we decomposed analyses of humeral morphology into whole-bone, diaphyseal and epiphyseal morphology. We tested for the presence of functional modules (epiphyses and diaphysis) in the humerus and the level of association between those. Furthermore, we assessed whether traditional metrics of wing aerodynamics are related to patterns and magnitudes of humeral morphological disparity. We hypothesise that the humerus is composed of two functional modules (diaphysis and epiphyses), one relating to ecology (epiphyses) and the other relating to body size (diaphysis). We also hypothesise that, given the strong association between differences in traditional aerodynamics and foraging strategies, AR and WL will also have a strong effect on epiphyseal morphology.

2 | METHODS

2.1 | Sample specimens

Our sample for this study comprised 56 adult specimens, selected to optimise coverage of phylogenetic (37 species, 20 families and both bat suborders), foraging behaviour (all broad foraging categories described for bats) and body size (10-fold range in body mass) diversity (Figure 1). This sample represents 95% of all modern families, 85% of dietary habits and all biomes where the order occurs. Six species were represented by four specimens each, one by two specimens and the rest by one specimen (Table S1). Alcohol-preserved specimens were sourced from the Western Australian Museum and research collections at the University of New South Wales (UNSW) and Institute of Ecology and Biological Resources of Vietnamese Academy of Science and Technology (IEBR; Table S1). Specimens sourced from IEBR were scanned at Musashino Art University using a microCT system (inspeXio SMX-90CT Plus, Shimadzu) with 90 kv source voltage and 100 mA source current at a resolution of 15 μm . Specimens sourced from UNSW were scanned at the same institution using a U-CT (Milabs, Utrecht) with 55 kV and 0.17 mA, ultrafocused setting at a resolution of 30–50 μm . Additional species were sampled from whole-body scans sourced from Digimorph (Table S1). 3D virtual models of the

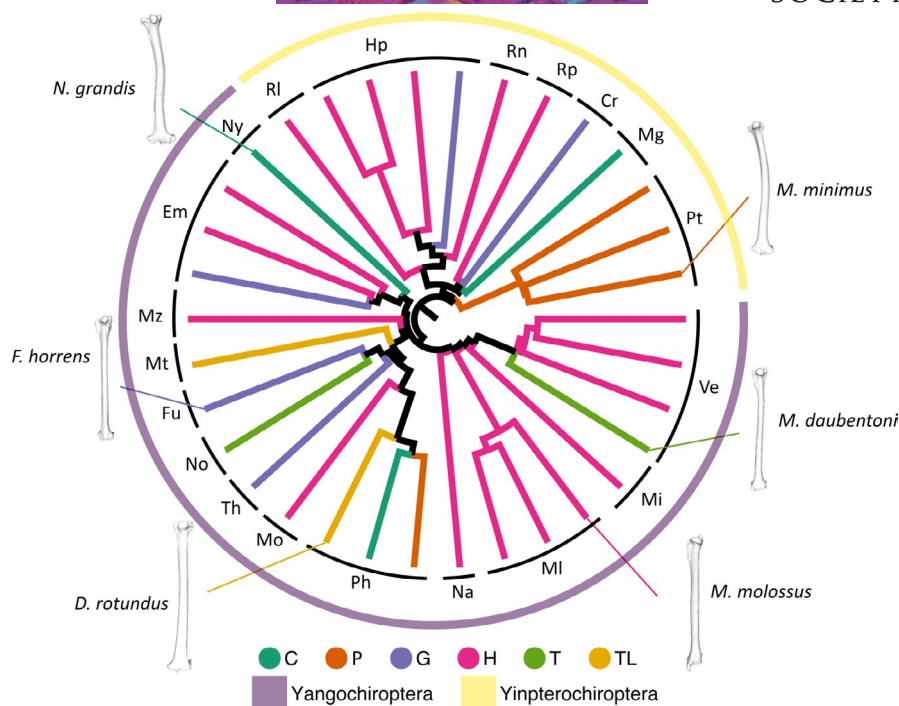


FIGURE 1 Phylogenetic relationships between sampled taxa based on Shi and Rabosky's (2015) phylogeny. Branch colours represent foraging guild categories (C= carnivores, P= phytophagous, G = gleaners, H = hawkers, T = trawlers and TL = terrestrial locomotion). Families represented in our sample: Craseonycteridae (Cr), Emballonuridae (Em), Furipteridae (Fu), Hipposideridae (Hp), Megadermatidae (Mg), Miniopteridae (Mi), Molossidae (MI), Mormoopidae (Mo), Mystacinidae (Mt), Myzopodidae (Mz), Natalidae (Na), Noctilionidae (No), Nycteridae (Ny), Phyllostomidae (Ph), Pteropodidae (Pt), Rhinolophidae (Ri), Rhinonycteridae (Rn), Rhinopomatidae (Rp), Thyropteridae (Th) and Vespertilionidae (Ve). 3D models of humeri illustrate humeral diversity in sampled taxa. Represented taxa clockwise from bottom left to bottom right: *Desmodus rotundus*, *Furipterus horrens*, *Nycteris grandis*, *Macroglossus minimus*, *Myotis daubentonii* and *Molossus molossus*

humeri were created by segmenting the raw DICOM data using the thresholding tool in MIMICS v. 20 software (Materialise NV, Leuven, Belgium). To control for differences between digitising protocols across specimens from different sources, all models were segmented manually by the lead author using raw data. In order to control for unwanted bilateral asymmetry affecting our analyses, only left humeri were digitised.

2.2 | Morphometric data

To quantify whole-bone humeral morphology, a landmarking protocol was developed using IDAV Landmark Editor (UC Davis, USA). The protocol comprised 221 landmarks and was implemented by the lead author to keep landmark placement consistent and avoid user bias error (Table S2; see López-Aguirre, 2020). Epiphyseal morphology was described with 31 homologous landmarks in traits found in all specimens (Figure 2). Proximal epiphysis was defined as the area containing the caput humeri, tuberculum majus and minus, crista pectoralis and tuberculi minoris. Distal epiphysis was defined as the area containing the fossae olecrani and radialis, spina entepicondylis, epicondylus lateralis and the condylus lateralis and medialis. Given the smooth curving surface of the diaphysis, diaphyseal morphology was described with 160 equidistant sliding semi-landmarks across four curves (40 landmarks per

curve) placed between homologous landmarks from the distal and proximal epiphyses (Figure 2). Thirty sliding semi-landmarks were placed along three curves (10 landmarks per curve) to describe the crista pectoralis, crista tuberculi minoris and crista tuberculi majoris. With this arrangement, we ensured that despite semi-landmarks not being homologous, the placement of the curves defining their positions along the humerus was homologous for all specimens.

2.3 | Phylogenetic, ecological and biological traits

To test different sources of variation in our morphometric data, we compiled information on three types of traits: phylogenetic, biological (i.e. body size) and ecological (i.e. foraging behaviour and diet) for all species. Phylogeny was codified at the familial level to test differences between families with convergent diets and foraging strategies (e.g. frugivory in phyllostomids and pteropodids and trawling in noctilionids and vespertilionids; see Table S3). Centroid size (CS), retrieved from the Generalised Procrustes Analysis of landmark coordinates (GPA; see statistical analysis section), was used as proxy for body size, implemented in 'gpgen' in the *Geomorph* R package (Adams et al., 2013; 2017). FGs were classified into six categories (trawling, hawking, gleaning, carnivory, phytophagy and terrestrial locomotion), combining traditional descriptions of

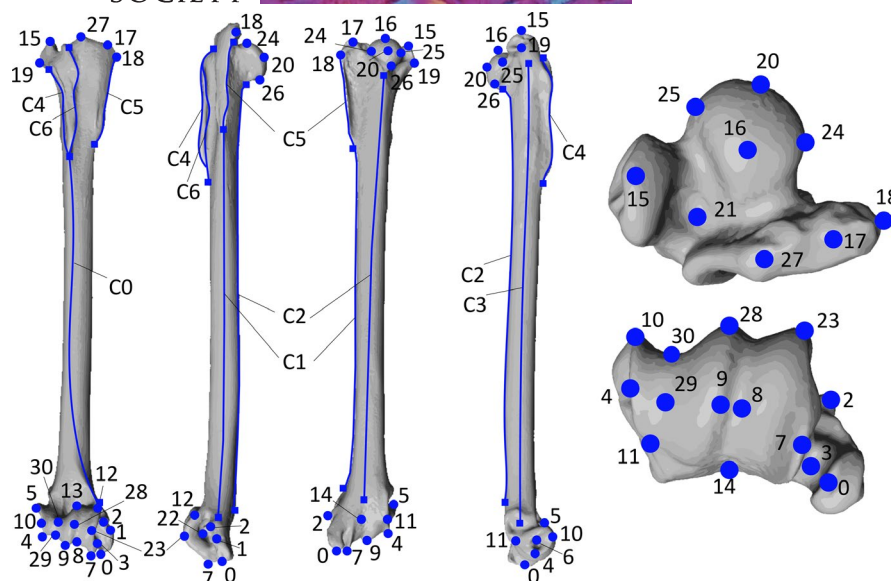


FIGURE 2 Landmarking protocol used to quantify humeral morphology. From left to right humeri are presented in anterior (far left), medial (centre left), posterior (centre right) and lateral (far right) views. Proximal (top right) and distal (bottom right) epiphyses are also presented. Homologous landmarks are represented by numbers 0–30 and curves used to place semi-landmarks are represented by C0–C5

foraging strategies and classifications of bat aerial guilds (Denzinger & Schnitzler, 2013; Norberg & Rayner, 1987). Our classification focuses on locomotory differences, rather than in echolocation-dependent aerial guild descriptions (see Denzinger & Schnitzler, 2013), emphasising foraging differences relevant to our study (Table S4). Phytophagy and carnivory were included as foraging strategies given that species with these diets show a plasticity in their foraging behaviours that is unique among bats, as well as the foodstuff they consume: hovering flight to perch-feeding in frugivores and nectarivores, and ground- and foliage-gleaning to perch-hunting in carnivores (Norberg & Rayner, 1987; Santana & Cheung, 2016). Dietary categories comprised phytophagy, insectivory, carnivory, piscivory, sanguivory and omnivory, and followed broad dietary classifications used in multiple studies (Arbour et al., 2019; Monteiro & Nogueira, 2011; Nogueira et al., 2009; Santana et al., 2012). Considering the existing interpretations of morphological adaptations for different FGs in bats based on aerodynamic properties of wing shape, we tested whether humeral morphology relates to patterns of WL and AR in our sample. WL and AR values were taken from the literature for 31 species in our sample (Bullen & McKenzie, 2001; Norberg & Rayner, 1987).

2.4 | Statistical analysis

For all statistical analyses, CS was log-transformed and species with multiple individuals were analysed based on the mean shape of the specimens using the 'mshape' function in *Geomorph* 3.2.1 for R 3.6.0 (Adams et al., 2013; 2017). For all phylogenetic analysis, Shi and Rabosky's (2015) species-level bat supermatrix phylogeny was used as the phylogenetic hypothesis of evolutionary kinship in our sample. To estimate how accurately our landmarking protocol captures

shape variation, we used the Landmark Sampling Evaluation Curve (LaSEC) approach, developed in the R package *LaMBDA* 0.1 (Watanabe, 2018). This approach estimates the fit of a 'parent' landmarking protocol from the original dataset by comparing it with the fit of subsampled landmarking protocols using Procrustes Sum of Squares. The original dataset was subsampled by sequentially adding one landmark at a time starting from 0 (each addition representing a new subsample), comparing the fit of each subsample to the parent protocol (Watanabe, 2018). An optimal landmarking protocol is expected to reach stationarity in its fit before the parental level of complexity is reached.

We tested patterns of humeral shape variation based on different evolutionary, ecological and biological hypotheses using Procrustes ANOVAs (PLM) with the 'procD.lm' function in *Geomorph* 3.2.1 (Adams et al., 2013; 2017). We compared the fit of four different statistical hypotheses against a null hypothesis in which shape is not correlated with any independent variable (shape ~1), using the 'anova' function in the *RRPP* 0.5.2 R package: differences based on body size (shape ~CS), phylogeny (shape ~suborder), foraging behaviour (shape ~FG) and diet (shape ~dietary category). Next, we tested the effect of evolutionary history on our four hypotheses of shape variation (Adams & Collyer, 2018b). For this, phylogenetic Procrustes ANOVAs (PGLS) were used to test how much of morphological variation of the humerus can be explained by diet, FG and body size (CS), after controlling for evolutionary kinship, using the 'procD.pgls' function in *Geomorph* 3.2.1 (Adams et al., 2013; 2017). Model fit comparison followed the one used for Procrustes ANOVA described above, using the 'anova' function in *RRPP* 0.5.2. PLM and PGLS were used in order to test the correspondence between humeral morphology and traditional wing shape aerodynamic properties (AR and WL) with the 'procD.lm' and 'procD.pgls' functions in *Geomorph* 3.2.1.

TABLE 1 Procrustes ANOVA (PLM) for different hypotheses of shape variation. Significance test was based on 1000 iterations

	ResDf	Df	RSS	SS	MS	Rsq	F	Z	p
Whole-bone									
log(CS)	32	1	0.338	0.009	0.009	0.026	0.841	-0.026	0.492
Family	14	19	0.134	0.213	0.011	0.614	1.174	0.572	0.264
Diet	28	5	0.260	0.087	0.017	0.250	1.866	1.808	0.027
FG	28	5	0.267	0.080	0.016	0.230	1.675	1.898	0.034
Diaphysis									
log(CS)	32	1	0.453	0.011	0.011	0.024	0.777	-0.135	0.529
Family	14	19	0.185	0.279	0.015	0.601	1.112	0.350	0.359
Diet	28	5	0.354	0.110	0.022	0.237	1.742	1.574	0.062
FG	28	5	0.364	0.100	0.020	0.217	1.548	1.560	0.063
Epiphysis									
log(CS)	32	1	0.027	0.002	0.002	0.074	2.546	2.480	0.019
Family	14	19	0.006	0.023	0.001	0.792	2.814	6.137	0.001
Diet	28	5	0.019	0.010	0.002	0.338	2.855	4.704	0.001
FG	28	5	0.019	0.009	0.002	0.330	2.753	4.795	0.001

TABLE 2 Kmult- statistic test of phylogenetic signal on shape data. Significance test was based on 1000 iterations

	K-	p	Z _{CR}
Whole-bone	0.9425	0.005	2.4979
Diaphysis	0.9341	0.009	2.1878
Epiphyses	1.0479	0.001	6.9649

The multivariate Kmult (K-) statistic was used to test whether morphological variation had a phylogenetic structure reflecting evolutionary relatedness, using the 'physignal' function in *Geomorph* 3.2.1 (Adams, 2014). K- evaluates the degree of phylogenetic signal in our dataset compared to what would be expected under a Brownian motion model of evolution based on 1000 iterations. Phylogenetic signal was tested for the whole-bone and each module separately.

We performed a Principal Component Analysis (PCA) to reduce the dimensionality of our data and visualise patterns of variation across taxa, reconstructing the morphospaces of humeral, diaphyseal and epiphyseal morphological variation, using the 'plotTangentSpace' function in *Geomorph* 3.2.1. Given the strong and significant phylogenetic signal in our dataset, we also reconstructed morphospaces of humeral, diaphyseal and epiphyseal morphological variation that accounted for phylogenetic relationships (Uyeda et al., 2015), using a phylogenetic PCA (pPCA) as implemented in the 'phyl.pca' function in the *phytools* 0.6-99 R package. To visualise the magnitude of shape variation across principal components (PCs) explained by individual landmarks, we estimated heatmaps of landmark shape variation by comparing the minimum and maximum of each PC, using *landvR* 0.3 (Guillaume & Weisbecker, 2019). Morphospaces occupied by each suborder were visualised to help identify possible convergent morphologies within FG.

2.5 | Morphological disparity, integration and modularity

Humeral shape disparity was quantified at whole-bone, diaphyseal and epiphyseal levels among FGs using Procrustes variance (i.e. sum of diagonal elements of covariance matrix divided by number of specimens by group) using the function 'morphol.disparity' from the R package *Geomorph* 3.2.1 (Adams et al., 2013; 2017). We used modularity and integration approaches to test the a priori hypothesis that the humerus represents two functional units (modules); one module for the diaphysis and the other for the epiphyses. Integration refers to the level of association in morphological variation within a structure, whereas modularity refers to how that association is distributed within the structure. Modularity reflects covariation being unevenly distributed within a structure, forming modules of highly correlated traits that show lower magnitudes of between-module covariation than within-module covariation. To assess whether the diaphysis and epiphyses of the humerus represent two independent modules, we implemented the 'modularity.test' function in *Geomorph* 3.2.1 (Adams et al., 2013; 2017), which quantifies the degree of modularity using the covariation ratio (CR) coefficient (Adams & Peres-Neto, 2016). Values <1 indicate greater within-module covariation relative to between-module (i.e. lower values provide greater support for modularity hypothesis). Statistical significance was assessed by comparing the observed CR value against a distribution of simulated CR values obtained by randomly assigning landmarks to either module for 1000 iterations. Lastly, to estimate the amount of covariation between the epiphyses and diaphysis modules, a two-block partial least squares (PLS) analysis was used, implemented in the 'integration.test' function in *Geomorph* 3.2.1, and its statistical significance was based on 999 iterations (Adams et al., 2013; 2017). Modularity and integration were also tested after accounting for phylogenetic relatedness using the 'phylo.modularity' and 'phylo.integration' functions in *Geomorph*.

TABLE 3 Phylogenetic Procrustes ANOVA for different hypotheses of shape variation. Significance test was based on 1000 iterations

	ResDf	Df	RSS	SS	MS	Rsq	F	Z	p
Whole-bone									
log(CS)	32	1	0.007	0.000	0.000	0.024	0.800	-0.107	0.518
Family	14	19	0.004	0.003	0.000	0.449	0.599	-1.840	0.971
Diet	28	5	0.005	0.001	0.000	0.218	1.560	1.297	0.108
FG	28	5	0.005	0.001	0.000	0.192	1.330	1.040	0.142
Diaphysis									
log(CS)	32	1	0.009	0.000	0.000	0.023	0.742	-0.207	0.553
Family	14	19	0.005	0.004	0.000	0.436	0.569	-1.953	0.978
Diet	28	5	0.007	0.002	0.000	0.209	1.477	1.123	0.144
FG	28	5	0.007	0.002	0.000	0.182	1.243	0.786	0.210
Epiphysis									
log(CS)	32	1	0.000	0.000	0.000	0.060	2.042	2.045	0.034
Family	14	19	0.000	0.000	0.000	0.668	1.483	2.419	0.011
Diet	28	5	0.000	0.000	0.000	0.319	2.621	4.386	0.001
FG	28	5	0.000	0.000	0.000	0.292	2.314	4.348	0.001

3 | RESULTS

3.1 | Landmarking accuracy

LaSEC analyses suggest that our landmarking protocol was effective in capturing humeral morphology at all levels (whole-bone, diaphyseal and epiphyseal): sampling curves show asymptotic trajectories reaching fit values of 1 before reaching parental level of landmarking complexity (Figure S1).

3.2 | Humeral morphological variation

Model comparison of shape variation based on PLM revealed that all four models tested (CS, family, diet and FG) performed better than the null model, with the exception of CS and family, which were not significant for whole-bone and diaphyseal shape variation (Table 1). On average, diet explained the highest proportion of shape variation (27.5%), followed by FG (27.88%). CS and family accounted for 4.09% and 79.2% of epiphyseal shape variation respectively. Diet ($Z_{\text{whole-bone}} = 1.81$, $Z_{\text{diaphysis}} = 1.57$) and FG ($Z_{\text{whole-bone}} = 1.90$, $Z_{\text{diaphysis}} = 1.56$) also had the highest effect sizes in whole-bone and diaphyseal shape variation, whereas family had the greatest effect on epiphyseal shape variation ($Z_{\text{epiphysis}} = 6.14$).

Tests of phylogenetic signal revealed a significant effect of evolutionary kinship on patterns of shape variation in our sample, suggesting that closely related taxa are morphologically more similar than expected under a Brownian motion model of evolution (Table 2). Both K - and Z_{CR} values were higher for epiphyseal shape, revealing a stronger phylogenetic constraint, whereas whole-bone and diaphyseal shape had similar values of K - and Z_{CR} .

Contrary to our PLM results, PGLS revealed divergent model fit in diaphyseal and whole-bone shape and similar model fit for

epiphyseal shape (Table 3). For whole-bone and diaphyseal shape, only diet and FG were marginally significant, accounting for 20.5% and 19.15% of shape variation, respectively. Contrastingly, CS and family were significant interaction terms only for epiphyseal shape variation. Family explained the highest proportion of epiphyseal shape variation (66.8%), followed by diet (31.9%), FG (29.2%) and CS (6%). Effect sizes revealed that diet (average $Z = 2.70$) and FG (average $Z = 2.75$) were the best models to explain shape variation at all levels. Given the close interaction between diet and FG (phytophagy and carnivory were also classified as foraging categories), and the functional association between foraging behaviours and wing morphology, all further analyses focused on FG.

WL was not correlated with humeral shape at any level of variation, whereas AR correlated significantly with epiphyseal shape (Table 4).

Levels of morphological disparity across different FG showed similarities between whole-bone and diaphyseal shape, and unique patterns of variation in epiphyseal shape disparity (Figure 3). For whole-bone and diaphyseal shape, phytophagy showed the highest magnitudes of morphological disparity (0.014 and 0.02 respectively), followed by gleaning (0.009 and 0.012 respectively), hawking (0.008 and 0.01 respectively), terrestrial locomotion (0.007 and 0.01 respectively) and carnivory (0.004 and 0.005 respectively). Trawling showed the lowest values of whole-bone and diaphyseal shape disparity (0.002 for both; Table 2). However, no statistical significance was found among pairwise comparisons (Supplementary Table 5). Hawking had the highest epiphyseal shape disparity (6.69E-04), followed by gleaning (5.89E-04), and terrestrial locomotion (4.56E-04), carnivory (4.08E-04), phytophagy (3.61E-04) and trawling with the lowest epiphyseal shape disparity (3.50E-04; Figure 3). Pairwise comparisons revealed that disparity values for hawking were significantly higher compared to carnivory, phytophagy and trawling (Table S5).

TABLE 4 Procrustes ANOVA for the hypotheses of covariation between humeral shape and wing aerodynamic properties (WL and AR). Significance test was based on 1000 iterations

	Df	SS	MS	Rsqr	F	Z	p
Whole-bone							
WL	1	0.007	0.007	0.023	0.676	-0.518	0.698
AR	1	0.010	0.010	0.030	0.909	0.012	0.513
Diaphysis							
WL	1	0.009	0.009	0.022	0.649	-0.543	0.704
AR	1	0.012	0.012	0.029	0.859	-0.071	0.544
Epiphyses							
WL	1	0.001	0.001	0.027	0.817	-0.297	0.597
AR	1	0.003	0.003	0.106	3.455	3.244	0.002

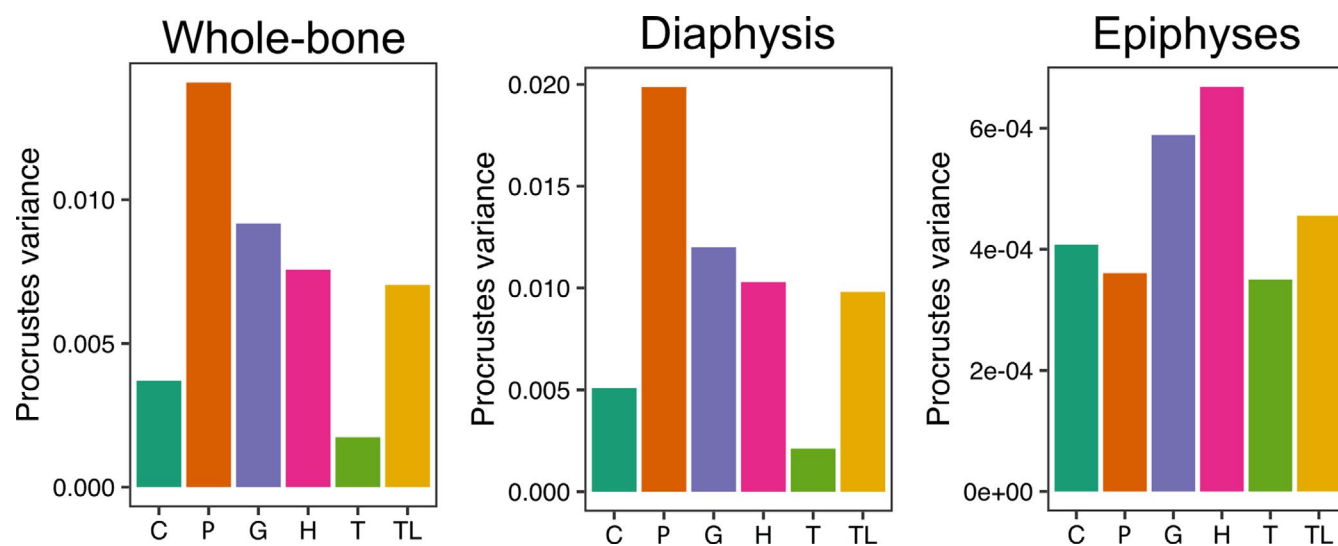


FIGURE 3 Humeral shape disparity of whole-bone (left), diaphyseal (centre) and epiphyseal (right) morphology. Shape disparity was decomposed based on foraging guild categories: C = carnivores, P = phytophagous, G = gleaners, H = hawkers, T = trawlers and TL = terrestrial locomotion

3.3 | Morphospace reconstruction

PCA of whole-bone humeral morphology resulted in the first four PCs of morphospace accounting for 69.34% of shape variance (Figure 4). PC1 primarily divides species between suborders, with a shape variation trend of increasing sigmoidal curvature of the diaphysis, as well as a reduction of the proximal projection of the tuberculum majus. Phytophagy and hawking showed the highest dispersion across PC2, whereas carnivory and trawling showed the lowest dispersion, driven by differences in the shape of the crista pectoralis. Phytophagy showed lower dispersion across PC3 (characterised by greater humeral torsion) and PC4 (changes in shape of tuberculum minus and spina entepicondylis). The first two components of pPCA revealed a greater overlap in morphospace between suborders, and lower dispersion within each FG. *Austronomus australis* expanded the distribution of Yangochiroptera both in PCA and pPCA.

The first four PCs of diaphyseal and epiphyseal morphospaces explained on average 64.03% of shape variation (71.39% and 56.68% respectively). Diaphyseal and whole-bone morphospaces

showed analogous patterns of variation across PC1 and PC2, with FGs distributed similarly across morphospace (Figures 4A,C and 5A,C). Pteropodid and phytophagous phyllostomids clustered on opposite sides of PC1 and PC2, whereas carnivores and trawlers clustered forming small morphospaces towards the centre (Figure S2). Higher dispersion of carnivores and gleaners was evident across PC3 and PC4. Suborders showed higher dispersion and overlap across the first four components of diaphyseal morphospace, compared to diaphyseal and whole-bone morphospaces. Moreover, FGs showed less overlap in epiphyseal morphospace, with carnivores and frugivores occupying non-overlapping sections of morphospace.

Phylogenetically corrected morphospaces of diaphyseal and epiphyseal shape show greater dispersion of suborders across morphospace, particularly in Yangochiroptera (Figure 6). In diaphyseal morphospace, Yinpterochiroptera and Yangochiroptera dispersed across opposite PCs (pPC1 and pPC2 respectively). Pteropodid and phytophagous phyllostomids occupied opposite ends of diaphyseal morphospace across pPC1 and pPC2, probably reflecting the sigmoidal shape diaphysis in pteropodids versus the straight shaft in phyllostomids. Gleaners

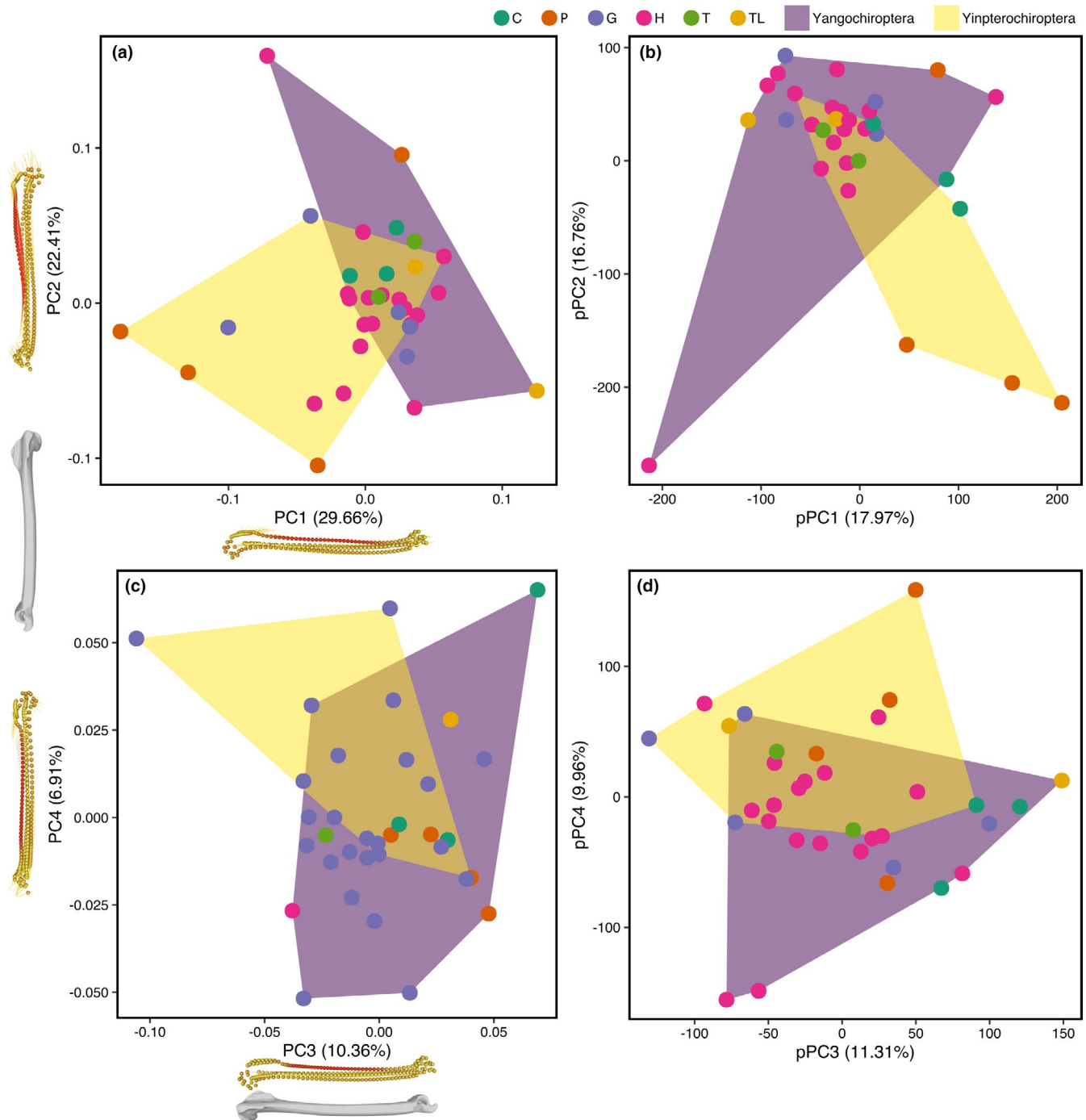


FIGURE 4 Morphospace (PCA, a and c) and phylogenetically corrected morphospace (pPCA, b and d) based on whole-bone shape data. Dot colours represent foraging guild categories (C = carnivores, P = phytophagous, G = gleaners, H = hawkers, T = trawlers and TL = terrestrial locomotion), and polygon colours suborder (purple = Yangochiroptera, yellow = Yinpterochiroptera). Landmark heatmaps of shape change represent magnitude of shape variation across each PC by comparing the minimum and maximum of each component. Humeri 3D models represent position of landmark heatmaps; red colours representing greater variation and yellow colours lower variation

and hawkers showed the highest overlap across pPC1 to pPC4, reflecting foraging plasticity and similarities in hunting styles between both groups. Carnivores and phytophagous species showed the highest discrimination across diaphyseal morphospace. FGs were relatively separated across pPCA diaphyseal morphospace relative to PCA morphospace. TL species clustered closer together in pPCA epiphyseal

morphospace, compared to whole-bone and diaphyseal morphospaces. Gleaners and hawkers shared similar patterns of variation once the effect of phylogeny was removed, overlapping more across pPC1–pPC4 than any other FG. Carnivores, phytophagous species, trawlers and terrestrial locomotors showed clear differences in diaphyseal morphospace once phylogenetic signal was removed.

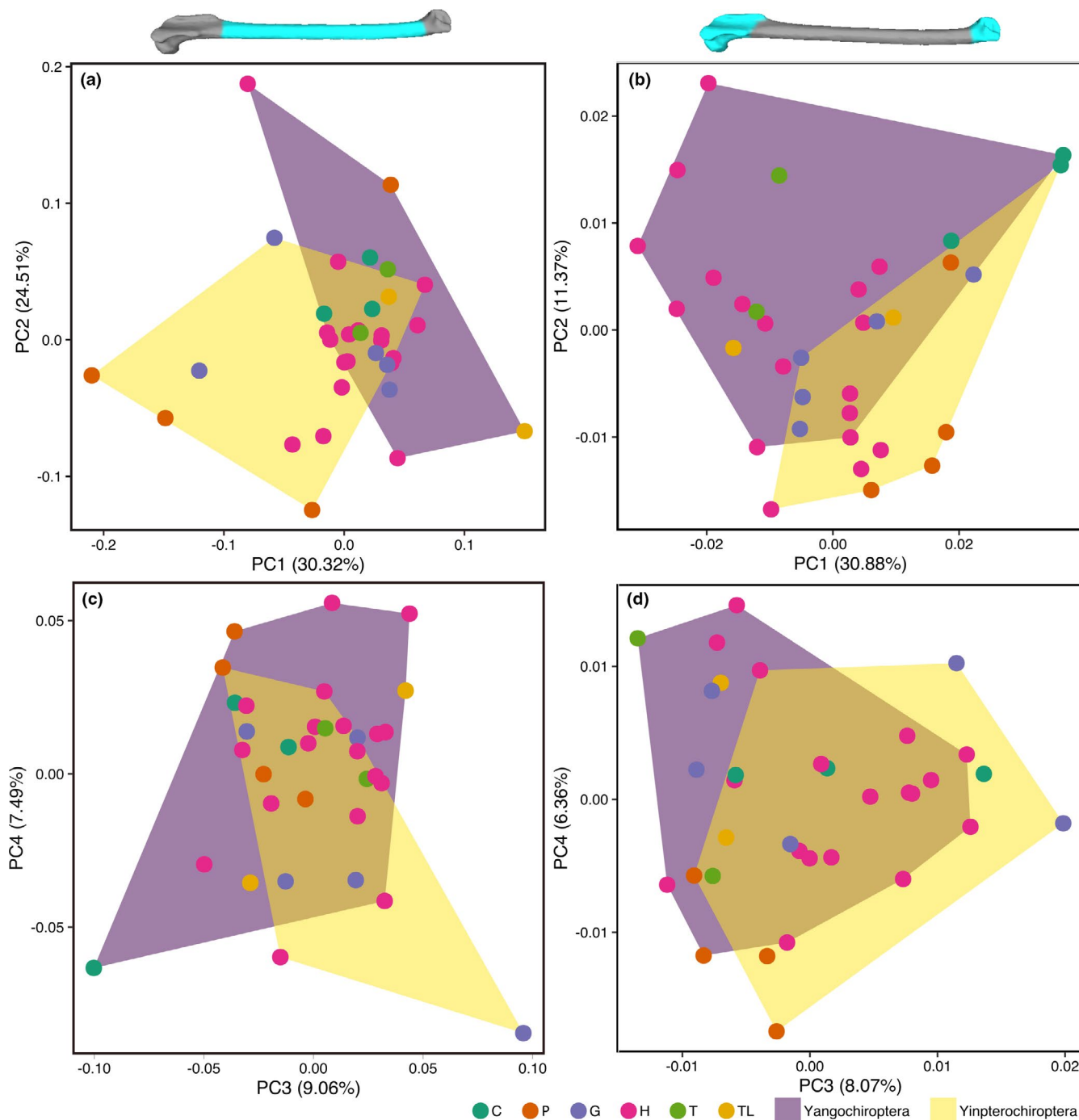


FIGURE 5 Diaphyseal (left) and epiphyseal (right) morphospaces of humeral morphology based on PCAs of shape data. Dot colours represent foraging guild categories (C = carnivores, P = phytophagous, G = gleaners, H = hawkers, T = trawlers and TL = terrestrial locomotion), and polygon colours suborder (purple = Yangochiroptera, yellow = Yinpterochiroptera)

3.4 | Morphological modularity and integration

Tests of modularity and phylogenetic modularity both rejected the null hypothesis of no modularity in humeral shape, supporting our hypothesis of a functional diaphysis and epiphyses modular partition. Modularity tests that accounted for phylogenetic relationships found greater support for our modularity hypothesis ($CR=0.67$, $p = 0.001$, $z = -13.66$) than those that did not ($CR = 0.79$,

$p = 0.001$, $z = -8.68$). Integration was also statistically significant for both tests, with phylogenetic integration showing a slightly lower magnitude of integration ($r = 0.83$, $p = 0.001$, $z = 4.53$) than non-phylogenetic integration ($r = 0.87$, $p = 0.001$, $z = 4.89$). PLS results of integration placed TL and phytophagous taxa at opposite ends of the axes, TL species occupying the positive extreme and phytophagous taxa the opposite extreme (Figure 7). The first axis of PLS explained 83% of shape covariation between modules.

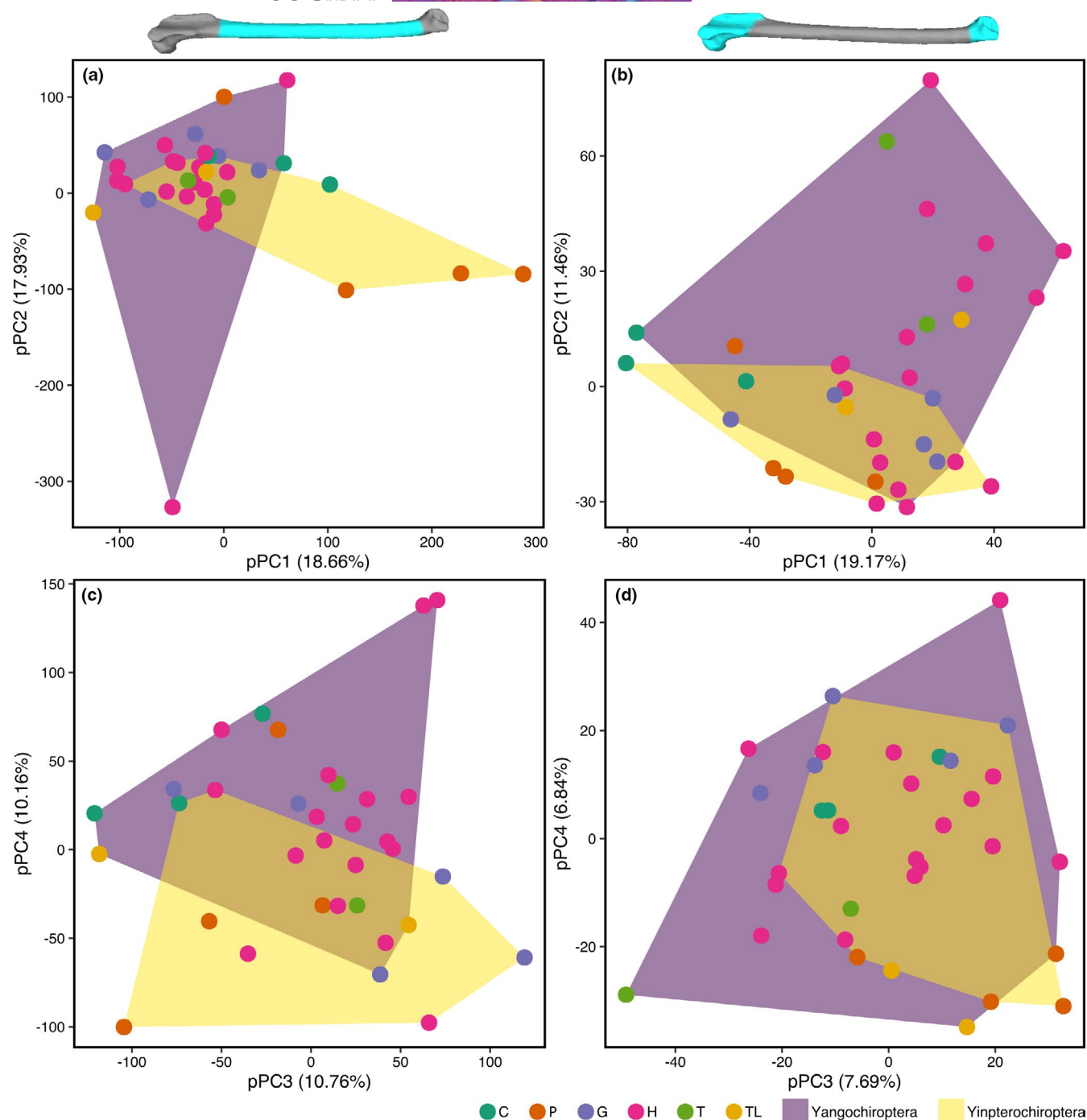


FIGURE 6 Diaphyseal (A and C) and epiphyseal (B and D) phylogenetically corrected morphospaces of humeral morphology based on pPCAs of shape data. Dot colours represent foraging guild categories (C = carnivores, P = phytophagous, G = gleaners, H = hawkers, T = trawlers and TL = terrestrial locomotion), and polygon colours suborder (purple = Yangochiroptera, yellow = Yinpterochiroptera)

4 | DISCUSSION

By combining GMM and phylogenetic comparative methods to study humeral morphology in bats, we found a strong association between humeral shape, ecology (i.e. diet and FG) and phylogeny, indicating that humeral morphological disparity has both an ecological and evolutionary signal. The magnitude of the effect of diet and FG in humeral shape mirrors patterns of cranial morphological

disparity in bats, providing evidence for a correspondence between cranial and postcranial morphological disparity (Arbour et al., 2019; Brokaw & Smotherman, 2020; Hedrick et al., 2019; Leiser-Miller & Santana, 2020; Monteiro & Nogueira, 2011). Our results reveal differences between epiphyseal and diaphyseal shape in their relationship to ecology and phylogeny. We found that ecology had a greater effect on epiphyseal morphology, whereas phylogeny and size (i.e. CS) had a greater effect on diaphyseal shape, also reflected in statistical

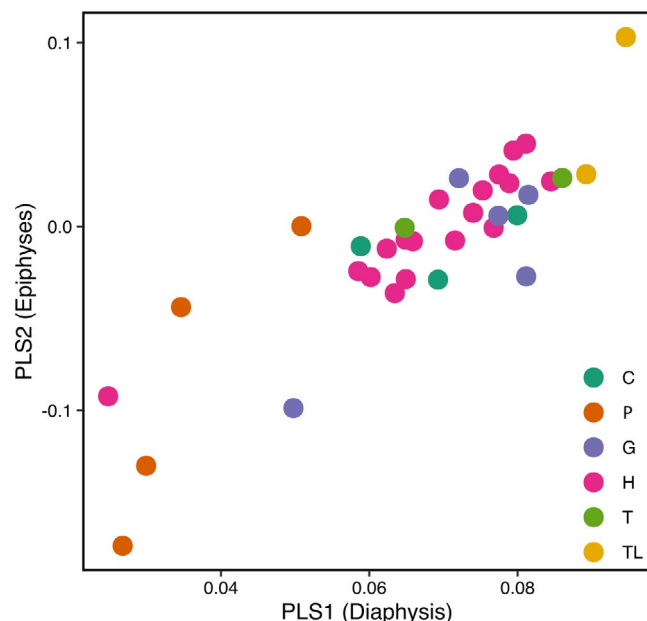


FIGURE 7 PLS biplot of first two axes of diaphyseal and epiphyseal shape covariation. Dot colours represent foraging guild categories (C = carnivores, P = phytophagous, G = gleaners, H = hawkers, T = trawlers and TL = terrestrial locomotion)

support for our modularity hypothesis dividing the humerus into two functional modules (diaphysis and epiphyses). Future studies could focus on further exploring the patterns found in this study in pteropodid bats to explore possible differences in larger-than-average bat species.

Integration tests found significant interaction between diaphyseal and epiphyseal shape variation with differing magnitude across FG. We found that only epiphyseal shape correlated with wing AR, traditionally used to study bat wing/flight capabilities (Amador, Almeida, et al., 2019; Amador, Simmons, et al., 2019; Norberg & Rayner, 1987; Rayner, 1988). Our results indicate multi-faceted patterns of shape variation in bats where ecology, size and phylogeny interplay to shape the modular morphology of the humerus (Kilbourne & Hutchinson, 2019; López-Aguirre et al., 2019). Moreover, we found a significant association between ecomorphological adaptations of the humeral epiphyses and wing shape, revealing the interplay between epiphyseal shape, wing shape and control of mobility during flight (Bergou et al., 2015; Boerma, Breuer, et al., 2019; Swartz et al., 2007).

4.1 | Drivers of humeral morphological variation

Forelimb ecomorphological diversity has been associated with the ecological and taxonomic diversification of mammals, showing an evolutionary trajectory of increasing forelimb disparity (Lungmus & Angielczyk, 2019). Our PLM results revealed a significant effect of evolutionary history and ecology on humeral shape at the three levels studied (i.e. whole-bone, diaphyseal and epiphyseal),

a result expected following studies of morphological variation in bats (Arbour et al., 2019; Brokaw & Smotherman, 2020; Monteiro & Nogueira, 2011; Rossoni et al., 2017), other mammals (Law, 2019) and other vertebrates (Gill et al., 2014; Hedrick et al., 2020; Vidal-García & Keogh, 2017; Wilson, 2013). Morphological adaptations of the humerus and shoulder joint in bats have proven informative, providing insight into the functional performance of bat species and the systematic arrangement of the order (Gaudioso et al., 2020; Hand et al., 2009; Schlosser-Sturm & Schliemann, 1995). Bat humeri exhibit a range of morphological, biomechanical and histological adaptations responding to demands for muscle insertion (enlarged crista pectoralis and crista tuberculi; Panyutina et al., 2015), shoulder and elbow joint mobility (tuberculum majus of proximal epiphysis and spinous process of distal epiphysis; Panyutina et al., 2015) and resistance to stress and strain (higher mineralisation compared to other wing bones and more circular diaphyseal cross-sectional geometry; Swartz & Middleton, 2008). Broadly speaking, functional demands driven by loading regimes and manoeuvrability exert multiple selective pressures across the humerus (López-Aguirre et al., 2019). The humeral diaphysis is functionally adapted to withstand torsion and bending stresses during flight (Swartz et al., 1992), whereas the humeral epiphyses show adaptations associated with control of wing-beat and manoeuvrability (Patel et al., 2013; Cubo & Casinos, 1998; Simons et al., 2011). Previous analyses of femoral and scapular morphological disparity in Yangochiropteran bats reveal a similar pattern to ours, detecting differences between major taxonomic groups, as well as between species with different ecologies (Gaudioso et al., 2020; Louzada et al., 2019). Foraging strategies have been strongly associated with differences in bat femoral morphology (Louzada et al., 2019), revealing parallel morpho-biomechanical traits between species with different foraging strategies (e.g. trawlers and walking bats). Similar functional demands between trawling and terrestrial locomotion could have led to the development of shared morpho-biomechanical adaptations (e.g. robust diaphyses) in these two foraging strategies (Louzada et al., 2019).

Support for different models of shape variation for diaphyseal and epiphyseal morphology (based on PGLS) may reflect multiple functional pressures acting on different parts of the humerus in bats. The significant effect of family in epiphyseal shape variation after controlling for phylogenetic kinship could indicate low convergence in shape between families with the same FG. The loss of statistical significance on the effect of diet and FG on whole-bone and diaphyseal shape variation after controlling for phylogenetic kinship suggests that unexplored sources of variation (e.g. biomechanical loading) could also affect humeral and diaphyseal shape variation (Swartz et al., 2007; Swartz et al., 1992). Morphological convergences between distantly related taxa with shared FG and additional sources of shape variation should be explicitly tested in future studies to further resolve patterns of humeral shape variation.

Greater effect size values and more than 30% of epiphyseal shape variation explained by diet and FG indicate that this region of the humerus could experience stronger selective pressures associated with adaptability to different foraging strategies. Descriptive

and comparative anatomy studies of the shoulder joint have identified three broad categories of shoulder joint specialisations linked to specific flight and locomotory capabilities (Schlosser-Sturm & Schliemann, 1995): (1) generalist shoulder joint with a single articular surface, commonly found in phytophagous pteropodid, (2) specialised shoulder joint with a single articular face described in mormoopid, noctilionid and emballonurid animalivores and (3) specialised shoulder joint with a secondary articulation reported in some noctilionoid families (e.g. Phyllostomidae, Thyropteridae and Furipteridae) and all vespertilionoid families studied (i.e. Natalidae, Molossidae and Vespertilionidae). Shoulder joint specialisations have a functional role in locomotory performance in limiting humeral rotation due to pronation during downstroke (Schlosser-Sturm & Schliemann, 1995). Specialisations of the shoulder joint imply adaptations of the proximal epiphysis of the humerus, indicating that humeral morphology can work as a proxy to study such adaptations (Hand et al., 2009; Schlosser-Sturm & Schliemann, 1995).

Statistically significant phylogenetic signal in our datasets follows similar patterns previously reported for cranial morphology in bats (Arbour et al., 2019; Hedrick et al., 2019). The magnitude of phylogenetic signal in postcranial morphological diversity in mammals remains unclear, with studies reporting a significant effect in carnivorans and marsupials (Janis et al., 2020; Martín-Serra et al., 2017; Martín-Serra et al., 2014), but non-significant at macroevolutionary scales (Lungmus & Angielczyk, 2019). Further studies are needed to understand the macroevolutionary patterns of morphological disparity in the mammalian postcranium and the role of ecology and phylogeny influencing those patterns.

We found epiphyseal shape to be highly correlated with AR (wing area) but found no significant correlation of humeral morphology with WL (weight divided by wing area). Considering the many studies using aerodynamic metrics of the wing to describe functional morphology in bats (Norberg & Rayner, 1987; Rayner, 1988), it is reasonable to expect that epiphyseal ecomorphology would be correlated with overall wing shape, reflecting daily functional demands on the shoulder and elbow joints that would act similarly at both scales. Epiphyseal morphology is directly involved in wingbeat as it controls movement, which in turn has a direct impact on the energetic cost (Riskin et al., 2012), flight speed and manoeuvrability of flight (Bergou et al., 2015; Boerma, Breuer, et al., 2019; Iriarte-Díaz et al., 2011). Recent studies in birds have posited that traditional metrics of wing morphology fall short in explaining the complexity of phenotypic adaptations for flight (Baliga et al., 2019), highlighting the need to apply novel and multi-disciplinary approaches to this question. Our results indicate that phenotypic adaptations for flight in bats are multi-faceted, acting at different scales (e.g. from single structure to functional unit), and that the implementation of different approaches can inform different aspects of those adaptations.

Strong similarities in whole-bone and diaphyseal morphospaces and levels of disparity reveal the influence of diaphyseal morphology on overall disparity in humeral shape. Across whole-bone and diaphyseal morphospaces, *Austronomus australis* separated from the rest of yangochiropterans, which tended to cluster together, revealing the

humeral morphology of this species adapted for higher speeds and minimising drag (Bullen & McKenzie, 2007; see Figure S2). Studies analysing flight speeds in a range of bat species have indicated that *A. australis* is able to reach faster-than-average flight speeds of up to 14 m s^{-1} (average speed of 6.76 m s^{-1} , ranging from 3.6 to 18 m s^{-1} ; see Bullen & McKenzie, 2007 and references therein). *A. australis*' flight speeds fall within the range of other fast-flying molossids such as *Tadarida brasiliensis* (14.3 m s^{-1} ; McCracken et al., 2016) and *T. teniotis* (13.9 m s^{-1} ; Marques et al., 2004). Gracile humerus and radius in *A. australis* have been associated with its interceptor foraging behaviour (fast aerial pursuit with low manoeuvrability in open areas), which depends on optimal generation of a leading-edge vortex (Bullen & McKenzie, 2007). Higher diaphyseal disparity in phytophagous species parallels the role that phytophagy had in the adaptive radiation of cranial phenotypes in bats (Arbour et al., 2019; Rossoni et al., 2017; Rossoni et al., 2019). Phytophagous phyllostomids and pteropodids occupied opposite regions of morphospace, suggesting divergent patterns of morphological specialisation for phytophagy that parallel the convergent evolution of frugivory and nectarivory in the two groups of bats (Datzmann et al., 2010; Monteiro & Nogueira, 2011; Rojas et al., 2012). Another possible explanation could be that, having similar diets, phyllostomids and pteropodids could have different foraging strategies that require further analyses (e.g. examining echolocation capabilities and use of feeding perches). Compared to other bats, pteropodid humeri show markedly simple diaphyses, with low cristae and an S-shaped shaft (Panyutina et al., 2015). Greater dispersion of gleaners and hawkers across diaphyseal morphospace (overlapping with most foraging strategies) could suggest lower evolutionary diversification, resulting in more generalist morphotypes. Ancestral reconstructions have theorised that insectivory and aerial hawking were the ancestral states in Chiroptera (Amador, Simmons, et al., 2019), which could indicate that modern gleaning and hawking morphotypes retain an ancestral, less divergent morphotype. Higher epiphyseal disparity in gleaners and hawkers could reflect a secondary aerodynamic diversification in bats associated with adaptations to manoeuvrability (Amador, Almeida, et al., 2019; Amador, Simmons, et al., 2019). Oligo-Miocene environmental changes coincide with the diversification of most modern bat families, linking the expansion of open-mosaic ecosystems with increased functional demands for manoeuvrability, along with sustained and fast flight (Amador, Almeida, et al., 2019; Amador, Simmons, et al., 2019). Carnivore and trawling species consistently clustered together with species of the same guild across morphospace, reflecting cranial morphological specialisations correlated with carnivory and piscivory in bats (Santana & Cheung, 2016).

4.2 | Morphological modularity and integration

Contrary to our study, most modularity hypotheses have focused on sets of bones that are tightly associated to form a single structure (e.g. cranium or complete limbs; Goswami & Polly, 2010; Hallgrímsson et al., 2004; Marroig et al., 2009; Martín-Serra et al., 2017; Martín-Serra et al., 2014; Porto et al., 2008; Santana & Lofgren, 2013), with

notable exceptions being studies on mandibular modularity (Garcia et al., 2014; Jójic et al., 2012; López-Aguirre et al., 2015). Within-bone modularity has been widely studied and demonstrated for mandibular morphology in mammals (Atchley & Hall, 1991; Hall, 2003; Zelditch et al., 2008; Polanski, 2011; Jójic et al., 2012), although the prevalence of mandibular modularity in other chordates remains to be tested (Parsons et al., 2012). Within-bone modularity has been reported in felid vertebrae, suggesting a developmental signal reflecting ontogenetic similarities (Randau & Goswami, 2017). Postcranial modularity in mammals has been studied between groups of bones representing functional units, such as the vertebral column in felids (Randau & Goswami, 2018), and the appendicular skeleton (Conaway et al., 2018; Diogo et al., 2019; Goswami et al., 2014; Martín-Serra et al., 2014). Special interest has been taken in studying developmental modularity and how it reflects the evolutionary history of mammals (Goswami et al., 2009; López-Aguirre et al., 2019a; Young et al., 2010).

Our study provides evidence for significant within-bone modularity in the appendicular skeleton, the first reported for any tetrapod. Differences in the morphological adaptations of the humeral diaphysis and epiphyses of fossil primates have been suggested to indicate a functional partitioning of the bone (Marchi et al., 2016). Epiphyseal and diaphyseal morphological specialisations have been associated with functional modularity in the appendicular skeleton of mammals (Goswami et al., 2014; Marchi et al., 2016). Epiphyseal adaptations in the humerus of bats have been linked to shoulder and elbow joint mobility, shaping the performance and manoeuvrability of the wing during flight and landing (Altenbach & American Society of Mammalogists, 1979; Boerma, Barrantes, et al., 2019; Boerma, Breuer, et al., 2019; Schlosser-Sturm & Schliemann, 1995; Vaughan, 1959). On the other hand, specialisations of diaphyseal morphology have been useful to describe functional adaptations to biomechanical loading stresses engendered during flight (Krause et al., 2014; López-Aguirre et al., 2019; Swartz et al., 1992; Swartz & Middleton, 2008). Novel locomotory and foraging strategies (e.g. terrestrial locomotion) in bats could have also canalised morphological adaptations in the epiphysis of the humerus (Hand et al., 2009; Norberg & Rayner, 1987; Riskin et al., 2006). At least eight ossification centres have been identified during humeral development in mammals (Kwong et al., 2014; Wisniewski et al., 2017). The humeral shaft is ossified prenatally in mammals (Wisniewski et al., 2017), whereas the epiphyseal plate remains cartilaginous at birth to allow longitudinal growth of the bone (Kwong et al., 2014). Multiple secondary ossification centres have been identified during the postnatal ossification of the epiphyses (Kwong et al., 2014), indicating that a developmental modularity hypothesis would be more complex than our diaphyseal-epiphyseal partition. Evidence of developmental modularity in the appendicular skeleton of mammals has been reported in primates and carnivorans (Conaway et al., 2018; Lawler, 2008; Martín-Serra et al., 2017; Martín-Serra et al., 2014; Young & Hallgrímsson, 2005).

Integration between the diaphysis and epiphyses varied between foraging strategies. Our PLS plot showed that phytophagous, carnivorous, walking and trawling species occupied non-overlapping morphospaces. Ecological differences between mammal taxa have

been suggested to structure the magnitude of morphological integration between functionally correlated traits (Makedonska et al., 2012). However, a similar test for postcranial integration in mammals is still missing at a broader scale. Dispersion of species with similar foraging strategies in our PLS plot does not reflect either size similarities or phylogenetic relationships (e.g. clustering of pteropodid and phyllostomid phytophagous and walking bats). Significant integration between diaphysis and the epiphyses of the humerus shows that ecomorphological adaptations shape morphology at different scales (within and between modules) within a single structure (Hallgrímsson et al., 2002; Young & Hallgrímsson, 2005; Young et al., 2010). Differences in patterns of integration between foraging strategies seem to reflect functional differences in manoeuvrability and loading.

5 | CONCLUSIONS

Our study explores the drivers (phylogenetic, ecological and biological) of within-structure morphological modularity and integration in bat humeri, elucidating the interaction between different types of traits exerting multiple selective pressures in a single bone. To our knowledge, this study is the first to find significant patterns of within-structure modularity in the appendicular skeleton of any tetrapod, highlighting the need to further explore decoupled patterns of phenotypic variation within single structures. Humeral morphological disparity was found to reflect the foraging strategies and diets of species, following previous findings on cranial morphology, and suggesting an interplay between cranial and postcranial morphological variation. Diaphyseal and epiphyseal morphology varied independently, the former better reflecting the effect of evolutionary kinship, and the latter the effect of diet, FG and size. Statistical support for a diaphysis-epiphyses modular partition of humeral shape variation also reinforces our hypothesis of decoupled patterns of morphological variation in bat humeri. Phytophagous bats showed greater diaphyseal disparity, whereas animalivores had greater epiphyseal disparity, suggesting a correlation between epiphyseal shape and control of manoeuvrability during flight. Also, we found a significant association between shape and AR only for the epiphyses, revealing an association between wing shape and epiphyseal morphology which governs range of motion for shoulder and elbow joints during flight.

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AUTHOR CONTRIBUTIONS

CL-A, SJH and LABW conceived the ideas and designed the methodology; CL-A, SJH, DK and VTT collected the samples; CL-A generated and analysed the data; CL-A, SJH and LABW led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are available on the Figshare Digital Repository at <https://doi.org/10.6084/m9.figshare.13025045.v1>.

OPEN RESEARCH BADGES



This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at <https://doi.org/10.5061/dryad.3xsj3txdh>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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