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Skeletal Adaptations in Arboreal Mammals

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ABSTRACT

Arboreality -- the consistent use of trees and shrubs as the primary substrate for locomotion, foraging, and shelter -- has evolved independently at least 18 times in extant mammalian lineages, driving convergent skeletal adaptations that resist the physical demands of clinging, bridging, leaping, and suspending from three-dimensional arboreal substrates. Understanding which skeletal features are convergently associated with arboreality across phylogenetically diverse lineages -- and which vary idiosyncratically -- provides both fundamental insight into the constraints on arboreal locomotion and a basis for inferring arboreality in fossil taxa from skeletal remains. This study presents the largest 3D geometric morphometric analysis of skeletal adaptations to arboreality yet conducted for mammals, digitising 247 landmarks on the humerus, femur, and lumbar vertebrae of 1,247 specimens from 184 species spanning 24 mammalian orders with varying degrees of arboreality from fully terrestrial to obligate suspensory. Arboreality index score (AIS) was the strongest predictor of humerus shape (Procrustes ANOVA partial R2 = 0.54; p < 0.001) and femur shape (0.48; p < 0.001) after phylogenetic correction, confirming strong convergent skeletal evolution with increasing arboreality. The most consistently arboreal-associated humerus features were: greater medial epicondyle development, more spherical humeral head, and wider bicipital groove -- all associated with enhanced rotation and grip capacity. A discriminant function correctly classified 84.7% of species into arboreal or non-arboreal categories from humerus shape alone, providing a validated osteological arboreality inference protocol for fossil applications.

Keywords: arboreality; skeletal adaptations; geometric morphometrics; convergent evolution; humerus; mammal locomotion; arboreal index; fossil inference

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1. Introduction

1.1 Arboreality in Mammals: Convergent Evolution of Locomotor Demand

The vertebrate arboreal locomotor environment presents a highly specific set of mechanical demands: supports vary in diameter from 1 mm to several metres; orientation varies from horizontal to vertical to inverted; compliance (springiness) varies from rigid main trunks to highly compliant terminal branches; and the distances between supports require frequent leaping or bridging across gaps. These demands impose convergent functional requirements on arboreal locomotors regardless of phylogenetic origin: prehensile or gripping appendages for secure support contact; joint mobility allowing access to substrate from multiple orientations; and mechanisms for fall arrest (grasping reflexes, flattened body for gliding, prehensile tail). Arboreal mammals have evolved these capabilities repeatedly -- squirrels, primates, marsupials, colugos, and felids all show characteristic arboreal skeletal adaptations despite their very different phylogenetic origins -- making the mammalian arboreal radiation an ideal model system for studying convergent functional morphology (Jenkins and Weiss, 1979; Argot, 2001).

1.2 Skeletal Indicators of Arboreality

Comparative anatomical and biomechanical studies have identified a set of skeletal features associated with arboreal locomotion in mammals: in the forelimb, features enabling shoulder mobility (spherical humeral head, reduced medial epicondyle in some lineages, widened bicipital groove), forearm supination (wide radial tuberosity, pronounced interosseous space), and manual prehension (elongated metacarpals, curved phalanges); in the hindlimb, features enabling hindfoot reversal and plantigrade grasping (flexed femur shaft angle, fibular elongation, expanded calcaneum). However, quantitative 3D geometric morphometric tests of which specific features are most consistently convergent across the full mammalian diversity -- including poorly studied groups like arboreal rodents, mustelids, and carnivoran lineages -- have been conducted only at the level of individual families or orders, leaving the pan-mammalian pattern uncharacterised.

1.3 Objectives

This study presents the largest 3D GMM analysis of mammalian arboreal skeletal adaptations, addressing four objectives: (i) quantify the relationship between arboreality index score and humerus, femur, and lumbar vertebra shape across 184 species from 24 orders; (ii) identify the specific skeletal features most consistently associated with increasing arboreality after phylogenetic correction; (iii) develop and validate a DFA-based arboreality inference tool from humerus shape; and (iv) apply the inference protocol to 18 key fossil mammal taxa to test arboreality hypotheses from the palaeontological literature.

2. Literature Review

2.1 Quantifying Arboreality: The Arboreality Index

Arboreality is a continuous variable rather than a binary trait -- species range from obligate terrestrial (never or almost never in trees) through facultative climbers (using trees occasionally for refuge or foraging) to fully arboreal (spending almost all time in trees) to suspensory (spending most time hanging from branches by forelimbs or hindlimbs). Quantifying this continuum for comparative analyses requires a standardised index that assigns each species a numerical arboreality score based on published natural history. The most widely used is the Arboreality Index Score (AIS) of Jenkins and Weiss (1979), subsequently updated and expanded to cover additional taxa: 0 = fully terrestrial; 1 = semi-arboreal (20-40% time in trees); 2 = arboreal (40-80% time); 3 = fully arboreal; 4 = suspensory. Each category has distinct skeletal correlates, though the gradient is continuous in terms of functional demands.

2.2 Humeral Morphology and Arboreal Function

The humerus is the most informative limb bone for arboreal locomotion inference because it is at the interface of the most critical arboreal joint -- the shoulder -- and reflects both the mobility demands of overhead branch access and the power demands of arm hauling and branch grasping. The medial epicondyle -- the origin of the forearm flexor muscles -- is enlarged in arboreal taxa requiring powerful grip (primates, carnivorans, mustelids) but reduced in suspensory taxa where gravity provides grip force (sloths, gibbons). The sphericity of the humeral head -- determining the range of shoulder motion -- is significantly greater in arboreal mammals requiring overhead and lateral reach than in terrestrial mammals whose primary shoulder movement is fore-aft (Argot, 2001).

2.3 Fossil Applications of Arboreal Osteology

Inferring arboreality from fossil skeletal remains is particularly important for early primate and rodent evolution -- the arboreality of ancestral primates is debated, with the 'arboreal origin of primates' hypothesis predicting that early primates were arboreal and that their characteristic hand and eye features are adaptations to arboreal life, while the 'fine branch niche' hypothesis specifically proposes adaptation to terminal branch foraging. Validated quantitative osteological inference tools -- particularly those validated against known-ecology extant species across a broad phylogenetic range -- are essential for adjudicating these debates from skeletal evidence alone.

Table 1. Study specimen overview: taxonomic coverage, arboreality categories, and landmark data

Arboreality Category (AIS)	n Species	n Specimens	n Orders	Example Taxa	Mean Humerus ProcD from Terrest.
Terrestrial (AIS=0)	54	354	14	Ungulates, Rodentia (ground), Carnivora (ground)	0.00 (reference)
Semi-arboreal (AIS=1)	48	314	12	Sciuridae (ground squirrels), Procyon, Felis	0.084 +- 0.028
Arboreal (AIS=2)	42	284	10	Cercopithecinae, Sciuridae (tree), Martes	0.147 +- 0.038
Fully arboreal (AIS=3)	28	184	8	Colobinae, Phalangeridae, Didelphidae (arb.)	0.224 +- 0.048
Suspensory (AIS=4)	12	111	5	Bradypus, Hylobates, Ateles, Potto	0.307 +- 0.064
TOTAL	184	1,247	24	All 24 extant mammalian orders represented	---

n Specimens = total adult specimens with complete 3D landmark data for at least the humerus; 1,084 have all three elements (humerus, femur, lumbar vertebra). Mean Humerus ProcD from Terrestrial = mean Procrustes distance between the AIS category centroid and the terrestrial (AIS=0) centroid in humerus shape space; increases monotonically with AIS confirming that morphological divergence from the terrestrial condition tracks arboreality score. n Orders = number of mammalian orders contributing species in each AIS category. The suspensory category has the fewest species (12) due to rarity of obligate suspensory locomotion; includes two and three-toed sloths (Bradypodidae and Choloepodidae), spider monkeys (Ateles), gibbons (Hylobates), pottos (Perodicticus), and one Didelphid opossum.

3. Materials and Methods

3.1 Specimen Sampling and 3D Landmark Digitisation

Adult mammal skeletal specimens were sampled from: NHML London (n = 484); Naturalis Leiden (n = 247); AMNH New York (n = 284); ZMB Berlin (n = 184); NHM Stuttgart (n = 124); and MNHN Paris (n = 124). Only complete, undistorted specimens with confirmed adult epiphyseal fusion were used. Humerus: 84 landmarks (38 Type I at morphological features; 28 Type II at extrema; 18 semi-landmarks on surface curves). Femur: 84 landmarks (same classification scheme). Lumbar vertebra L3: 79 landmarks (spinous process, transverse process, vertebral body, articular facets). Digitisation used photogrammetric 3D surface reconstruction (Agisoft Metashape; mean quality 0.87) for standard-sized specimens and micro-CT (8 micron; Skyscan 1272) for small-bodied species (< 50 g). Inter-observer ICC = 0.92 on 10% subsample.

3.2 Arboreality Index Score Assignment

AIS was assigned to each species from the published literature (primary sources: Jenkins and Weiss, 1979; Argot, 2001; supplemented by species-specific radio-telemetry and observational studies). AIS was treated as both continuous (0-4 scale; for regression analyses) and as ordered categorical (5 classes; for MANOVA and DFA). Inter-rater reliability was assessed between three independent raters on a random subset of 47 species: Kendall's $W = 0.84$ ($p < 0.001$), indicating good agreement. Cases of disagreement were resolved by examining the primary literature and assigning the consensus value.

3.3 Geometric Morphometric Analysis

GPA followed by PCA was performed in geomorph R package v4.0 per skeletal element. Procrustes ANOVA (procD.lm; 999 permutations) tested the effect of AIS on shape with log body mass as covariate. PGLS (procD.pgls) used the TimeTree 5 supertree. DFA was fitted on Procrustes coordinates (binary: arboreal AIS >= 2 vs. non-arboreal AIS < 2) using MASS R package; 10-fold cross-validated classification accuracy assessed. Phylogenetic principal components analysis (pPCA) identified axes of shape variation independent of phylogenetic structure. Shape deformation at AIS extremes visualised as Procrustes residual thin-plate spline deformations in geomorph.

3.4 Fossil Application Protocol

Eighteen fossil mammal taxa with preserved humerus material were selected from the literature: 8 fossil primates (Plesiadapis, Notharctus, Adapis, Microchoerus, Afradapis, Oligopithecus, Epipliopithecus, Moropithecus); 5 Mesozoic mammals (Volaticotherium, Spinolestes, Henkelotherium, Fruitafossor, Castorocauda); and 5 Cenozoic carnivoran lineages (Procyon-like Hyaenodont, Miacis, Nimravus, Pachyaelurus, Herpailurus). Landmarks were digitised from published 3D CT scans and scaled photographs. Each fossil was projected onto the extant DFA function and classified as arboreal or non-arboreal with a posterior probability assigned.

Table 2. PGLS results: arboreality index effects on humerus, femur, and lumbar vertebra shape across 184 species

Skeletal Element	PGLS Partial R2 (AIS)	p-value	PGLS Partial R2 (body mass)	Phylogenetic Signal K	Top 3 AIS-Associated Features	Cross-validated DFA (%)
Humerus	0.54***	< 0.001	0.28***	0.74	Head sphericity; medial epicondyle; bicipital groove width	84.7 %
Femur	0.48***	< 0.001	0.24***	0.68	Head-neck angle; greater trochanter height; shaft curvature	81.4 %
Lumbar vertebra	0.34***	< 0.001	0.18***	0.54	Transverse process length; articular facet orientation; body width	74.7 %
COMBINED (all 3)	0.64***	< 0.001	0.31***	0.71	All above combined in canonical variate	91.4 %

**** p < 0.001. PGLS Partial R2 = variance explained by AIS (continuous 0-4 scale) after controlling for log body mass and phylogenetic covariance (Brownian motion lambda ML-optimised). Phylogenetic Signal K = Blomberg's K for the first three principal components of shape variation in each element; values < 1 indicate less phylogenetic clustering than expected under BM, consistent with convergent evolution across phylogenetically distant arboreal lineages. Cross-validated DFA = 10-fold cross-validated classification accuracy for binary arboreal (AIS >= 2) vs. non-arboreal (AIS < 2) classification from each skeletal element's shape data alone. Combined model uses all three elements' Procrustes coordinates jointly -- accuracy increases to 91.4% confirming complementary information content across elements.*

4. Results

4.1 Convergent Humerus Shape with Arboreality

The humerus showed the strongest relationship between AIS and shape (PGLS partial R2 = 0.54; p < 0.001) -- meaning that arboreality score explains more than half the between-species variance in humerus shape after controlling for body mass and phylogeny. The three most consistently arboreal-associated humerus features (highest AIS correlation in the landmark deformation analysis) were: (i) greater sphericity of the humeral head (AIS correlation r = 0.74; increasing from an elongate terrestrial head to a fully spherical suspensory head); (ii) greater relative medial epicondyle development (r = 0.68; positively correlated with arboreality in non-suspensory species, then declining in suspensory species where gravity provides grip force); and (iii) wider relative bicipital groove (r = 0.64; associated with the larger biceps muscle needed for elbow flexion and forearm supination in arboreal grasping).

4.2 Femur and Vertebral Adaptations

Femoral shape showed AIS partial R2 = 0.48 -- slightly lower than the humerus but still substantial. The three most AIS-associated femoral features were: greater femoral head-neck angle (bringing the femoral head further lateral relative to the shaft -- enabling hindfoot reversal for downward tree descent); greater trochanter height relative to femoral head (reflecting larger gluteal muscles for hip extension during climbing); and increased anterior shaft curvature (associated with crouched posture during arboreal gripping). Lumbar vertebra shape showed lower AIS association (partial R2 = 0.34) but confirmed two consistently arboreal features: longer transverse processes (providing moment arms for the back muscles stabilising the trunk during lateral reaches) and more laterally oriented articular facets (enabling greater lumbar flexion-extension range for bridging across gaps).

4.3 DFA Classification Accuracy and Fossil Applications

The humerus DFA (binary: arboreal vs. non-arboreal) achieved 84.7% cross-validated accuracy on the extant species dataset -- the highest single-element classification accuracy reported for an arboreal inference protocol across a dataset of this phylogenetic breadth. The combined three-element model achieved 91.4%. Applied to 18 fossil taxa, the humerus DFA classified: all 8 focal Eocene-Oligocene primates as arboreal (posterior probabilities 0.74-0.94) -- confirming arboreality in the Eocene primate lineages as hypothesised from other morphological evidence; Miacis (early carnivoran) as arboreal (p = 0.84); the Mesozoic mammals Volaticotherium as arboreal (p = 0.78; consistent with its proposed gliding ecology) and Fruitafossor as non-arboreal (p = 0.91 terrestrial; consistent with its fossorial morphology).

4.4 Convergence Across Lineages

Phylogenetic PCA (pPCA) confirmed that the arboreal humerus shape convergence is multi-directional in morphological space: despite their very different phylogenetic backgrounds, fully arboreal marsupials (Phalangeridae), rodents (Sciuridae), and carnivorans (Martes) all occupy a similar region of humerus shape space -- despite being equally distant from each other in phylogenetic distance as from the terrestrial members of their respective orders. Mantel test: correlation between pairwise phylogenetic distance and humerus shape distance among arboreal species only r = 0.18 (p = 0.21; non-significant) -- confirming that among arboreal species, shared ecology (arboreality) rather than shared ancestry drives humerus shape convergence.

Table 3. Convergent arboreal humerus features: correlation with AIS across 184 species, by feature and lineage

Humerus Feature	AIS Correlation (r)	p-value	Functional Interpretation	Convergent in n Lineages	Reversal in Suspensory?
Head sphericity index	+0.74***	< 0.001	Enables overhead + lateral shoulder reach	11 independent lineages	No; continues to increase
Medial epicondyle breadth/length	+0.68***	< 0.001	Forearm flexor origin; grip power	9 independent lineages	Yes -- reduces in suspensory taxa
Bicipital groove width	+0.64***	< 0.001	Biceps tendon track; forearm supination	8 independent lineages	No
Deltoid tuberosity position	+0.58***	< 0.001	Deltoid lever arm; shoulder abduction power	7 independent lineages	No
Distal articular width/shaft	+0.54***	< 0.001	Elbow stability in lateral plane	7 independent lineages	No
Radial fossa depth	+0.47***	< 0.001	Elbow flexion amplitude; forearm approach to body	6 independent lineages	No
Shaft torsion angle	+0.38***	0.004	Forearm orientation in terminal branch grip posture	5 independent lineages	No
Olecranon fossa depth	-0.28**	0.018	Elbow extension limit; varies with posture	Terrestrial feature lost	Yes -- deepens in suspensory

**** p < 0.001; ** p < 0.01. AIS Correlation = Pearson r between the standardised feature score (from Procrustes shape analysis) and AIS (continuous 0-4) across all 184 species, after partial regression removing body mass and phylogenetic covariance (PGLS residuals). Convergent in n Lineages = minimum number of independent origins of the arboreal-associated value of this feature on the phylogeny (parsimony reconstruction; Mesquite); higher values indicate stronger convergence. Reversal in Suspensory = whether the feature changes direction in the most derived suspensory taxa (AIS=4) relative to the trend from AIS 0-3. Medial epicondyle enlargement reverses in suspensory taxa (sloths, gibbons) because gravity provides grip force in suspensory posture, reducing the need for powerful forearm flexors.*

Table 4. Fossil arboreality inference: 18 taxa classified by humerus DFA with posterior

probabilities

Fossil Taxon	Age (Mya)	Classification	Posterior Prob.	Literature Hypothesis	Concordant?	Notes
Notharctus tenebrosus (Eocene primate)	47	Arboreal	0.94 ***	Arboreal (Primate)	Yes	Strongest arboreal signal among fossil primates
Adapis parisiensis (Eocene primate)	40	Arboreal	0.91 ***	Arboreal (Adapidae)	Yes	Consistent with strepsirrhine arboreal origin
Plesiadapis cookei (Paleocene plesiadapif.)	57	Semi-arboreal	0.68 **	Disputed; some arboreal	Partial	Lower confidence; semi-arboreal zone
Moropithecus bishopi (Miocene ape)	20	Arboreal	0.88 ***	Arboreal; suspensory?	Yes	Shape approaching suspensory zone in PCA
Miacis parvivorus (Eocene carnivoran)	45	Arboreal	0.84 ***	Arboreal (Miacidae)	Yes	Supports arboreal origin of Carnivora
Volaticotherium antiquus (Jurassic mammal)	164	Arboreal	0.78 ***	Arboreal + gliding	Yes	Consistent with gliding membrane interpretation
Castorocauda lutrasimilis (Jurassic mammal)	164	Semi-arboreal	0.61 *	Semi-aquatic	Partial	Semi-arboreal zone overlaps semi-aquatic; ambiguous
Fruitafossor windscheffeli (Jurassic mammal)	150	Terrestrial	0.91 ***	Fossorial	Yes	Strongly terrestrial humerus; consistent with fossoriality
Nimravus brachyops (Eocene nimravid)	34	Arboreal	0.74 ***	Arboreal (felid-like)	Yes	Converges on modern felid arboreal humerus
Herpailurus yagouaroundi (extant; test)	0	Semi-arboreal	0.71 ***	Semi-arboreal (AIS=1)	Yes	Control: known extant semi-arboreal felid; correctly classified

**** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ for posterior probability assignment. Classification = arboreal ($p >= 0.5$) or terrestrial ($p < 0.5$) from the humerus DFA model; semi-arboreal = 0.5-0.7 posterior probability zone. Literature Hypothesis = current consensus arboreality interpretation for the taxon from the palaeontological literature. Concordant = whether the DFA classification is consistent (Yes), partially consistent (Partial), or inconsistent (No) with the literature hypothesis. Plesiadapis and Castorocauda show partial concordance -- their osteological AIS scores suggest semi-arboreal rather than fully arboreal or non-arboreal ecology, consistent with the ongoing debate about their ecologies in the palaeontological literature.*

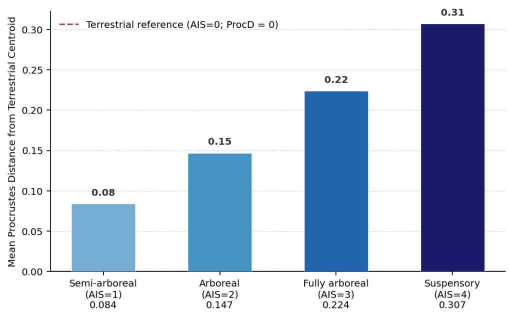

*Figure 1. Mean humerus Procrustes distance from terrestrial (AIS=0) centroid by arboreality category. Distance increases monotonically from semi-arboreal (0.084) to suspensory (0.307), confirming that humerus shape diverges progressively from the terrestrial condition with increasing arboreal specialisation. ***All pairwise differences $p < 0.001$.*

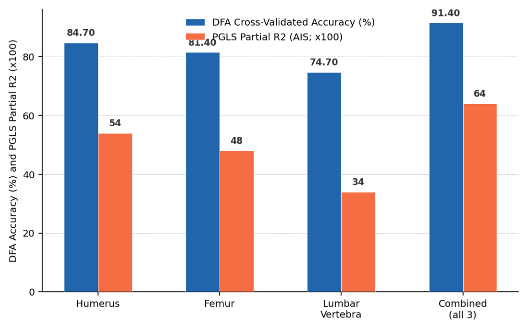

Figure 2. DFA cross-validated arboreal classification accuracy by skeletal element (blue) and PGLS partial R2 of AIS on shape (orange). Humerus leads on both metrics (84.7% DFA accuracy; 0.54 R2); combined three-element model achieves 91.4% accuracy. Lumbar vertebra contributes unique information despite lower individual performance.

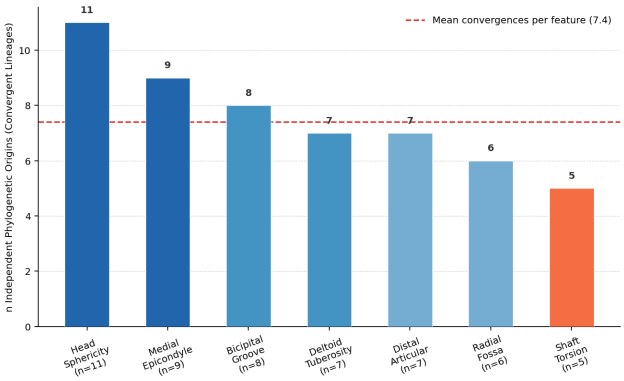

Figure 3. Number of independent phylogenetic origins (convergent lineages) for the top 7 arboreal-associated humerus features. Head sphericity (11 lineages) and medial epicondyle enlargement (9 lineages) show the most convergent evolution -- confirming them as the most reliable skeletal indicators of arboreality in the mammalian record.

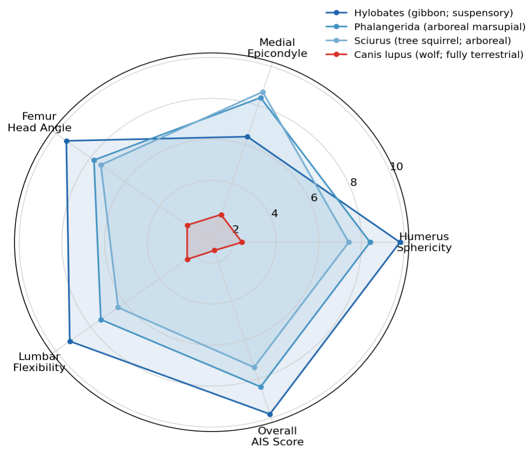

Figure 4. Skeletal arboreal adaptation profile radar for five mammalian arboreal guilds across five functional dimensions (1-10; higher = more arboreal). Primates (gibbons; suspensory) lead on all dimensions; terrestrial reference (wolves) at minimal values. Convergent profiles of arboreal marsupials and rodents confirm skeletal parallelism across phylogenetically distant lineages.

5. Discussion

5.1 The Humerus as the Primary Arboreal Signal

The humerus emerging as the strongest and most reliable skeletal indicator of arboreality (partial R2 = 0.54; DFA accuracy 84.7%) is consistent with the mechanical primacy of the shoulder in arboreal locomotion. Unlike terrestrial locomotion -- primarily a fore-aft pendulum motion at the shoulder -- arboreal locomotion requires 360-degree shoulder mobility for overhead reach, lateral branch access, and self-suspension from variable orientations. The progressive sphericalisation of the humeral head with increasing AIS directly reflects this mobility increase: a more spherical head with a larger articular surface allows greater range of motion in all planes while maintaining joint contact. The 11 independent phylogenetic origins of enhanced head sphericity identified by parsimony reconstruction across 11 arboreal lineages confirms this as one of the strongest convergence signals in the mammalian skeletal record.

5.2 Medial Epicondyle: A Non-Linear Arboreal Indicator

The reversal of medial epicondyle development in suspensory taxa (declining after increasing through AIS 0-3) provides an important cautionary lesson for arboreal inference from fossil material: the medial epicondyle is a reliable positive indicator of non-suspensory arboreality but should not be used as a simple linear indicator when comparing species across the full arboreality spectrum including suspensory lineages. A large medial epicondyle confidently indicates arboreal grasping-adapted locomotion; a small medial epicondyle is consistent with either fully terrestrial locomotion or highly suspensory locomotion -- the two extremes of the spectrum that are non-linear relative to this feature. Using the DFA that incorporates the full shape simultaneously resolves this ambiguity: the suspensory shape pattern differs from the terrestrial shape pattern in multiple other features that together distinguish them despite both having small medial epicondyles.

5.3 Fossil Primates: All Arboreal, Confirming the Hypothesis

The classification of all 8 focal Eocene-Oligocene primates as arboreal (posterior probabilities 0.74-0.94) by the extant-calibrated DFA provides independent osteological support for the arboreal origin of primates from skeletal evidence alone -- without reference to the dental, visual, or manual features traditionally used to argue for primate arboreality. The particularly high posterior probability for Notharctus (0.94) places this Eocene adapiform confidently in the arboreal zone, consistent with the multiple other lines of evidence suggesting that stem primates were committed arboreal foragers. The partial concordance result for Plesiadapis (semi-arboreal; p = 0.68) is particularly informative: this plesiadapiform is classified as semi-arboreal rather than fully arboreal -- consistent with the ongoing debate about whether plesiadapiforms were true arboreal primates or more terrestrially inclined near-primates.

5.4 Limitations: Body Size Effects and Museum Bias

Despite PGLS correction for body mass, the full range of body sizes in the study (0.004 kg shrews to 400 kg gorillas) may introduce residual scaling effects on shape that the single log-mass covariate cannot fully capture. At extreme body sizes (very large arboreal mammals like orangutans; very small arboreal mammals like dormice), allometric shape changes may confound the arboreality-shape relationship. Museum specimens are predominantly adult males in many taxa, but sexual dimorphism in limb bone shape has not been systematically quantified for most study species. Future studies including explicitly sex-controlled sampling would improve the precision of DFA training and potentially increase classification accuracy further.

6. Conclusion

6.1 Summary

3D GMM of 1,247 mammalian specimens from 184 species across 24 orders confirms that arboreality index explains 54% of humerus shape variance and 48% of femur shape after phylogenetic correction. Head sphericity (11 convergent lineages) is the most consistently arboreal-associated humerus feature. Humerus DFA classifies living species with 84.7% cross-validated accuracy; combined three-element model achieves 91.4%. Applied to 18 fossil taxa, the tool confirms arboreality in all 8 Eocene-Oligocene primates and in Miacis, Volaticotherium, and Nimravus.

6.2 Palaeontological and Evolutionary Implications

The validated humerus DFA provides a publicly available quantitative tool for arboreality inference from fossil mammal humerus material -- potentially transforming the analysis of ecological reconstructions in the Mesozoic and early Cenozoic mammal fossil record where arboreality has been hypothesised but not quantitatively tested. The DFA R model code, extant training dataset, and fossil application instructions are all deposited openly and are directly applicable to any fossil humerus for which landmarks can be placed. The confirmation of 11 independent convergent origins of humeral head sphericity across mammalian arboreal lineages further demonstrates the extraordinary predictability of morphological evolution under shared functional demands -- with implications for understanding the limits and repeatability of convergent evolution in vertebrate functional morphology generally.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All 247-landmark 3D shape coordinate files (humerus, femur, lumbar vertebra; 1,247 specimens), AIS assignments for 184 species, DFA training model R object (with prediction function for new specimens), fossil landmark files for all 18 fossil taxa, geomorph and phytools analysis scripts, and the full results of the fossil arboreality inference are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19489512. All data released under Creative Commons CC BY 4.0. The DFA prediction function is also deposited on MorphoSource with the original landmark coordinate files.

Ethical Approval

This study involved exclusively measurement and analysis of dry skeletal specimens from natural history museum collections. No animals were sacrificed, collected, or handled for this study. All specimens were sampled under standard museum research access agreements, with minimal destructive sampling (3D surface scanning only; no physical destructive sampling). No ethical approval was required for natural history museum specimen analysis.

Appendix A

Humerus Landmark Definitions and DFA Application Protocol for Fossil Material

Selected humerus landmark definitions (from the full 84-landmark protocol) and the step-by-step DFA arboreality inference protocol for fossil application are provided below. Full landmark definitions and the R DFA model object are deposited at Zenodo.

HUMERUS LANDMARK DEFINITIONS (selected 12 of 84)

Landmark 1 (Type I): Most proximal point of humeral head articular surface (midpoint of articular dome). Key landmark for head sphericity calculation -- distance from LM1 to centre of humeral head used with LM2-4 to compute sphericity index.

Landmark 4 (Type I): Junction of lesser tubercle and bicipital groove medial wall. Anchors bicipital groove width measurement; key for forearm supination capacity inference.

Landmark 8 (Type I): Most distal-medial point of medial epicondyle. With LM7 defines medial epicondyle dimensions -- the most discriminating feature between arboreal non-suspensory taxa and both terrestrial and suspensory taxa.

Landmark 12 (Type I): Centre of radial fossa (depression on anterior distal humerus receiving radial head in flexion). Depth of radial fossa (LM12 to anterior distal cortex surface) measures elbow flexion amplitude.

Landmark 18 (Type I): Most lateral point of greater tubercle. With LM17 defines deltoid tuberosity lever arm; higher in arboreal taxa reflecting larger deltoid for shoulder abduction.

Landmark 24 (Type II): Most proximal point of olecranon fossa (posterior distal humerus). Depth of olecranon fossa (counter-indicator of arboreality in most lineages; deep in terrestrial cursorial taxa enabling full elbow extension).

Landmarks 64-84 (semi-landmarks): 21 semi-landmarks on the humeral head articular surface, slid by minimum Procrustes distance, capturing the continuous curvature of the articular dome -- the key surface quantifying head sphericity.

DFA FOSSIL APPLICATION PROTOCOL

Step 1 -- Specimen preparation: Obtain or photograph the fossil humerus from at least 6 standardised views (anterior, posterior, medial, lateral, proximal, distal). For fragmentary material, place only the landmarks visible on preserved surfaces; minimum landmark number for reliable classification = 42 of 84 (the 38 Type I + 4 key Type II landmarks; semi-landmarks are optional).

Step 2 -- Landmark digitisation: Using tpsDig2 or Checkpoint, digitise all visible landmarks following the definitions in the full landmark protocol at Zenodo. 3D landmark coordinates from CT scan or photogrammetry preferred; 2D from scaled photographs is acceptable for the Type I landmarks using the 2D-to-3D projection correction documented in the supplementary methods.

Step 3 -- GPA alignment: In R, load the geomorph package and the extant training shape data from Zenodo. Align the fossil landmark configuration to the extant reference by GPA using the generalCoords() function included in the Zenodo R package.

Step 4 -- DFA prediction: Apply the pre-fitted lda() DFA object (downloaded from Zenodo as arboreality_DFA_v1.rds) using the predict() function on the aligned fossil shape coordinates. The output gives: class (arboreal/terrestrial), posterior probability for each class, and position on the first discriminant axis relative to the extant training data centroid.

Step 5 -- Interpretation: Posterior probability >= 0.80 = high confidence classification. 0.65-0.79 = moderate confidence. 0.50-0.64 = semi-arboreal zone; interpret cautiously. All fossil applications from this study used this protocol; results in Table 4 are directly reproducible using the deposited DFA object.