



Functional associations between support use and forelimb shape in strepsirrhines and their relevance to inferring locomotor behavior in early primates



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ABSTRACT

The evolution of primates is intimately linked to their initial invasion of an arboreal environment. However, moving and foraging in this milieu creates significant mechanical challenges related to the presence of substrates differing in their size and orientation. It is widely assumed that primates are behaviorally and anatomically adapted to movement on specific substrates, but few explicit tests of this relationship in an evolutionary context have been conducted. Without direct tests of form–function relationships in living primates it is impossible to reliably infer behavior in fossil taxa. In this study, we test a hypothesis of co-variation between forelimb morphology and the type of substrates used by strepsirrhines. If associations between anatomy and substrate use exist, these can then be applied to better understand limb anatomy of extinct primates. The co-variation between each forelimb long bone and the type of substrate used was studied in a phylogenetic context. Our results show that despite the presence of significant phylogenetic signal for each long bone of the forelimb, clear support use associations are present. A strong co-variation was found between the type of substrate used and the shape of the radius, with and without taking phylogeny into account, whereas co-variation was significant for the ulna only when taking phylogeny into account. Species that use a thin branch milieu show radii that are gracile and straight and have a distal articular shape that allows for a wide range of movements. In contrast, extant species that commonly use large supports show a relatively robust and curved radius with an increased surface area available for forearm and hand muscles in pronated posture. These results, especially for the radius, support the idea that strepsirrhine primates exhibit specific skeletal adaptations associated with the supports that they habitually move on. With these robust associations in hand it will be possible to explore the same variables in extinct early primates and primate relatives and thus improve the reliability of inferences concerning substrate use in early primates.

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1. Introduction

It is widely argued that over the past 65 million years or more of primate evolution, primates underwent a significant change in the functional role of the forelimb. Primate locomotor evolution thus involved a decrease in the weight-bearing role of the forelimb

relative to the hind limb by shifting weight support toward the hind limb and using the forelimb to a greater degree as a grasping and manipulative organ (Jones, 1916; Stern, 1976; Schmitt and Lemelin, 2002; Patel et al., 2015). This evolutionary shift is thought to be associated with a movement of early primates into an arboreal environment (Jenkins, 1973) associated with thin branches (Cartmill, 1972, 1974a,b, 1992). This idea assumes changes in forelimb morphology that can be tracked in both extant and fossil primates. Nonetheless, few studies have tested the relationship

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between forelimb anatomy and the size of substrates habitually used in extant primates. Without such a test and clear relationships between form and function (Bock and van Wahlert, 1965; Kay and Cartmill, 1977) we cannot hope to infer substrate use in extinct primates like *Carpolestes* (Bloch and Boyer, 2002), *Cantius* (Rose and Walker, 1985), Fayum primates (Conroy, 1976), and the many and diverse primates of the Miocene (Rose, 1989). The goal of the present study is to fill that gap and to ask if there are features of the forelimb that are clearly associated with the size and orientation of the substrates primates use so that if such relationships exist they can be used to better infer behavior in extinct primates and test hypotheses about primate locomotor evolution in future studies.

Although many authors consider the evolution of primates to be intimately linked to the colonization of the arboreal niche (Jones, 1916; Cartmill, 1972, 1992; Jenkins, 1972; Ravosa and Dagosto, 2007; Ross and Martin, 2007; Sussman et al., 2013), there remains disagreement about the specific size and quality of the substrates used by early primates and little anatomical data linking limb anatomy to substrate that can be applied to the earliest primate fossils. The exploitation of terminal branches is thought to be a strong selective force driving the evolution of the anatomy of modern primates (Cartmill, 1972; Charles-Dominique, 1975; Sussman, 1991). The study of the fossil record of early primates shows that they display morphological features (e.g., nails on digits instead of claws, orbital convergence and frontation, and prehensile hands and feet) that are thought to have evolved early on as a response to living and moving in a terminal-branch environment, which is characterized by substrates of varying diameter and orientation and an overall high degree of spatial complexity (Dunbar and Badam, 2000; Bloch and Boyer, 2002; Schmitt and Lemelin, 2002).

Several hypotheses have been proposed to explain the evolution of the morphological features typical of early primates (e.g., nails on digits instead of claws, orbital convergence and frontation, and prehensile hands and feet; Cartmill, 1972, 1974a,b; Szalay and Dagosto, 1988; Rasmussen, 1990; Godinot, 1991, 2007; Sussman, 1991; Sussman et al., 2013). The visual predation hypothesis suggests that primate ancestors hunted on thin branches and thus developed long and clawless fingers that were used to catch insects without losing stability on narrow supports (Cartmill, 1972, 1992; Kirk et al., 2003). Another hypothesis suggested the coevolution of morphological features of early primates with grasping and leaping ability (Szalay and Dagosto, 1988). Along the same lines, Godinot (1991, 2007) proposed that the insect predation hypothesis was a driving factor in primate evolution suggesting that the morphological features of early primates were well adapted specifically for catching insects, rather than being associated with the size of support traveled to catch these insects, or to retrieve terminal food sources such as fruits and nectar as suggested by the angiosperm coevolution hypothesis (Sussman and Raven, 1978; Sussman, 1991). With the exception of the grasp-leaping hypothesis and the insect predation hypothesis, all other hypotheses seem to agree on the importance of the use of thin branches as a locomotor milieu for early primates. In addition, experimental studies of locomotor behavior in primates and marsupials show broad support for an association between several specialized aspects of primate gait and locomotion on relatively thin supports (Cartmill et al., 2002, 2007; Schmitt and Lemelin, 2002; Lemelin and Schmitt, 2007; Young, 2012; Karantanis et al., 2015). The diameter and orientation of the support has been demonstrated to significantly impact the locomotor behavior of an animal, and has been well studied in primates, other mammals, and other vertebrates (see for example Prost, 1965; Vilensky and Larson, 1989; Meldrum, 1991; Vilensky et al., 1994; Schmitt, 1998, 1999, 2003; Hirisaki et al., 2000; Schmitt and Hanna, 2004; Higham and Jayne, 2004; Spezzano and Jayne, 2004; Nyakatura et al., 2008;

Stevens, 2008; Shapiro and Young, 2010, 2012; Channon et al., 2011; Foster and Higham, 2012; Herrel et al., 2013; Shapiro et al., 2014; Karantanis et al., 2015; Toussaint et al., 2015). For example, thin branches such as twigs and foliage are more flexible and less stable than wide supports, creating challenges for animals in maintaining grip, avoiding support failure, and controlling unwanted pitch (Dunbar and Badam, 2000; Cartmill et al., 2002, 2007). Consequently arboreal species must be able to adapt their locomotor behavior in order to reduce the risk of falling. Moreover, for supports narrower than the width of the body, an active muscular torque needs to be generated by the animal to counter the tendency to topple sideways at the slightest loss of balance (Napier, 1967; Cartmill, 1985; Dunbar and Badam, 2000).

If narrow substrates were indeed a defining feature of the milieu of early primates, then it is reasonable to assume that extant primates will show morphological adaptations of the limbs, optimizing locomotion on these types of substrates. In addition, as primates moved into the terminal branch environment they would have developed a strong pedal grip that allowed the forelimbs to be at least partially decoupled from their role in locomotion, and to play a role in reaching beyond the sagittal plane, and grasping and manipulating objects (Patel et al., 2015). The increased non-locomotor role of the forelimb is thought to be associated with a change in peak forelimb forces (Jones, 1916; Stern, 1976; Kimura et al., 1979; Demes et al., 1994) with reduced loading of the forelimb relative to the hind limb, a pattern accentuated in highly arboreal species (Reynolds, 1985; Schmitt, 1999; Schmitt and Hanna, 2004; Wallace and Demes, 2008). This functional differentiation of forelimb and hind limb in both loading and manipulation should then be reflected in an increased mobility of the forearm in species moving and foraging on narrow supports, and more robust and constrained forelimbs in species using wider substrates as the forelimbs maintain their role in locomotion. This hypothesis is supported by studies examining isolated forelimb elements (see as examples Napier and Davis, 1959; Conroy, 1976; Feldesman, 1982; Fleagle and Kay, 1987; Schmitt, 1996; Kay et al., 2004), but these studies were limited by the range of information about forelimb shape available at the time, and by the frequent absence of strepsirrhine primates in the comparative sample. This latter point is especially relevant since strepsirrhine primates represent examples of some of the earliest nodes of primate evolution (Horvath et al., 2008) and they have a long history of locomotion in complex arboreal environments, many of which involve animals habitually moving on thin and flexible supports. Testing for relationships between substrate use and forelimb in strepsirrhines is a necessary step in developing understanding of form–function relationships which can be applied to the fossil record (Kay and Cartmill, 1977).

In this study, we investigate the co-variation between the shape of the long bones of the forelimb and the support used (type and orientation) in strepsirrhine primates. To do so, we perform a shape analysis on entire long bones of the forelimb (humerus, ulna, and radius) as well as on their extremities (proximal and distal articulations/epiphyses) and examine co-variation with data on substrate use and orientation collected by Oxnard and collaborators (1990). This allows us to test hypotheses about coevolution between the shape of each long bone of the forelimb and support use. We predict that bones of the forearm (ulna and radius) will show a stronger pattern of co-variation with substrate use than the humerus, due to both their distal position and the associated movements of pronation–supination, allowing positioning of the hand for grasping and complex manipulation movements. In addition, we describe the shape of the long bones of the forelimb associated with the use of a thin branch milieu and large supports in extant species, allowing better future inferences on substrate use in extinct species.

2. Material and methods

2.1. Sample and data collection

The data set was composed of the forelimb long bones (humerus, ulna, and radius) of 92 individuals belonging to four species of Cheirogaleidae, one species of Daubentoniidae, four species of Galagidae, four species of Indridae, six species of Lemuridae, one species of Lepilemuridae, and three species of Lorisidae (Table 1, Supplementary Online Material [SOM] Table S1). For each species, the number of specimens ranged from two to six (Table 1). Where possible, specimens were of wild caught origin and equal numbers of males and females were included. Specimens were obtained from the Anatomie Comparée, Muséum national d'Histoire naturelle (MNHN), Paris, France, the research collection at Brunoy, France, the Royal Museum for Central Africa, Tervuren, Belgium, and from the Smithsonian National Museum of Natural History, Washington, District of Columbia. See SOM Table S1 for a complete list of the specimens used in the analyses. All the bones were digitized using a Breuckmann 3D surface scanner at the MNHN. This surface scanner allows the acquisition of the 3D surface of the bone at high resolution using white light fringes (StereoSCAN^{3D} model with a camera resolution of five megapixels).

2.2. Quantification of shape using 3D geometric morphometrics

The shape of each forelimb long bone is complex and cannot be adequately represented using a traditional landmark-based approach (Fabre et al., 2013a,b, 2014, 2015a,b). Consequently, a three-dimensional (3D) sliding-semilandmark procedure (Bookstein, 1997; Gunz et al., 2005) was used to quantify their morphology, and especially their articulations and diaphyses. Through this procedure, semilandmarks are allowed to slide along the curves and surfaces that are predefined while minimizing the bending energy, and are thus transformed into geometrically (i.e., spatially) homologous landmarks that can be used to compare shapes (Parr et al., 2012).

Anatomical landmarks and sliding-semilandmarks of curves were obtained on 3D surface scans of each bone using the software package Idav Landmark (Wiley et al., 2005), while the library

'Morpho' (Schlager, 2013) in R (Hornik, 2015) was used to perform the sliding-semilandmark procedure. To do so, we first created a template for each long bone and its extremities following the method of Cornette et al. (2013). The landmark schemes for the humerus, ulna and radius are given, respectively, in Tables 2–4, and Figures 1, 2 and 3. Next, each specimen is first defined by homologous landmark coordinates. All curves are constrained by

Table 2
Anatomical and semilandmarks used in this study (humerus).

2a) Summary of landmarks			
Landmark type	Number of landmarks		
	Whole humerus	Proximal articulation	Distal articulation
Anatomical	27	9	11
Semilandmarks on curves	99	26	26
Semilandmarks on surfaces	265	35	61
2b) Definition of anatomical landmarks			
Landmark ^a	Definition		
	E	P	D
1	–	1	Most medial and distal point of the posterior part of the trochlea
2	–	2	Most medial and proximal point of the posterior side of the trochlea
3	–	3	Point of maximum of curvature of the olecranon fossa
4	–	4	Most lateral and proximal point of the posterior side of the trochlea
5	–	5	Most lateral and distal point of the posterior side of the trochlea
6	–	6	Most lateral and proximal point of the anterior side of the capitulum
7	–	–	Most distal point of the anterior side of the supracondylar foramen
8	–	7	Most proximal point of contact between the trochlea and the capitulum
9	–	–	Most proximal point of the anterior side of the supracondylar foramen
10	–	8	Most proximal point of the anterior side of the trochlea
11	–	9	Most proximal point of the medial epicondyle
12	–	10	Most distal point of the medial epicondyle
13	–	11	Most distal point of the lateral epicondyle
14	–	–	Most proximal point of insertion of the lateral supracondylar ridge on the diaphysis
15	–	–	Most proximal point of insertion of the medial supracondylar ridge on the diaphysis
16	–	–	Most distal point of the deltopectoral crest
17	1	–	Most distal point of the humeral head
18	2	–	Most lateral point of contact between the humeral head and the greater tubercle
19	3	–	Most medial point of contact between the humeral head and the greater tubercle
20	4	–	Most lateral point of contact between the humeral head and the lesser tubercle
21	5	–	Most medial point of contact between the humeral head and the lesser tubercle
22	6	–	Most proximo-lateral point of the greater tubercle
23	7	–	Most proximo-medial point of the greater tubercle
24	8	–	Most proximo-lateral point of the lesser tubercle
25	9	–	Most proximo-medial point of the lesser tubercle
26	–	–	Most proximal point of the posterior side of the supracondylar foramen
27	–	–	Most distal point of the posterior side of the supracondylar foramen

^a D – distal articulation of the humerus; E – entire humerus; P – proximal articulation of the humerus.

Table 1
Specimens used in the analyses with family, species name, and sample size (n).

Family	Species	n
Cheirogalidae	<i>Cheirogaleus major</i>	2
	<i>Microcebus murinus</i>	5
	<i>Mirza coquereli</i>	2
	<i>Phaner furcifer</i>	5
	<i>Daubentonia madagascariensis</i>	4
Galagidae	<i>Sciurocheirus alleni</i>	3
	<i>Galagoides demidoff</i>	3
	<i>Galago senegalensis</i>	4
Indridae	<i>Otolemur garnettii</i>	2
	<i>Avahi laniger</i>	5
	<i>Indri indri</i>	5
	<i>Propithecus diadema</i>	3
	<i>Propithecus verreauxi</i>	5
Lemuridae	<i>Eulemur fulvus</i>	4
	<i>Eulemur mongoz</i>	4
	<i>Hapalemur griseus</i>	6
	<i>Hapalemur simus</i>	2
	<i>Lemur catta</i>	5
	<i>Varecia variegata</i>	5
Lepilemuridae	<i>Lepilemur ruficaudatus</i>	5
Lorisidae	<i>Loris tardigradus</i>	3
	<i>Nycticebus coucang</i>	5
	<i>Perodicticus potto</i>	5

Table 3
Anatomical and semilandmarks used in this study (ulna).

3a) Summary of landmarks			
Landmark type	Number of landmarks		
	Whole ulna	Proximal articulation	Distal articulation
Anatomical	25	12	8
Semilandmarks on curves	97	39	15
Semilandmarks on surfaces	311	60	64

3b) Definition of anatomical landmarks			
Landmark ^a			Definition
E	P	D	
1	1	–	Most distal point of the radial notch
2	2	–	Most lateral point between the radial and the trochlear notches
3	3	–	Point of maximum of concavity in the disto-lateral side of the trochlear notch
4	4	–	Point of maximum of concavity in the proximo-lateral side of the trochlear notch
5	5	–	Point of maximum of concavity in the proximo-medial side of the trochlear notch
6	6	–	Point of maximum of concavity in the disto-medial side of the trochlear notch
7	7	–	Most medial point between the radial and the trochlear notches
8	8	–	Point of maximum of concavity between the radial and trochlear notches
9	9	–	Most antero-medial point of the olecranon
10	10	–	Most antero-lateral point of the olecranon
11	11	–	Most postero-lateral point of the olecranon
12	12	–	Most postero-medial point of the olecranon
13	–	1	Most proximal point of the distal radial notch
14	–	2	Most lateral point of the distal radial notch
15	–	3	Most distal point of the distal radial notch
16	–	4	Most medial point of the distal radial notch
17	–	5	Most anterior point of the styloid process
18	–	6	Most lateral point of the styloid process
19	–	7	Most posterior point of the styloid process
20	–	8	Most medial point of the styloid process
21	–	–	Most distal point of the interosseous crest
22	–	–	Point of maximum of convexity of the interosseous crest
23	–	–	Most proximal point of the interosseous crest
24	–	–	Most proximal point of the anterior ridge of the diaphysis of the ulna
25	–	–	Most distal point of the postero-lateral ridge of the diaphysis of the ulna

^a D – distal articulation of the ulna; E – entire ulna; P – proximal articulation of the ulna.

homologous landmarks (Gunz et al., 2005). Based on the homologous landmarks and curves taken on the specimen, all the sliding-semilandmarks (curve and surface sliding-semilandmarks) of the template were projected onto the new specimen using a thin plate spline deformation (Gunz and Mitteroecker, 2013). Finally, spline relaxation was performed, with both sliding and relaxation repeated iteratively until the bending energy was minimized (Schlager, 2013), following which the landmarks of all specimens can be compared using traditional geometric morphometric methods. All the spline relaxation steps were performed using the library ‘Morpho’ (Schlager, 2013) based on the algorithm of Gunz and collaborators (2005), which is implemented in R (Hornik, 2015). Ultimately, four thin-plate spline relaxations were performed, the first relaxation performed against the template, and the three others against Procrustes consensus calculated using the data from the previous iteration. Once all landmark data were obtained, a generalized Procrustes superimposition (Rohlf and Slice, 1990) was performed using the library ‘Geomorph’ (Adams and

Table 4
Anatomical and semilandmarks used in this study (radius).

4a) Summary of landmarks			
Landmark type	Number of landmarks		
	Whole radius	Proximal articulation	Distal articulation
Anatomical	21	6	11
Semilandmarks on curves	49	16	32
Semilandmarks on surfaces	252	49	24

4b) Definition of anatomical landmarks			
Landmark ^a			Definition
E	P	D	
1	1	–	Most proximo-lateral point of the anterior side of the radial head
2	2	–	Point at the middle of the fovea
3	3	–	Most latero-proximal point of the radial head
4	4	–	Point of maximum of curvature of the postero-lateral side of the radial head
5	5	–	Most latero-distal point of the radial head
6	6	–	Point of maximum of curvature of the antero-medial side of the radial head
7	–	1	Point of insertion of the styloid process on the diaphysis
8	–	2	Most lateral point of the styloid process
9	–	3	Point of maximum of curvature on the anterior side of the scaphoid articulation
10	–	4	Tip of the styloid process
11	–	5	Point of maximum of curvature on the posterior side of the scaphoid articulation
12	–	6	Most distal point of the groove for extensor digitorum
13	–	7	Most disto-medial point of the lunar facet
14	–	8	Most proximal point of the groove for extensor digitorum
15	–	9	Most posterior point of the distal ulnar notch
16	–	10	Most proximal point of the distal ulnar notch
17	–	11	Most anterior point of the distal ulnar notch
18	–	–	Most proximo-lateral of the tuberosity
19	–	–	Most disto-lateral of the tuberosity
20	–	–	Most disto-medial of the tuberosity
21	–	–	Most proximo-medial of the tuberosity

^a D – distal articulation of the radius; E – entire radius; P – proximal articulation of the radius.

Otarola-Castillo, 2013) in R (Hornik, 2015). Finally, a mean shape was calculated for each species using the Procrustes coordinates and this was used in all further analyses.

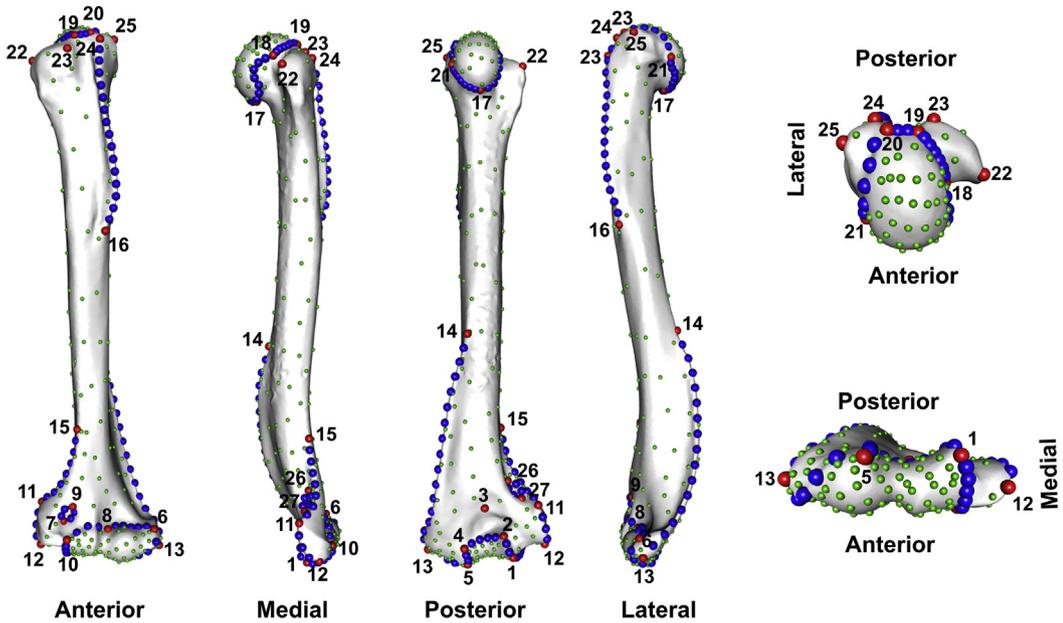
2.3. Allometry

Size and shape are tightly linked. Consequently, shape tends to be influenced by size, termed allometry (Gould, 1966; Klingenberg, 1996). To assess the allometry in our data set, we performed a regression of the mean of the log of the centroid size of each species on the mean shape of each species for each long bone and its extremities using the function ‘procD.lm’ of the ‘Geomorph’ package (Adams and Otárola-Castillo, 2013) in R (Hornik, 2015).

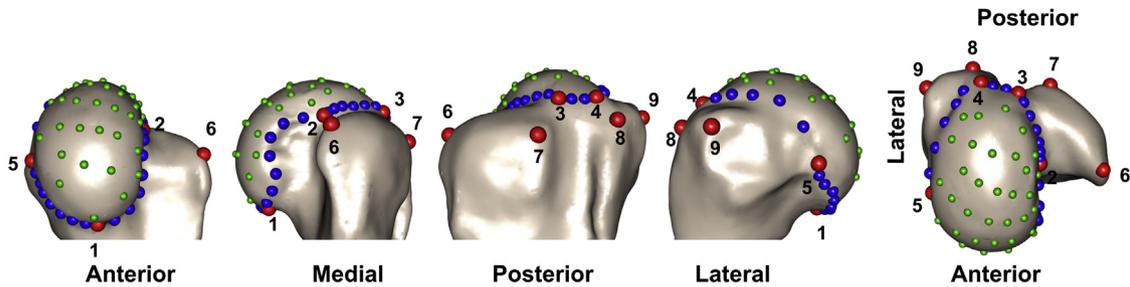
2.4. Quantification of supports

The support data set used comes from Oxnard et al. (1990), who (from detailed descriptions) developed standardized scores for size and orientation of supports used by strepsirrhines, ranging from 0 (never used) to 10 (used exclusively) (Table 5). A principal component analysis (PCA) was performed on these scores to visualise the variation in substrate use in strepsirrhines for the entire support utilization repertoire.

Entire humerus dataset



Proximal articulation of humerus dataset



Distal articulation of humerus dataset

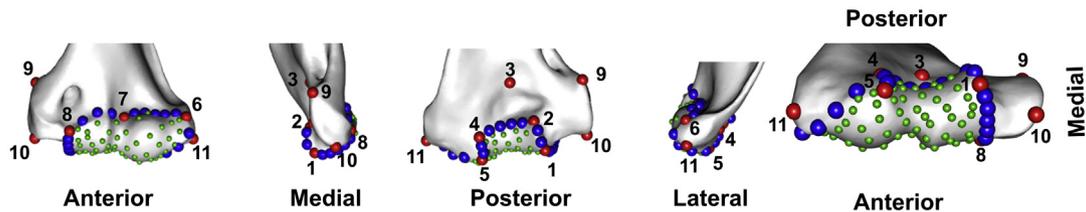


Figure 1. Humerus landmarks used in our analyses. Red points represent anatomical landmarks (see numbers/definitions in Table 2), blue points represent semilandmarks of curves, and light green points represent surface semilandmarks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

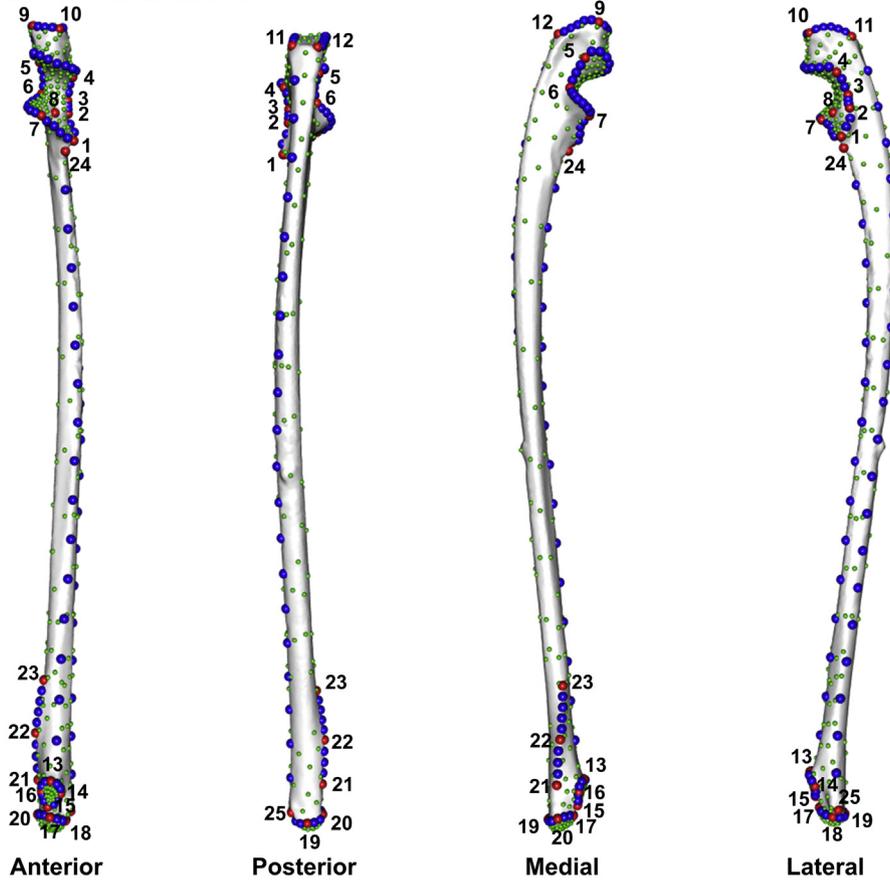
2.5. Phylogenetic signal

The phylogenetic tree of strepsirrhines used in all our comparative analyses (Fig. 4) was obtained from the 10kTrees Project (Arnold et al., 2010). Specifically, we used a consensus tree of Version 1 (Matthews et al., 2010) with branch lengths obtained from a Bayesian phylogenetic analysis. It uses four mitochondrial genes and one autosomal gene from GenBank as a backbone, and branch lengths are proportional to geological time. Full details of

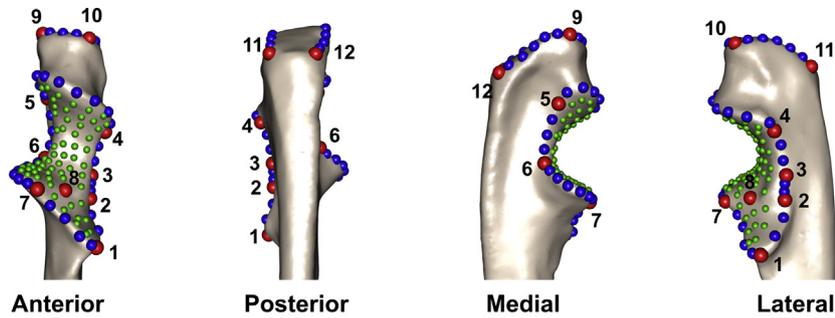
the phylogenetic reconstruction and the tree are provided in Arnold et al. (2010) and Matthews et al. (2010).

To estimate phylogenetic signal in forelimb shape and in support utilization scores (Table 5) we used a randomization test following the method of Blomberg et al. (2003) and the extended methods of Adams (2014). A multivariate K-statistic (Adams, 2014) was calculated based on the mean of the Procrustes coordinate of each species for the shape of each long bone as well as on residual shape and support utilization scores, using the

Entire ulna dataset



Proximal articulation of ulna dataset



Distal articulation of ulna dataset

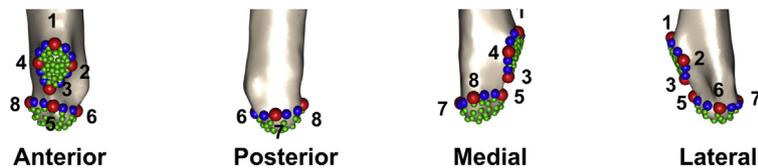
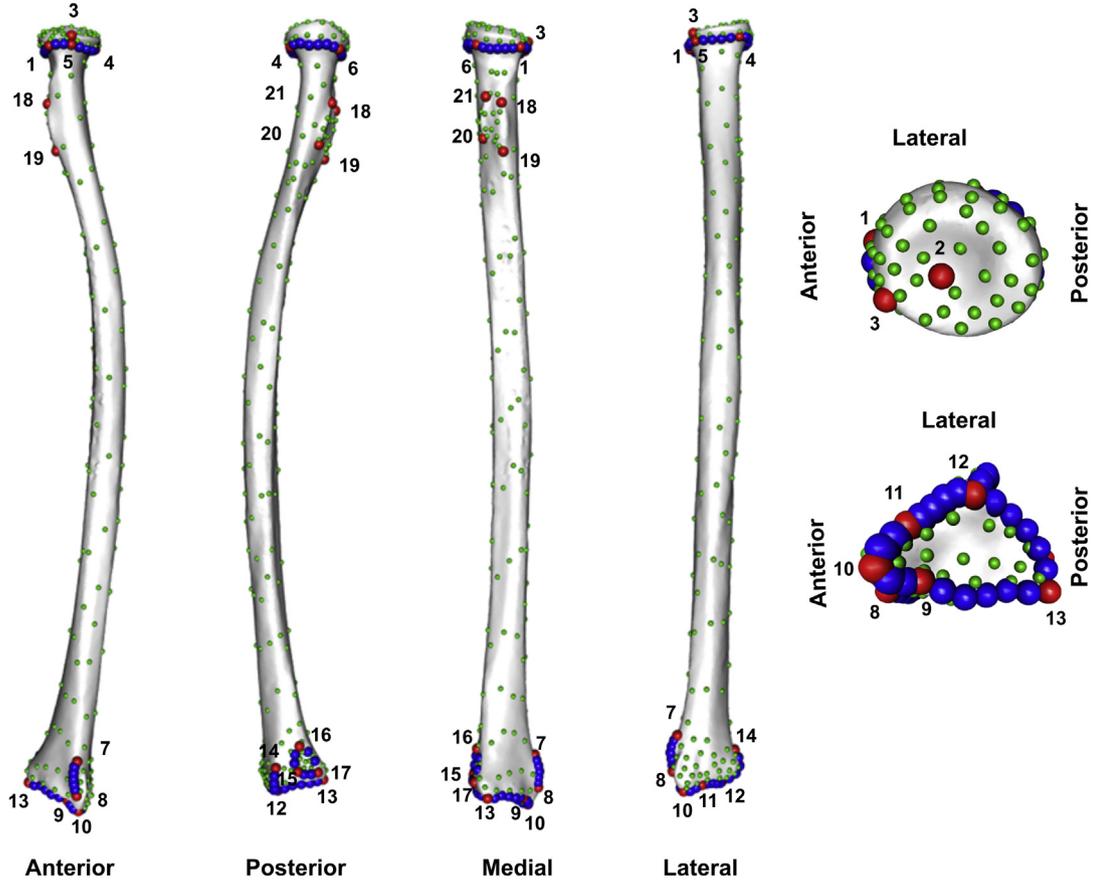


Figure 2. Ulna landmarks used in our analyses. Red points represent homologous landmarks (see numbers/definitions in Table 3), blue points represent semilandmarks of curves, and light green points represent surface semilandmarks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

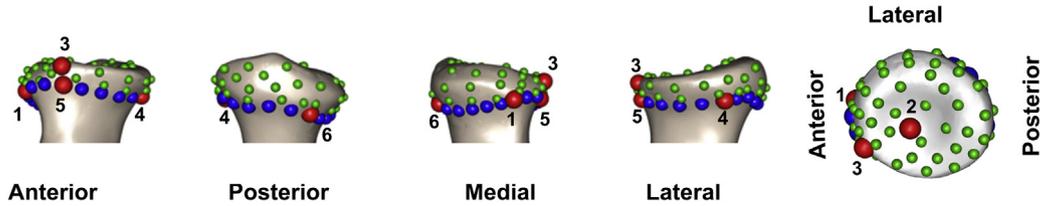
'geomorph' library (Adams and Otárola-Castillo, 2013) in R (Hornik, 2015). The higher the K-value is, the stronger the phylogenetic signal. A K-value of one corresponds to character evolution under Brownian motion. A K-value greater than one

indicates a strong phylogenetic signal, which means that traits are entirely conserved within the phylogeny. Conversely, a K-value close to zero means that the phylogenetic signal is weak (Blomberg et al., 2003).

Entire radius dataset



Proximal articulation of radius dataset



Distal articulation of radius dataset

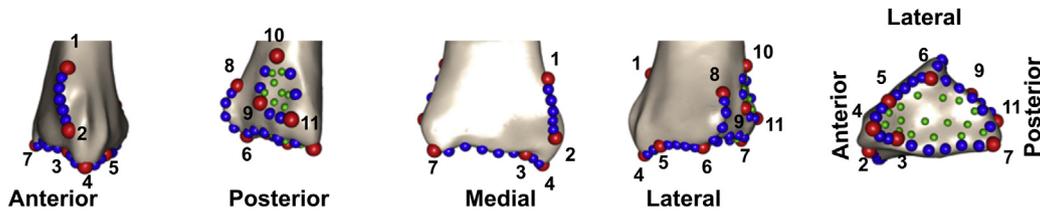


Figure 3. Radius landmarks used in our analyses. Red points represent homologous landmarks (see numbers/definitions in Table 4), blue points represent semilandmarks of curves, and light green points represent surface semilandmarks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.6. Co-variation of forelimb and locomotion

We performed a two-block partial least squares (2B-PLS; Rohlf and Corti, 2000) implemented in the library Geomorph (Adams and Otárola-Castillo, 2013) in R (Hornik, 2015) to study co-variation between support utilization and the shape of each bone of the forelimb. A covariance matrix was calculated from two blocks

representing the variation of the two objects (support utilization versus humeral shape, proximal articulation of humeral shape, distal articulation of humeral shape, ulnar shape, proximal articulation of ulnar shape, distal articulation of ulnar shape, radius shape, proximal articulation of radial shape, and distal articulation of radial shape). A PLS correlation coefficient between the two blocks of the PLS scores (r_{PLS}) was calculated to estimate the degree

Table 5
Oxnard's scores for support use (Oxnard et al., 1990).^a

Family	Species	Horizontal supports	Vertical supports	Small supports	Large supports
Cheirogaleidae	<i>Cheirogaleus major</i>	4.4	2	1.6	4.2
	<i>Microcebus murinus</i>	4	3.7	7	2
	<i>Mirza coquereli</i>	3.8	2.8	2	3
	<i>Phaner furcifer</i>	4	2.8	2.8	3
Daubentoniidae	<i>Daubentonia madagascariensis</i>	4	3	2	3
Galagidae	<i>Sciurocheirus alleni</i>	1.8	8	1.8	2.3
	<i>Galagooides demidoff</i>	3.8	5.2	7	2
	<i>Galago senegalensis</i>	4	2.6	1.6	2
	<i>Otolemur garnettii</i>	8	2.3	2	3.8
Indriidae	<i>Avahi laniger</i>	3.2	4.5	2	4.5
	<i>Indri indri</i>	3	5	1.8	4.4
	<i>Propithecus diadema</i>	3.4	3.5	3	4.5
	<i>Propithecus verreauxi</i>	3.4	3.5	3	4.5
Lemuridae	<i>Eulemur fulvus</i>	7.1	1.3	1.6	2.4
	<i>Eulemur mongoz</i>	7.1	1.2	1.6	2.4
	<i>Hapalemur griseus</i>	1	5	2	3
	<i>Hapalemur simus</i>	1	5	2	3
	<i>Lemur catta</i>	4.5	2.6	2	3.5
	<i>Varecia variegata</i>	4	1.6	1.8	3
Lepilemuridae	<i>Lepilemur ruficaudatus</i>	1.4	5.5	1.2	4.5
Lorisidae	<i>Loris tardigradus</i>	4	4	5	2.7
	<i>Nycticebus coucang</i>	6	2.4	3.2	3.2
	<i>Perodicticus potto</i>	4.2	4.2	1.8	4.5

^a Scale for scoring: 0 = never; 0.1–0.2 = rarely; 0.5 = sometimes; 1 = quite often; 2 = commonly; 4–5 = mostly; 6 = dominated by; 8 = almost exclusively; 10 = exclusively. Small supports include twigs and leaves, large supports are branches (see Oxnard et al., 1990).

of co-variation using the function 'pls2b' in R in the Morpho library (Schlager, 2013) following the method PLS (Bookstein et al., 2003). The pls2b function uses 3D landmark data after superimposition and performs a singular warps analysis (Bookstein et al., 2003). A significance test was obtained by 1000 permutations of the landmarks in one block relative to those of the scores of support use in the other, and a sampling distribution of coefficients is obtained by resampling. The P_{95} -value is calculated by comparison of the observed PLS coefficient to those obtained after resampling. The significance of each linear combination was assessed by comparing the singular value (PLS coefficient) to those obtained from permuted blocks. If the PLS coefficient was higher than those obtained from permuted blocks, its associated P_{95} -value was considered to be significant.

As species share some part of their evolutionary history, they cannot be treated as independent data points. Thus, we also conducted 2B-PLS in a phylogenetic framework (Felsenstein, 1985; Harvey and Pagel, 1991) using the phylogeny described above (Fig. 4) and the 'phylo.integration' function (Adams and Felice, 2014) in the Geomorph library of R (Adams and Otárola-Castillo, 2013). This function allows us to quantify the degree of co-variation between the mean shape of each species for each long bone and the support utilization while accounting for phylogeny using a partial generalised least squares algorithm under a Brownian motion model of evolution (Adams and Felice, 2014). Evolutionary PLS scores were calculated from the two blocks of phylogenetically corrected data, and the evolutionary correlation between the two blocks of the PLS scores (r_{PLS}) was evaluated. Partial least squares correlation significance was assessed using phylogenetic permutation, where the shape or the support use values for all species for one block are permuted on the tips of the phylogeny. The derived correlation scores were obtained from the permuted data sets and can be compared to the observed value.

2.7. Shape visualization

In order to visualise shape change at each extreme of the 2B-PLS and phylogenetic 2B-PLS, a thin-plate spline (TPS) deformation of a

3D-scanned long bone was performed. These functions allowed us to visualize the shape at each extreme of the 2B-PLS axis, and thus to evaluate the kind of bone shape that is associated with support use. This deformation is a smooth interpolation function that maps points of one form to corresponding points on another one and has the advantage of integrating the displacements at all landmarks relative to all others (Rohlf and Corti, 2000; Zelditch et al., 2012). Partial warps are used to represent the shape by supplying a basis for the tangent space of all possible shape differences (Zelditch et al., 2012). Details of the computation of partials warps are given in Bookstein (1989, 1991) and Rohlf (1993). Shape visualization of the effect of support use on each long bone of the forelimb was performed using the 'plsEffects', 'warp.mesh' and 'shade3d' functions of the 'rgl' (Adler and Murdoch, 2012) and 'Morpho' (Schlager, 2013) libraries in R using thin-plate spline deformations of a 3D-scanned long bone of an *Avahi laniger* specimen (MNHN 1880-2506).

2.8. Regression of long bones shapes on small support scores

To estimate the shape of long bones of species that use predominantly small supports (such as twigs or foliage, as defined by Oxnard et al., 1990) we performed a regression of the shape of each long bone as well as its extremities on substrate use scores in R using the function "procD.lm".

3. Results

3.1. Support use

Figure 5 depicts how the support data set derived from Oxnard et al. (1990) discriminates strepsirrhine species in our data set. The first two principal components account for 86.1% of the overall variation. The first axis displays species that tend to use vertical supports, which are situated along the negative part of the axis. In contrast, species that tend to use horizontal supports are situated along the positive part of the axis. On the second axis, the negative part of the axis characterizes species that tend to use narrow

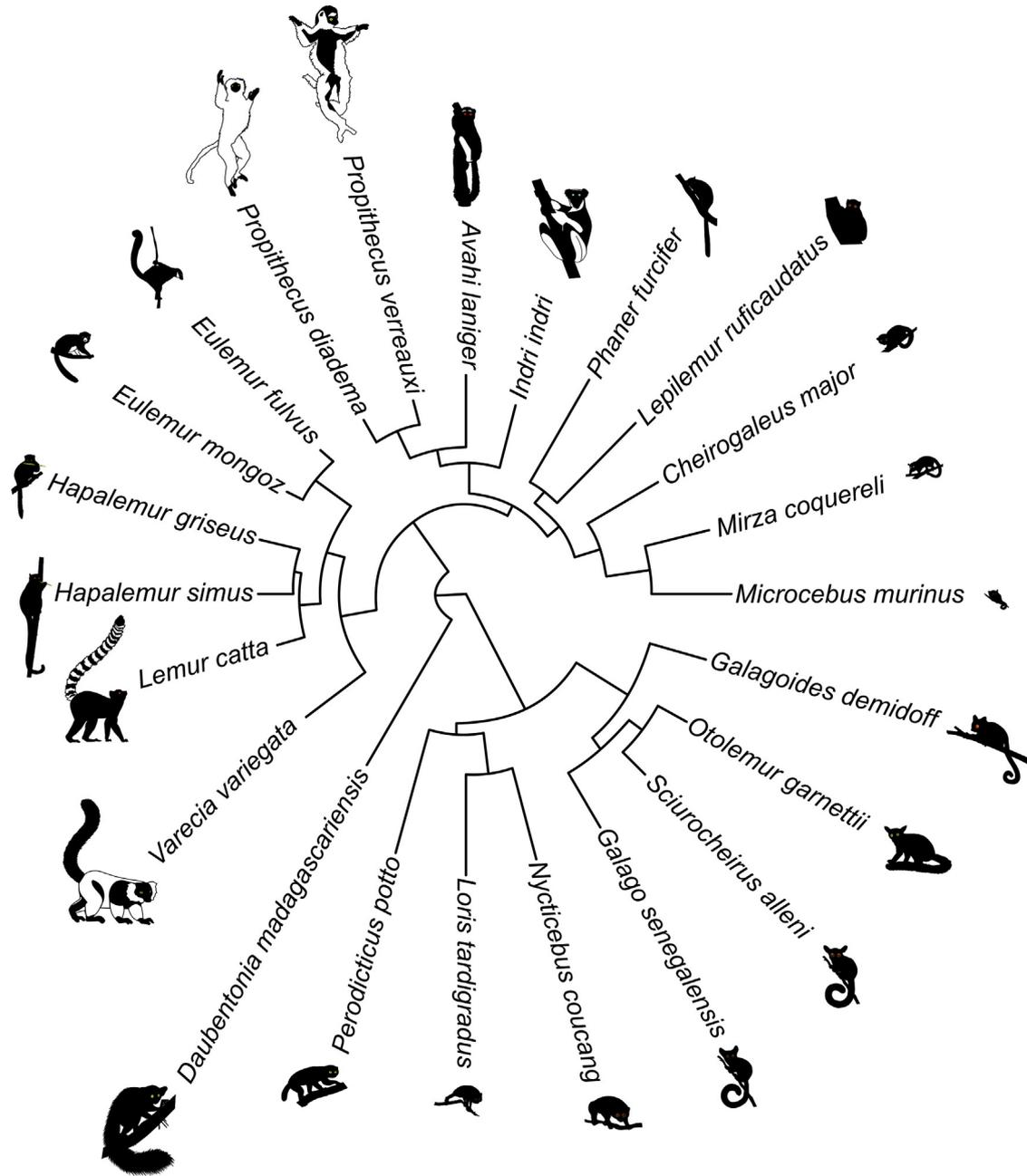


Figure 4. The phylogenetic relationships of the strepsirrhine species used in this study, derived from the 10kTrees Project website (Arnold et al., 2010).

supports, whereas the positive part of the axis characterizes species that use large supports (Table 6).

3.2. Allometry

Logged centroid size had a significant relationship with the shape (Table 7) of the entire humerus ($R^2 = 0.15$, $p_{rand} = 0.009$) and its proximal articulation ($R^2 = 0.09$, $p_{rand} = 0.045$) but not on the distal articulation ($R^2 = 0.057$, $p_{rand} = 0.22$). Logged centroid size also had a significant relationship with the shape of the entire ulna ($R^2 = 0.13$, $p_{rand} = 0.035$), but not its proximal ($R^2 = 0.08$, $p_{rand} = 0.1$) and distal articulations ($R^2 = 0.07$, $p_{rand} = 0.1$; Table 7). There was a significant relationship between logged centroid size and proximal ($R^2 = 0.13$, $p_{rand} = 0.007$) and distal ($R^2 = 0.13$, $p_{rand} = 0.003$) articulations of the radius (Table 7), but not with the entire radius

($R^2 = 0.07$, $p_{rand} = 0.14$). Further analyses were performed on shape coordinates as well as on the residuals for each long bone.

3.3. Phylogenetic signal

The results of the multivariate K-statistic calculated on the shape data, to evaluate the strength of phylogenetic signal in explaining variation within forelimb long bone anatomy and substrate use scores, are given in Table 8. These results show a significant phylogenetic signal between 0.5 and 1 in the shape of each long bone of the forelimb, which is moderately strong. It highlights the importance of taking phylogeny into account in further analyses. The result of the multivariate K-statistic calculated on the substrate use scores was not significant (Table 8), suggesting that substrate use is not structured by phylogenetic relationships.

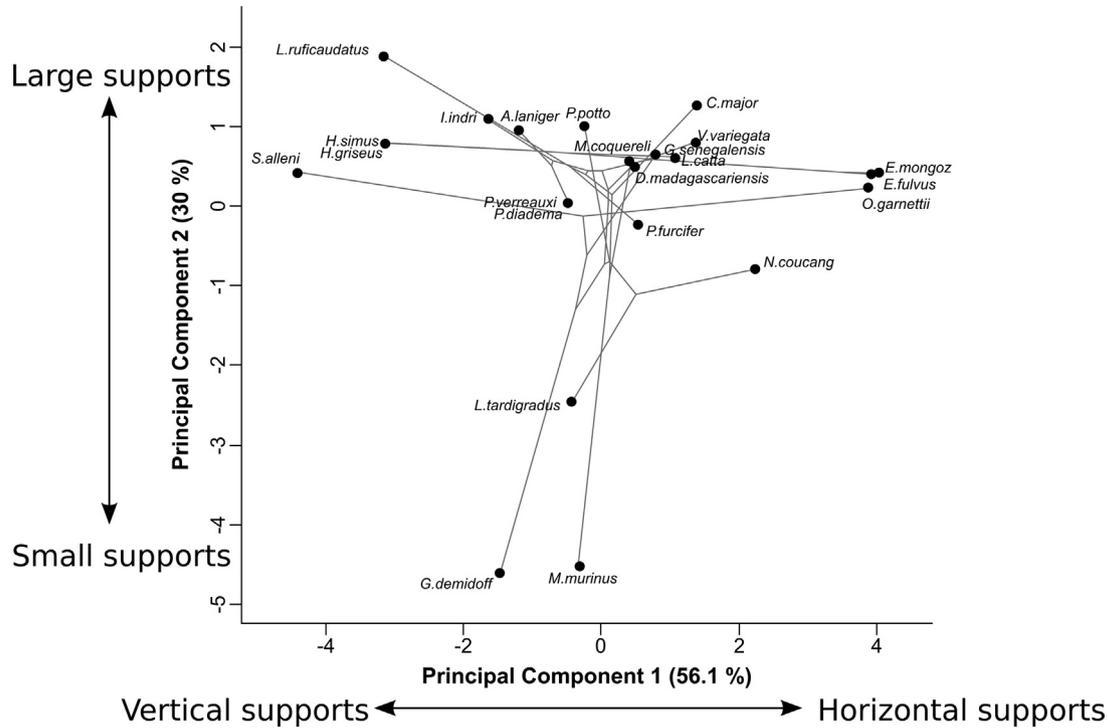


Figure 5. Results of the principal components analysis performed on support data. The scatter plot illustrates the position of the different species on the first two principal components. The first principal component tends to separate species depending on whether they use vertical or horizontal supports, whereas the second principal component tends to separate species depending on whether they use small or large supports. Loadings for each variable can be found in Table 6.

Table 6
Loadings of the Principal Component Analysis performed on the support data.

Variable	Principal component 1	Principal component 2	Principal component 3
Horizontal support	0.7518	-0.1468	0.5936
Vertical support	-0.6531	-0.08	0.6484
Small support	-0.0765	-0.9412	0.0007
Large support	-0.0477	0.2933	0.4765

3.4. Co-variation between forelimb long bone shapes and support use

3.4.1. Humerus There was no significant co-variation between humeral shape and support use (Table 9, SOM Fig. S1A, C, E), nor between entire humerus shape residuals and support use (Table 9, SOM Fig. S2A, C, E). This remained true even when taking phylogeny into account (Table 9, SOM Figs. S1B and S2B). However, when phylogeny was taken into account there was significant co-variation between both proximal and distal articulations and support utilization for shape and shape residuals (Tables 9 and 10, Fig. 6A, B, SOM Fig. S1D, F, and SOM

Table 7
Results of the regression analysis of centroid size and forelimb long bone shape.^a

Bone	Part	R ²	p
Humerus	Entire	0.15	0.009
	Proximal	0.09	0.045
	Distal	0.057	0.22
Ulna	Entire	0.13	0.035
	Proximal	0.08	0.1
	Distal	0.07	0.1
Radius	Entire	0.07	0.14
	Proximal	0.13	0.007
	Distal	0.13	0.003

^a Significant results are indicated in bold.

Fig. S2D, F). Thus, there is a significant degree of co-variation between humeral articular shape and support utilization when the phylogeny is taken into account, as is evident, for example, when comparing the sister taxa *Otolemur garnettii* and *Sciurocheirus alleni*. Occupying a portion of the scatterplot associated with habitual use of horizontal and large supports (Fig. 6), *O. garnettii* displays a relatively round humeral head that is at the same level as the greater and lesser tubercles, both of which are relatively well developed (Fig. 6A). It also shows a relatively more rounded capitulum with a narrower trochlea, and a medial epicondyle and lateral epicondylar ridge that are relatively well developed and posteriorly oriented (Fig. 6B). In contrast, *S. alleni*, which occupies a portion of the scatterplot associated with the use of large and vertical supports (Fig. 6), displays a relatively oval-shaped humeral head that is higher than the greater and lesser tubercles, both of which are also relatively less developed than in *O. garnettii* (Fig. 6A). *Sciurocheirus alleni* also shows a relatively elongated capitulum

Table 8
Results of the multivariate K-statistic for long bone shape and substrate use.^a

Bone	Part	Procrustes coordinates		Shape residuals coordinates	
		Kmult	p	Kmult	p
Humerus	Entire	0.69	0.001	0.63	0.003
	Proximal	0.65	0.001	0.62	0.001
	Distal	0.78	0.001	0.74	0.001
Ulna	Entire	0.7	0.001	0.69	0.001
	Proximal	0.93	0.001	0.87	0.001
	Distal	0.77	0.001	0.75	0.001
Radius	Entire	0.71	0.001	0.68	0.001
	Proximal	0.54	0.002	0.48	0.016
	Distal	0.68	0.001	0.61	0.001
Substrate use scores		0.38	0.44	NA	NA

^a Significant Kmults are indicated in bold.

Table 9Results of the 2B-PLS and the phylogenetic 2B-PLS between each long bone of the forelimb and the supports used.^a

Bone	Part	2B-PLS between shape and substrate		Phylogenetic 2B-PLS between shape and substrate		2B-PLS between shape residuals and substrate		Phylogenetic 2B-PLS between shape residuals and substrate	
		Γ_{PLS}	p	Γ_{PLS}	p	Γ_{PLS}	p	Γ_{PLS}	p
Humerus	Entire	0.55	0.15	0.55	0.18	0.57	0.15	0.57	0.13
	Proximal	0.55	0.53	0.74	0.03	0.55	0.4	0.72	0.05
	Distal	0.56	0.43	0.78	0.04	0.51	0.6	0.79	0.01
Ulna	Entire	0.47	0.28	0.62	0.02	0.52	0.28	0.66	0.01
	Proximal	0.53	0.2	0.67	0.2	0.4	0.7	0.72	0.08
	Distal	0.45	0.5	0.72	0.02	0.52	0.19	0.73	0.02
Radius	Entire	0.63	0.05	0.7	0.01	0.61	0.06	0.69	0.01
	Proximal	0.71	0.06	0.55	0.53	0.67	0.82	0.57	0.48
	Distal	0.59	0.56	0.77	0.04	0.58	0.61	0.77	0.04

^a Significant co-variations are indicated in bold.**Table 10**

Summary of the significant PLS loadings of the different types of supports used in relation to the different anatomical parts of the long bones.

Bone	Part of the bone and figure associated	Horizontal	Vertical	Small	Large
Phylogenetic 2B-PLS Humerus	Proximal part (Fig. 6A)	-0.68	0.7	0.12	-0.1
	Residuals proximal part (Fig. S2D)	-0.69	0.71	0.09	-0.07
	Distal part (Fig. 6B)	0.7	-0.6	-0.1	0.1
	Residuals distal (SOM Fig. S2F)	-0.7	0.65	0.01	-1.1
Phylogenetic 2B-PLS Ulna	Entire shape (Fig. 7A)	0.61	-0.66	-0.33	0.25
	Entire residuals (SOM Fig. S4B)	0.59	-0.66	-0.39	0.21
	Distal part (Fig. 7B)	0.69	-0.71	-0.004	0.08
	Residuals distal (SOM Fig. S4F)	0.69	-0.71	-0.02	0.07
2B-PLS Radius	Entire shape (Fig. 8A)	-0.33	0.65	0.6	-0.11
Phylogenetic 2B-PLS Radius	Entire shape (Fig. 8B)	-0.6	0.67	0.33	-0.19
	Entire residuals (SOM Fig. S6B)	-0.63	0.67	0.35	-0.18
	Distal part (Fig. 8C)	0.74	-0.63	-0.2	0.18
	Residuals distal part (SOM Fig. S6F)	0.74	-0.64	-0.06	0.14

with a more developed trochlea, as well as a medial epicondyle and lateral epicondyles that are relatively well developed (Fig. 6B).

3.4.2. Ulna There was no significant co-variation between ulnar shape or shape residuals and support use (Table 9 and SOM Fig. S3A, C, E). The same was true when phylogeny was taken into account for the proximal articulation of the ulna (Table 9 and SOM Figs. S3D, S4D). However, there was strong evolutionary co-variation when phylogenetic relationships among taxa are taken into account for shape and shape residuals of the entire ulna, as well as its distal articulation (Tables 9 and 10, Fig. 7A, B, SOM Fig. S3B, F and SOM Fig. 4B, F). Again, for example, the ulna of *O. garnettii* (Fig. 7), has a relatively broad and tall olecranon process that is medially oriented, a large medial flange of the trochlear notch, a diaphysis that is curved in the middle and, a distal end with a radial notch that is oriented relatively distally, a relatively broad styloid process, and a relatively short pronator flange (Fig. 7A). The distal articulation of the ulna shows an ulnar notch that is relatively less rounded and less developed and the styloid process is relatively broader and flatter (Fig. 7B). In contrast, *S. alleni*, has an ulna with a relatively narrow, short and straight olecranon process, a relatively small medial flange of the trochlear notch, a diaphysis that is curved proximally, and a distal end with a radial notch that is oriented relatively ventrally, a relatively narrow styloid process, and a short pronator flange (Fig. 7A). The distal ulnar notch is relatively rounded and prominent and the styloid process is relatively narrower and more angular (Fig. 7B).

3.4.3. Radius There was no significant co-variation between proximal and distal articulation shape or shape residuals and support use in the radius (Table 9, SOM Fig. S5B, E, SOM Fig. S6C, E). However, there was significant co-variation between radial shape and the orientation and size of supports (Tables 9 and 10,

Fig. 8A, SOM Fig. S5A, and SOM Fig. S6A). The scatterplot of the 2B-PLS (Fig. 8A) shows that species, including *Loris tardigradus*, *Galagoides demidoff* and *S. alleni*, that occupy the positive space are mainly nocturnal and use narrow supports. In contrast, taxa using large supports with a gradient from vertical (*A. laniger*, *Indri indri*, *Propithecus verreauxi*, *Propithecus diadema*, *Lepilemur ruficaudatus*, *Haplemur griseus* and *Haplemur simus*) to horizontal (*Eulemur mongoz*, *Eulemur fulvus*, *O. garnettii*, *Cheirogaleus major*, *Varecia variegata*) are observed running from the middle of the scatterplot through to the maximum negative side of the axis. Species that tend to use narrow supports display a relatively gracile radius (Fig. 8A) with a relatively straight diaphysis. Species using narrow supports also display relatively small, round, and smooth articulations (radial head, ulnar notch and articulations for lunate and scaphoid) with a styloid process that is largely absent. The articular surface for the scaphoid is relatively small with a triangular shape in comparison to the articulation for the lunate, which is larger with a trapezoidal shape. In contrast, species situated at the negative part (Fig. 8A) of the scatterplot (mainly species using wide supports oriented horizontally) display a radius which is more robust, with a relatively curved diaphysis. They also display relatively large articulations (with a radial head that is less rounded, a wide distal ulnar notch and wide articulations for the lunate and scaphoid) and a well-developed styloid process. The articular surface for the scaphoid is relatively small and curved with a triangular shape, in comparison to the articulation for the lunate which is larger and flatter with a square shape.

There was no significant co-variation between the proximal articulation of the radius (shape and shape residuals) and support use when phylogeny was taken into account (Table 9, SOM Fig. S5D, and SOM Fig. S6D). However, co-variation between support use and entire radial shape, and shape residuals, as well as the distal

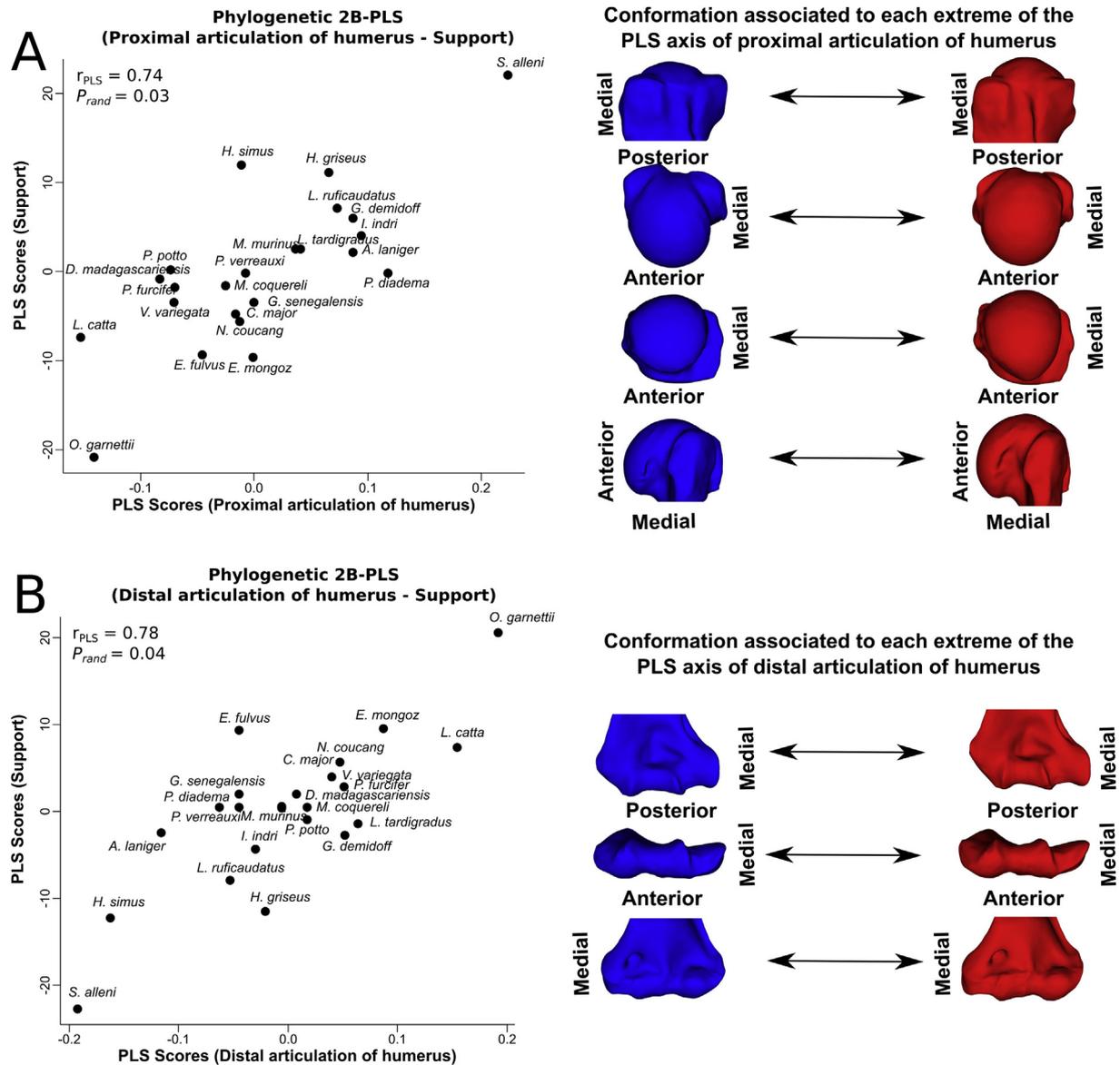


Figure 6. Results of the phylogenetic 2B-PLS between the humerus and the supports used. **A**, Scatter plot of the first PLS axis describing shape co-variation between the proximal articulation of the humerus and the supports used taking phylogeny into account. Proximal humeral shapes (left side) associated with each minimum and maximum of co-variation are illustrated in blue and red, respectively, to the right of the scatter plot. **B**, Scatter plot of the first PLS axis describing shape co-variation between the distal articulation of the humerus and the supports used taking into account the phylogeny. Distal humeral shapes (left side) associated with each minimum and maximum of co-variation are illustrated in blue and red, respectively, to the right of the scatter plot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

articulation of the radius (shape and shape residuals) was highly significant when the phylogenetic relationships among species were taken into account (Tables 9 and 10, Fig. 8B, SOM Fig. S5B, F, and SOM Fig. S6B, F). As observed for the ulna, a strong difference in support use and forelimb shape between species that are closely related can be observed, with *S. alleni* mainly using vertical supports whereas *O. garrettii* mainly uses horizontal ones (Fig. 8B and C). The positive part of the scatterplot corresponds to a radius which is relatively more gracile and straighter with smaller articulations (Fig. 8B). The end of the radius is flat with a styloid process that is poorly developed (Fig. 8B). In contrast, the negative part of the scatterplot corresponds to a relatively more robust and curved radius with wider and bigger articulations. The distal part of the radius is more angular with a styloid process that is well developed (Fig. 8B). The negative part of the scatterplot corresponds to a distal articulation of the radius with a narrower distal articulation for the scaphoid that is posteriorly more developed, with the groove for

the extensor digitorum also being relatively less developed (Fig. 8C). In contrast, species in the positive part of the scatterplot display a distal articulation of the radius that is broader, the scaphoid articulation is less developed posteriorly, and the groove for the extensor digitorum is relatively more developed (Fig. 8C).

3.5. Effect of small substrate use on forelimb long bone shape

There was a significant correlation between the use of small supports and the shape of the entire radius, but no significant associations in proximal and distal radial elements, the humerus, or the ulna (Table 11).

4. Discussion

The goals of this study were to test whether substrate use and forelimb anatomy co-vary in living strepsirrhine primates, and to

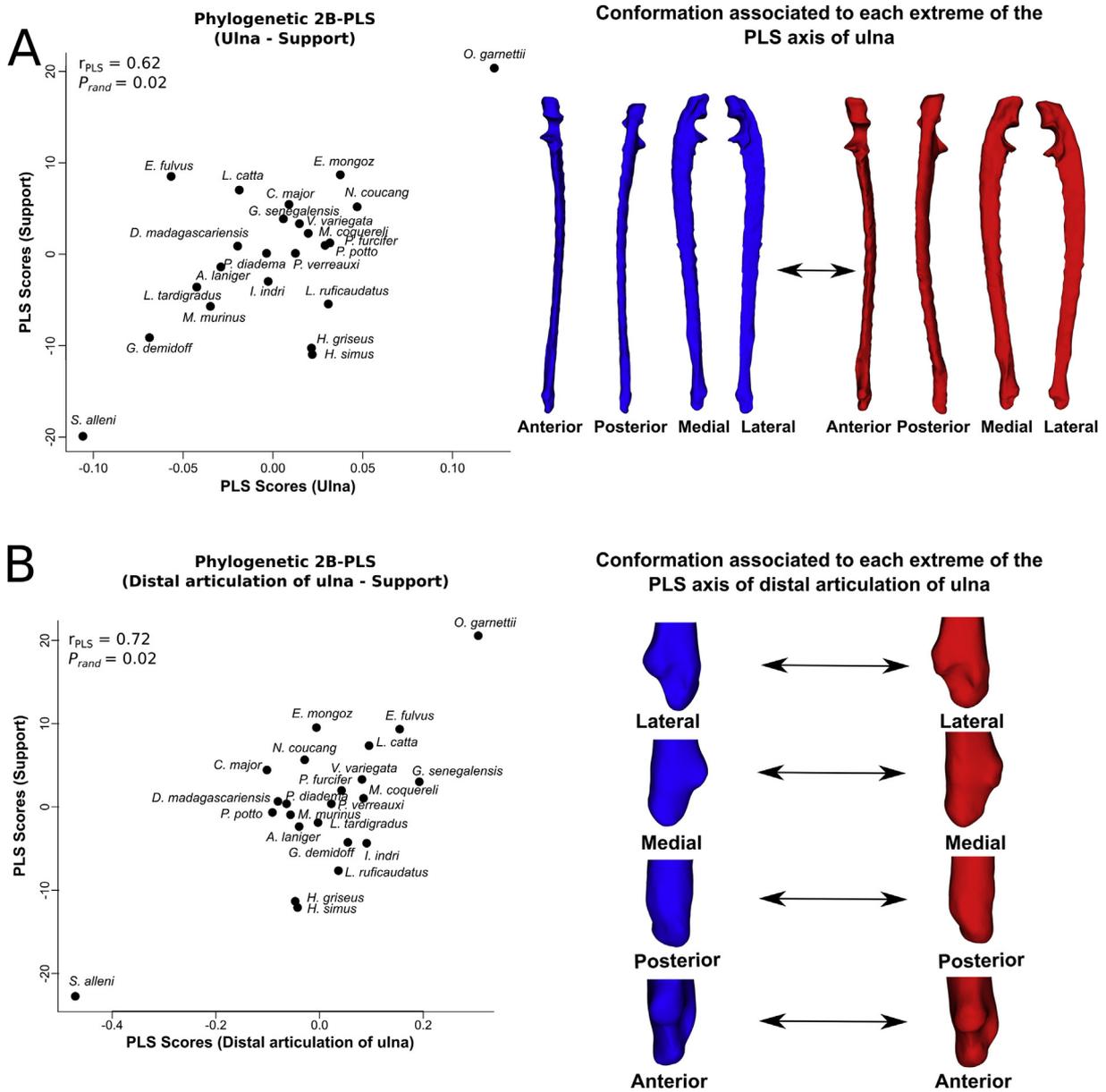


Figure 7. Results of the phylogenetic 2B-PLS between the ulna and the supports used. **A.** Scatter plot of the first PLS axis describing shape co-variation between the ulna and the supports used taking phylogeny into account. Ulnar shapes (left side) associated with each minimum and maximum of co-variation are illustrated in blue and red, respectively, to the right of the scatter plot. **B.** Scatter plot of the first PLS axis describing shape co-variation between the distal articulation of the ulna and the supports used taking phylogeny into account. Distal ulnar shapes (left side) associated with each minimum and maximum of co-variation are illustrated in blue and red, respectively, to the right of the scatter plot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

examine the strength of that relationship when phylogeny and body mass are accounted for. Our results show that patterns of substrate use in strepsirrhines are not influenced by phylogeny, highlighting the fact that these animals have independently evolved into different support niches. This result is also evident when examining the principal component analysis with a phylogenetic tree mapped onto the scatterplot. This plot shows a strong overlap between clades, but with a clear distribution of species depending on the type of substrate they used. Nonetheless, our results show that the shape of the forelimb contains a significant phylogenetic signal, implying that species that are closely related are more similar to one another than might be expected by chance. This result has already been highlighted in several studies on cranial anatomy (Fleagle, 2013; Baab et al., 2014). However, even when phylogeny is accounted for there remains a strong relationship

between the substrate used and the shape of the long bones of the forearm, although the patterns are not uniform across bones and bone regions. In the following discussion of the functional demands that may have been at the origin of the specific changes in limb shape observed in relation to support use we refer extensively to Table 12 and Figure 9 depicting the origins and insertions of the major limb muscles in strepsirrhines.

4.1. Humerus

Primates that predominantly use horizontal supports during locomotion tend to show adaptations of the humerus that are related to the avoidance of sideways toppling. Strepsirrhine species using horizontal supports have broader greater and lesser tubercles than species using vertical supports. These large tubercles allow the

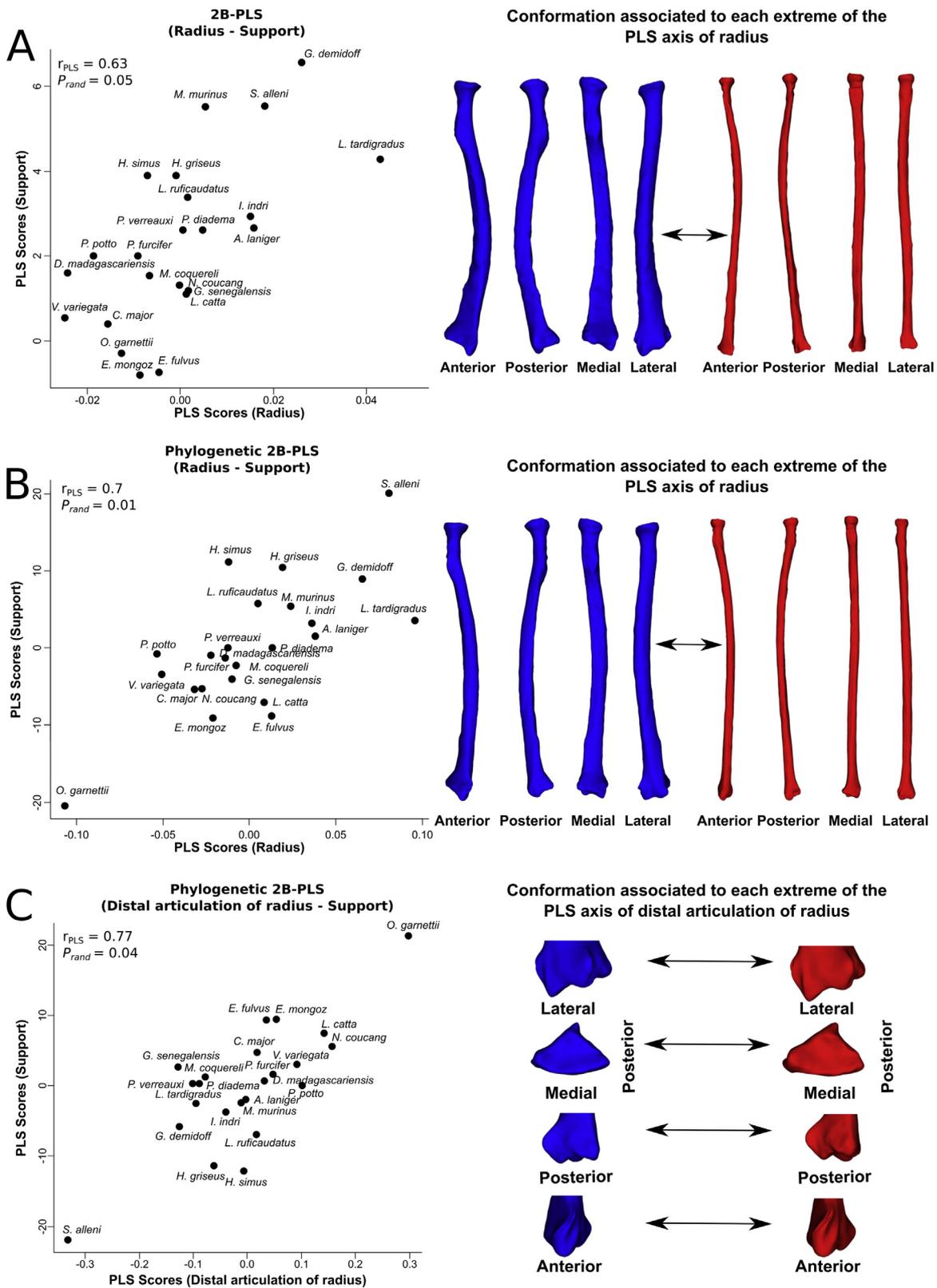


Figure 8. Results of the 2B-PLS and the phylogenetic 2B-PLS between the radius and the supports used. **A**, Scatter plot of the first PLS axis describing shape co-variation between the radius and the supports. Radial shapes (left side) associated with each minimum and maximum of co-variation are illustrated in blue and red, respectively, to the right of the scatter plot. **B**, Scatter plot of the first PLS axis describing shape co-variation between the radius and the supports used taking phylogeny into account. Radial shapes (left side) associated with each minimum and maximum of co-variation are illustrated in blue and red, respectively, to the right of the scatter plot. **C**, Scatter plot of the first PLS axis describing shape co-variation between the distal articulation of the radius and the supports used taking phylogeny into account. Distal radial shapes (left side) associated with each minimum and maximum of co-variation are illustrated in blue and red, respectively, to the right of the scatter plot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 11

Results of the regression analysis testing the effect of use of small substrates on the forelimb long bone shape.

		R ²	p-value ^a
Humerus:	Entire	0.09	0.09
	Proximal	0.06	0.16
	Distal	0.06	0.12
Ulna:	Entire	0.06	0.19
	Proximal	0.09	0.06
	Distal	0.062	0.1
Radius:	Entire	0.12	0.04
	Proximal	0.077	0.1
	Distal	0.049	0.3

^a Significant correlations are indicated in bold.

insertion of more strongly developed supraspinatus and the subscapularis muscles, respectively, providing more stability at the shoulder joint (Table 12, Fig. 9). They also display a round humeral head that does not project above the tubercles, which also promotes stability. This is likely important during quadrupedal locomotion in trees (Larson and Stern, 1989; Whitehead et al., 2005; Salton and Sargis, 2008; Fleagle, 2013). In contrast, the proximal articulation of the humerus of species using vertical supports shows greater and lesser tubercles that do not project above the humeral head, allowing greater freedom of movement of the shoulder, which can be important for control of rotational force during landing after leaping (Demes et al., 1996).

The distal articulation of the humerus in strepsirrhine species that mainly use horizontal supports displays a trochlea that is less wide than seen in taxa using vertical supports. The shape of the trochlea is often thought to be related to the flexion and extension ability of the forelimb at the elbow joint (Argot, 2001). A less convex trochlea may then reflect a higher stabilization of the forelimb during extension (Jenkins, 1973; Argot, 2001) and may help to stabilize the forearm when the animal is moving on horizontal supports. In the species included in our study that use vertical substrates, the trochlea is less convex, which may reflect a less-stabilised humeroulnar joint, allowing the ulna to 'rock' on the humerus during supination (Taylor, 1974; Argot, 2001). Furthermore, the lateral and medial epicondyles are posteriorly oriented in species in our study that use horizontal substrates. The epicondyles

are the attachment sites for extensor and flexor muscles of the hand and the wrist (Fig. 9, Table 12). In general, a medial epicondyle that is posteriorly oriented will decrease the medial torque induced by the flexors at the elbow joint (Jenkins, 1973; Argot, 2001). Thus, when reorienting the epicondyle, the torque may be decreased because the flexors pass behind rather than to the medial side of the trochlea (Argot, 2001), decreasing flexion of the digits and, thus, the grip on the substrate in species using horizontal supports in comparison to those using vertical ones (Congdon and Ravosa, 2016). In contrast, the medial epicondyle of species using vertical substrates is prominent and may reflect well-developed flexor musculature of the digits (Argot, 2001), with strong flexion of the digits increasing frictional force between the hand and support during climbing on vertical substrates (Jenkins, 1973; Stalheim-Smith, 1984; Argot, 2001). Thus, stronger digit flexion may be necessary to increase the grip on the substrate in order to maintain the position of an animal on a vertical support (Congdon and Ravosa, 2016).

4.2. Ulna

The shape differences associated with the ulna of a strepsirrhine species using horizontal supports are a relatively broader and taller olecranon process that is medially oriented. In contrast, species using vertical supports have a relatively narrower and smaller olecranon process, which is straight. The olecranon process is the area of insertion of the main elbow extensor, the triceps brachii (Fig. 9 and Table 12), which can play a role in extension and retraction of the forelimb as a whole (Leach, 1977; Argot, 2001; Fabre et al., 2015a). Nonetheless, the triceps is often categorized as an anti-gravity muscle, providing stability in flexed postures rather than necessarily generating motion (Jouffroy and Stern, 1990). Thus, species that mainly use horizontal supports and need to maintain elbow angle against the action of gravity, may have a larger triceps brachii (Oxnard, 1963; Argot, 2001), which is reflected in a broader and taller olecranon. In contrast, species using vertical supports may flex their elbows to remain close to the substrate and resist pitch, and have a smaller triceps and consequently display a shorter and smaller olecranon process (Jolly, 1967; Walker, 1974; Cartmill, 1974b; Argot, 2001; Preuschoft, 2002; Johnson, 2012). When looking at only the distal articulation of

Table 12

General origin and insertion of the muscles depicted in Figure 9 and their hypothesized action (modified from Jouffroy, 1962 and Moore et al., 2013).

Muscle	Origin	Insertion	Hypothesized action
Triceps brachii complex	Axillary border of the scapula (only long head), diaphysis of humerus (medial and lateral heads)	Olecranon of ulna	Elbow extension, shoulder extension, forelimb retraction
Brachialis	Posterolateral aspect of proximal humerus	Medial aspect of coronoid process of ulna	Elbow flexion
Biceps brachii	Coracoid process of scapula and from glenoid cavity until the bicipital groove of humerus	Bicipital tuberosity of radius	Elbow flexion
Pronator teres	Medial epicondyle of humerus	Distal half of the medial side of radius	Pronation of forearm
Flexors of the forearm, wrist and digits:	Medial epicondyle of the humerus and medio-proximal ulna	Carpal bones and digits on ventral side of the hand:	Flexion of hand, wrist and digits
- 1, flexor carpi radialis	- 1, medial epicondyle of humerus	- 1, ventral side of trapezium and second metacarp	
- 2, flexor digitorum	- 2, medial epicondyle of the humerus	- 2, phalanges of digits II to V	
- 3, flexor carpi ulnaris	- 3, humeral and ulnar origin	- 3, pisiform and base of V metacarpal	
Extensors of the forearm, wrist and digits:	Lateral epicondyle of the humerus	Carpal bones and digits on dorsal side of the hand:	Extension of the forearm, wrist and digits:
- 1, extensor carpi radialis		- 1, second and third metacarpals	- 1, extension of digits
- 2, extensor digitorum communis		- 2, phalanges of digits II to V	- 2, extension of digits
- 3, extensor carpi ulnaris		- 3, between dorsal surface of ulniform and fifth metacarp	- 3, extension of wrist and hand
Pronator quadratus	Pronator flange of ulna	Pronator flange of radius	Pronation of forearm

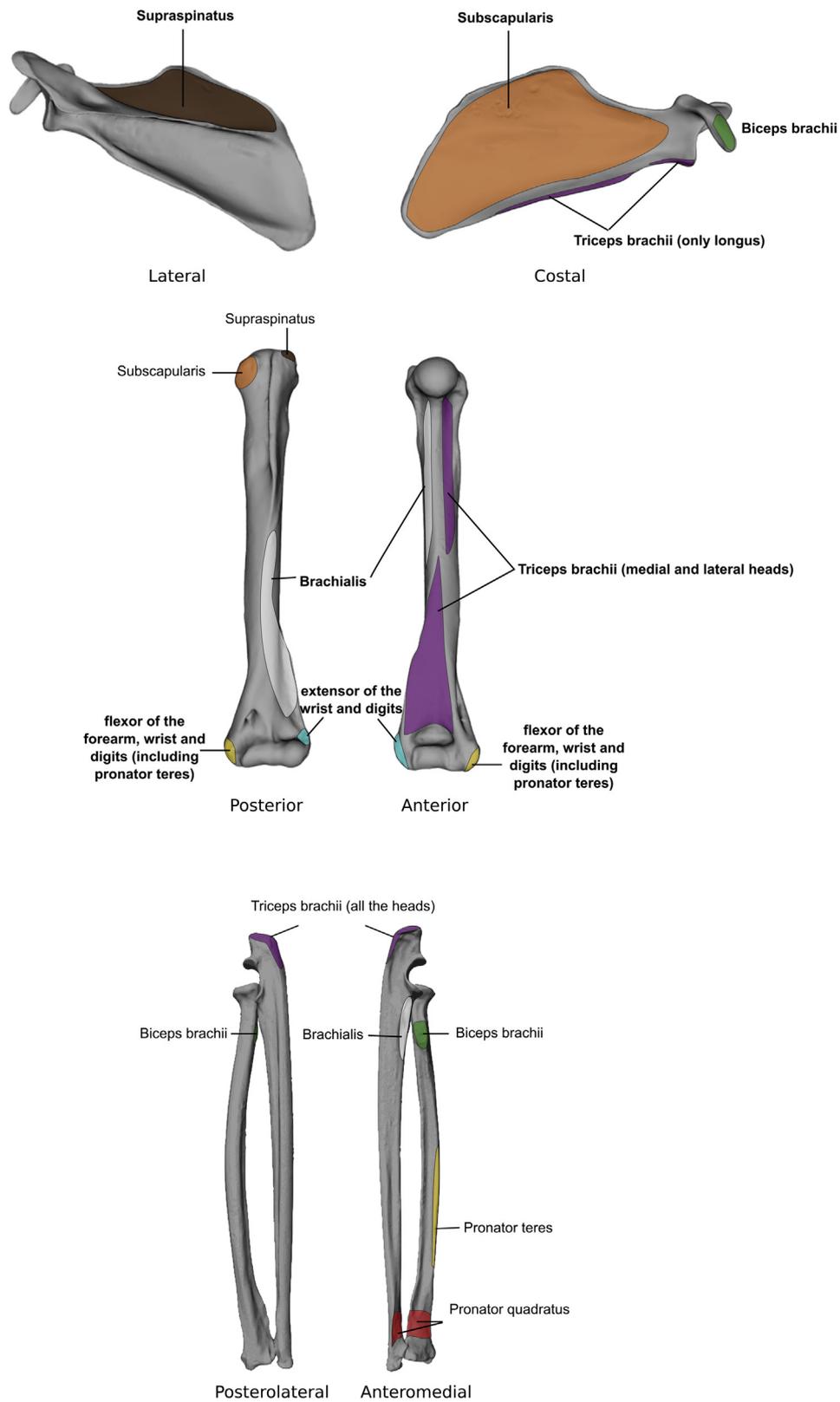


Figure 9. Schematic figure illustrating the origins and insertions of muscles discussed in the manuscript for the left scapula, humerus, radius and ulna (modified from Jouffroy, 1962). Colors represent different muscles inserting on the long bones of the forelimb and the scapula. Definition of the muscle origins, insertions, and their suggested actions can be found in Table 7. Muscle names in bold indicate muscle origins, whereas their insertions are indicated in normal font.

the ulna, species using vertical substrates, have a better defined articular facet than species using horizontal substrates. This more definite articular surface on which the carpal bones can slide could provide a greater mobility of the wrist in species that use vertical substrates (Taylor, 1974).

Strepsirrhine species utilizing horizontal supports also more frequently display a larger medial flange of the trochlear notch of the ulna in comparison to those using vertical supports much more. The medial orientation of the medial flange can have an important role in stabilization of the elbow during the flexion of the forelimb (Rose, 1989) during locomotion on horizontal supports. The diaphysis of species that utilize more horizontal supports is curved posteriorly in its mid-part, whereas it is only posteriorly curved in the proximal part and straight for the rest of the diaphysis in species that utilize vertical supports. Previous studies have suggested that the curvature of the long bones of the forelimb can affect the distribution and magnitude of stresses by reducing bending stresses in bones subjected purely to axial loading and by increasing bending stresses in eccentrically end-loaded bones (Schwartz, 1990). It has also been argued that curvature may affect the geometry of supinator muscles insertions (Fig. 9 and Table 12) on the forelimb (Lanyon, 1980; Schwartz, 1990). Thus, the curvature of the diaphysis can be a mechanism to provide more space to accommodate expanded muscle bellies (Lanyon, 1980; Schwartz, 1990). In this sense, the shape and robusticity of the forelimb bones carries two, not mutually exclusive, signals. The robust and highly curved bones of the species that habitually use relatively large horizontal supports may reflect relatively high loading (Schmitt, 2003) on larger substrates. That shape configuration may also reflect larger muscles both in terms of load and muscle attachment area. Regardless, the configuration suggests that animals that habitually use large horizontal supports have limbs that reflect relatively high loading by muscles or substrate reaction forces compared to those that habitually move on small and non-horizontal supports.

4.3. Radius

The radius of strepsirrhine species using horizontal supports is relatively robust in comparison to the relatively more gracile radius of those using vertical supports. Gracile limbs have been interpreted in previous studies (Cartmill, 1985; Argot, 2001; Sargis, 2002; Samuels and Van Valkenburgh, 2008) as giving much more mobility to the limb in arboreal species. In the present context, where two species (*O. garnettii* and *S. alleni*) are arboreal but differ in their support use, we can hypothesize that species using more horizontal supports need to have a greater stability of the forelimbs, whereas species using mainly vertical supports may rely more on their hind limbs for stability. The other major shape change that can be seen between strepsirrhine species using vertical supports and those using horizontal supports is located at the distal end of the radius. Species using horizontal supports display a radial styloid process with a well-developed and a large and concave distal articulation, whereas in vertical climbers the styloid process is not well developed and the distal articulation is small and flat. This feature may be described as allowing greater mobility of the wrist as in species using narrow supports. Thus, species using vertical supports tend to show greater mobility of the hand, as do species using narrow supports, whereas species using wider supports tend to show a morphology conferring a greater stability. Moreover, when looking at only the distal part of the radius, species using horizontal substrates show a broader articulation with the carpal bones in comparison to those mainly using vertical ones. A broader articulation has been previously interpreted as playing an important role in load-bearing at the proximal part of the wrist, providing

greater stabilization of the wrist and restriction of its degree of movement (Saltan and Sargis, 2008). Thus, again, species using wider and horizontal supports tend to show a morphology more related to the stability of the forelimb.

Our radius analyses show a gradient from species that use narrow substrates (*L. tardigradus*, *Microcebus murinus* and *G. demidoff*) to those that use large ones. Furthermore, of the species that use large substrates, another gradient – of species using vertical substrates to horizontal ones – is evident. This result is interesting from a paleontological perspective as it suggests that the radius may be an especially informative bone for inferring the type of support used in extinct taxa. For example, *L. tardigradus* (a specialist user of narrow supports) displays a radius with a straight and gracile diaphysis, whereas the radius is strongly curved and robust in *V. variegata* (a species using wide and horizontal supports). Previous studies have interpreted a strongly curved diaphysis as a way to increase the power of forearm and hand in pronated posture, including increasing the power of pronator teres (Fig. 9) (Whitehead et al., 2005). The distal articulation of the radius of *V. variegata* is deeply concave, with a radial styloid process that is well developed in comparison to that of *L. tardigradus*. The deeply concave distal articulation with a highly developed styloid process has been interpreted by Jenkins and Fleagle (1975) as a feature that provides stability and constrains movement, with the ridge playing a supportive role for the concave surface. In contrast, the distal end of the radius as observed in *L. tardigradus*, which shows an articulation with a reduced carpal that is flat and triangular and a poorly-developed radial styloid process, is close to that observed in humans, and has been suggested to enhance wrist mobility (Cartmill and Milton, 1977). These results highlight the fact that species using narrow supports tend to show greater mobility of the hand whereas species using wider supports tend to show features enhancing stability.

4.4. Ulna/radius

The ulna and radius are likely to be strongly morphologically integrated (Fabre et al., 2014), as they interact functionally by allowing rotation of the radius relative to the ulna, and have two joints in common, as well as being joined via an interosseous membrane (Rose, 1989, 1993; Argot, 2001, 2003; Patel, 2005; Fabre et al., 2014). It is interesting to note that co-variation with locomotor supports is higher in the radius, the bone which is the more mobile of the two and responsible for the motions of pronation and supination and, in concert with the ulna, flexion and extension. Pronation–supination movements play an important role in many animal behaviors, including grasping, hunting, and locomotion (Gonyea, 1978; Rose, 1988, 1993; Iwaniuk et al., 1999; Argot, 2001, 2003; Fabre et al., 2013a, 2014). Arboreal animals, including strepsirrhines, need to maintain balance and stability in an arboreal environment.

The proximal posterior convexity of the radius of *S. alleni* and other vertical climbers is at the opposite side of the insertion of the muscles biceps brachii and brachialis, flexors of the forearm (Fig. 9 and Table 12). This convexity could be explained by the larger limb flexors that need to pull the center of mass of the animal up against gravity in vertical climbers (Taylor, 1971; Argot, 2001). Species that predominantly use horizontal supports show a shorter and more medially developed pronator flange in comparison to those that predominantly use vertical supports that have a relatively taller but less medially developed one. The pronator flange is where the pronator quadratus muscle originates (Fig. 9 and Table 12). This muscle then inserts on the distal posterior end of the radius. This muscle seems to mainly maintain the integrity of the antebrachium near the carpus, because forces applied on this distal part of the

forearm (which is mobile) can cause separation of the radius and the ulna relative to each other (Argot, 2001). Thus, it may help to stabilize the forearm when the animal is moving and seems to be more pronounced in species using mainly horizontal supports.

4.5. Contribution of this study to the narrow branch hypothesis

The evolutionary origin of early primates seems to be tightly linked to a complex arboreal environment. Irrespective of the main hypotheses proposed (e.g., visual predation [Cartmill, 1972, 1974a,b, 1992; Kirk et al., 2003], insect predation [Godinot, 1991, 2007], angiosperm coevolution [Sussman and Raven, 1978; Sussman, 1991], predation and angiosperm coevolution [Rasmussen, 1990]), all suggest the importance of narrow support use. Our results show that the long bones of the forearm coevolve with the type of support, at least in strepsirrhines. Our results also show that parts of the bone (proximal and distal end of the humerus and distal end of the ulna and radius) have probably adapted to the type of support, which is promising for making ecological inferences for extinct species often represented by incomplete remains. This means that aspects of the shape of the long bones, even when incomplete, may be used to infer the type of locomotor support used by a species. Characterizing the shapes of the long bones of the forearm in detail can help to infer the supports used by extinct species and test hypotheses on the role of thin branches in primate origins. Considering the association that exists between the proximal and distal end of the humerus, the entire radii and ulnae of extant primates and the type of supports used, and that the morphology of the radii and ulnae of notharctines share many similarities with *Lepilemur* and *Propithecus*, we could hypothesize that notharctines probably used medium sized supports that were neither completely vertical nor horizontal. This seems to indicate that these early primates were generalists.

Given the suggested importance of the narrow branch milieu in the origins of the defining anatomical features of primates we also specifically investigated the effect of narrow supports on forelimb shape and found that this effect is significant only for the radius. The radial shape associated with small support use is one that is gracile overall. A clear example of this is the radius of *L. tardigradus* with a relatively straight diaphysis, a flat distal radial end with a reduced articulation with the carpals and a triangular and poorly-developed radial styloid process. These features are thought to enhance wrist mobility (Cartmill and Milton, 1977), highlighting the fact that species using narrow supports tend to show a greater mobility of the hand. The radius is also similar to that described for “brachiating” species (Cartmill and Milton, 1977) that employ cautious arboreal locomotion and show a great range of limb motion. Given the observed impact of substrate diameter on radial shape it would be interesting to include stem primates (Plesiada-piformes) and the earliest members of crown primates (Omomyiformes or Adapiformes) in a future study, to better understand their evolution in, and adaptations to, the arboreal environment.

5. Conclusions

In summary, our study shows that the shape of the long bones of the forelimb in strepsirrhines is influenced by phylogeny, which may be of interest for systematic studies. However, even if the morphology of the forelimb of strepsirrhines is constrained by phylogeny, our results show that support use adaptations are marked for the radius, the ulna and their distal articulations, in addition to the proximal and distal articulations of the humerus. Furthermore, the long bones of the forearm, which allow pronation–supination and thus enable the hands to adapt their position depending on the substrates used in an arboreal environment,

show a strong coevolution with substrate properties. The evolution of morphology in association with substrate use is strongest for the radius, which is mainly involved in mobility of the forelimb. Thus, functional constraints associated with the mobility of the lower arm appear to be driving the evolution of these long bones and the types of substrates used. Finally, this study is promising for testing hypothesis pertaining to the use of the thin branch milieu in early primates and shows that the radius might be an especially informative bone in this context. With these robust associations in hand, it will be possible to explore the same variables in extinct early primates and primate relatives to infer substrates most consistently used and address long-standing debates about the ecological context of primate locomotor evolution.

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Supplementary Online Material

Supplementary online material related to this article can be found at <http://dx.doi.org/10.1016/j.jhevol.2017.03.012>.

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