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Do constraints associated with the locomotor habitat drive the evolution of forelimb shape? A case study in musteloid carnivorans

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Abstract

Convergence in morphology can result from evolutionary adaptations in species living in environments with similar selective pressures. Here, we investigate whether the shape of the forelimb long bones has converged in environments imposing similar functional constraints, using musteloid carnivores as a model. The limbs of quadrupeds are subjected to many factors that may influence their shape. They need to support body mass without collapsing or breaking, yet at the same time resist the stresses and strains induced by locomotion. This likely imposes strong constraints on their morphology. Our geometric morphometric analyses show that locomotion, body mass and phylogeny all influence the shape of the forelimb. Furthermore, we find a remarkable convergence between: (i) aquatic and semi-fossorial species, both displaying a robust forelimb, with a shape that improves stability and load transfer in response to the physical resistance imposed by the locomotor environment; and (ii) aquatic and arboreal/semi-arboreal species, with both groups displaying a broad capitulum. This augments the degree of pronation/supination, an important feature for climbing as well as grasping and manipulation ability, behaviors common to aquatic and arboreal species. In summary, our results highlight how musteloids with different locomotor ecologies show differences in the anatomy of their forelimb bones. Yet, functional demands for limb movement through dense media also result in convergence in forelimb long-bone shape between diverse groups, for example, otters and badgers.

Key words: 3D geometric morphometrics; body mass; comparative analysis; convergence; ecomorphology.

Introduction

The limbs of mammals are complex functional and integrated units that are shaped by demands on motion and force, yet are constrained by phylogeny, developmental programming and integration. At minimum, limbs need to support weight without breaking or collapsing, yet at the same time they must resist the stresses and strains induced by locomotion and other behaviors. This is particularly so for the forelimb, which is the principal support of body mass during locomotion in most quadrupedal mammals (Reynolds, 1985; Schmitt & Lemelin, 2002; Hanna et al. 2006; Raichlen et al. 2009). The forelimbs are also used for diverse functions other than locomotion, including hunting, mating and grooming. In contrast, the hind limbs are mainly used for locomotion as they provide most of the power needed for locomotion (Ewer, 1973; Martín-Serra et al. 2014a). Forelimb morphology has been suggested to be a good indicator of ecology because the forelimb is of crucial importance in climbing, swimming and digging, and thus is expected to show a strong functional signal, as suggested by previous authors (Ewer, 1973; Gambarayan, 1974; Gonyea, 1978; Van Valkenburgh, 1985, 1987, 1988; Taylor, 1989; Argot, 2001, 2003a,b, 2004; Andersson, 2003, 2004a, 2005; Schutz & Guralnick, 2007; Samuels & Van Valkenburgh, 2008; Flores & Díaz, 2009; Halenar, 2011; Walmsley et al. 2012; Meloro et al. 2013; Samuels et al. 2013; Ercoli et al. 2014; Martín-Serra et al. 2014b).

In this study, we focus on the long bones of the forelimb (humerus, ulna and radius) and more precisely on their articulation at the elbow, because this articulation allows

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both movements of flexion–extension and pronation–supination (Jenkins, 1973; Ivanco et al. 1996; Iwaniuk & Whishaw, 1999, 2000; Iwaniuk et al. 1999, 2000; Argot, 2001), yet at the same time needs to provide stability and support. As such, the morphology of the elbow joint likely reflects a compromise phenotype depending on the lifestyle specializations of the taxa under study. The goal of this study is to understand the adaptive nature of the morphology of the forelimb long bones in relation to locomotion, while taking into account effects of body mass, and doing so in an explicit phylogenetic framework. Thus, we can investigate convergence in forelimb shape resulting from evolutionary adaptations in species living in environments with similar selective pressures, and test if this results in similarities in phenotypic features such as limb bone shape. We selected the Musteloidea as our model group because of its great species diversity, encompassing over 82 species, more than one-third of all living carnivorans (Wilson & Mittermeier, 2009; Hunter & Barrett, 2011). Musteloids are also ecologically diverse and show a remarkable diversity of locomotor modes (ranging from arboreal species such as the kinkajou and the red panda that climb, to specialized swimmers species such as the sea otter) and diet (from strictly herbivorous species such as the red panda, to strictly carnivorous such as the wolverine; Wilson & Mittermeier, 2009; Hunter & Barrett, 2011). Moreover, musteloids are geographically widespread and occupy an extreme range of habitats. As a consequence of their adaptation to these different kinds of habitats over geological time, they display an exceptional disparity and diversity of form and function. For example, they show variation in size spanning three orders of magnitude (from 45 g for the weasel to 45 kg for the sea otter), with little or no change in limb posture (Fabre et al. 2013a). In addition, their phylogeny is well studied and well resolved (Flynn et al. 2005; Koepfli et al. 2007, 2008; Sato & Wolsan Minami, 2009; Sato et al. 2012; Eizirik et al. 2010; Slater et al. 2012). The musteloidea includes four families: Mephitidae (including skunks and stink badgers; four genera, 12 species); Mustelidae (including badgers, otters, weasels and their relatives; 22 genera, 57 species); Procyonidae (including coatis, raccoons, the kinkajou and their relatives; six genera, 12 species); and Ailuridae (which is represented by a sole living representative, the red panda).

Here, we use surface 3D geometric morphometric methods to investigate the morphology of the long bones and the elbow joint. This allows us: (i) to test if lifestyle influences the shape of the forelimb long bones and articulations of musteloids; and (ii) to investigate the morphological adaptations related to lifestyle. We predict that species with aquatic and semi-fossorial lifestyles will have a forelimb shape that is more robust with a more stable articulation due to the resistance imposed by their locomotor environment (Hildebrand, 1985, 1988; Hildebrand & Goslow, 2001; Moore et al. 2013; Samuels et al. 2013). In contrast, we predict that arboreal and semi-arboreal species will have forelimb long bones that facilitate pronation–supination with the articulations favoring mobility rather than stability (Rose, 1988, 1993; Fabre et al. 2013b; Samuels et al. 2013).

Materials and methods

Materials

The sample is composed of the three long bones of the forelimb of 81 individuals belonging to 20 species of mustelids, one species of ailurid, eight species of procyonids and four species of mephitids. For each species, the number of specimens ranged from one to seven (Table S1). All specimens were adults and predominantly of wild-caught origin. Equal numbers of males and females were included where possible. Specimens were obtained from the following collections: Mammifères et Oiseaux, Muséum National d'Histoire Naturelle, Paris, France; the Naturhistorisches Museum, Basel, Switzerland; the Harvard Museum of Comparative Zoology, Cambridge, Massachusetts, USA; and the Smithsonian National Museum of Natural History, Washington, District of Columbia, USA (see Table S2 for a complete list of the specimens used in the analyses). Bones were digitized using a Breuckmann 3D surface scanner at the Muséum National d'Histoire Naturelle, Paris, France (white-light fringe StereoSCAN^{3D} model with a camera resolution of 1.4 megapixels).

Geometric morphometrics

The shape of the long bones of the forelimb is complex and cannot be adequately represented using a traditional landmark-based approach. Consequently, a 3D sliding-landmark procedure (Bookstein, 1997; Gunz et al. 2005) was used to better quantify the morphology of these long bones, and especially their articulations. Through this procedure, sliding-semi-landmarks on surfaces and curves are transformed into geometrically (i.e. spatially) homologous landmarks that can be used to compare shapes. Semi-landmarks are allowed to slide along the curves and surfaces that are predefined while minimizing the bending energy. Landmarks and curves were obtained using the software package IDAV LANDMARK (Wiley et al. 2005), while EDGEWARP3D 3.31 (Bookstein & Green, 2002) was used to performed the sliding-semi-landmark procedure. To do so, we first created a template representing the entire variation of the musteloid data set following the method of Cornette et al. (2013). In this procedure, each specimen is first defined by homologous landmark coordinates, which consisted of 21 landmarks for the humerus (Fig. S1; Table S3), 19 landmarks for the ulna (Fig. S1; Table S4) and 13 landmarks for the radius (Fig. S1; Table S5). Based on the homologous landmarks, all the sliding-landmarks of the template are warped onto the new specimen while minimizing the bending energy. Next, the warped sliding-semi-landmarks are projected onto the predefined curves and surfaces of the new specimen. The curves consist of the distal surfaces of the articulation of the humerus, and the proximal and distal articulation surface of the radius and ulna (Fig. S1). Finally, spline relaxation must be performed. Both sliding and relaxation are repeated iteratively until the bending energy is minimized. At the end of this procedure, 306 landmarks (21 anatomical landmarks and 285 sliding-landmarks) for the humerus, 165 landmarks (13 anatomical landmarks and 152 sliding-landmarks) for the radius, and 330 landmarks (19 anatomical landmarks and 311 sliding-landmarks) for the ulna are used to describe the shape of each bone and its articulation (Fabre et al. 2013a,b, 2014). After this operation has been performed for each data set, the landmarks of all specimens can be compared using traditional morphometric methods.

Once all landmark data were obtained, a generalized Procustes superimposition (Rohlf & Slice, 1990) was performed using the package RMORPH (Baylac, 2012) in R (R Core Team, 2014). Finally, a principal component analysis (PCA) was performed on the shape data to evaluate the distribution of the species in morphospace. The factor scores of those factors describing 70% of the overall shape variation were subsequently used as input for our comparative analyses.

Phylogeny

The phylogenetic tree used in our analyses is based on the family-level phylogeny of Carnivora from Eizirik et al. (2010) as a backbone upon which time-calibrated molecular phylogenies for each family are appended (Slater et al. 2012). Full details of the phylogenetic reconstruction and the tree are provided in the supporting information of Slater et al. (2012). For our analyses, we pruned the tree so that only species represented in our data set remained (Fig. 1). This tree was used in all comparative analyses, and branch lengths are proportional to geological time.

Phylogenetic signal

To estimate the phylogenetic signal in forelimb long-bone shape, 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 Procrustes coordinate for each bone of the forelimb using the 'GEOMORPH' library (Adams & Otarola-Castillo, 2013) in R (R Core Team, 2014). Next, a univariate *K*-statistic was calculated for the first four PCs of the humerus, the first three PCs of the ulna, and the first two PCs of the radius using the 'PICANTE' library in R (Kembel et al. 2010). The higher the *K*-value is, the stronger the phylogenetic signal. A *K*-value of 1 corresponds to character evolution under Brownian motion and indicates some degree of phylogenetic signal. A *K*-value > 1 indicates a strong phylogenetic signal, which means that traits are conserved within the phylogeny. Conversely, a *K*-value close to 0 means that phylogenetic signal is weak, indicating strong morphological convergence. We finally also mapped the phylogeny onto the morphospace using the polymorphospace function in R (R Core Team, 2014) implemented in the 'PHYTOOLS' library (Revell, 2012; Figs 2–4; S2–S4).

ANCOVAs and phylogenetic ANCOVAs

Because species share their evolutionary history, they cannot be considered as independent data (Felsenstein, 1985). Consequently, phylogenetic comparative analyses need to be performed to test differences between groups. These analyses take into account shared ancestry in explaining patterns of shape diversity. Thus, a phylogenetic analysis of covariance (Garland et al. 1993) with body mass as covariate was used to test whether lifestyle influences the shape of the forelimb long bones. Prior to analyses, body mass data were retrieved from the literature (Fabre et al. 2013a; Table S1) and $\log_{10}$-transformed. We defined five categories of lifestyles following Nowak (2005), Wilson & Mittermeier (2009), Hunter & Barrett (2011), Samuels et al. (2013) and Fabre

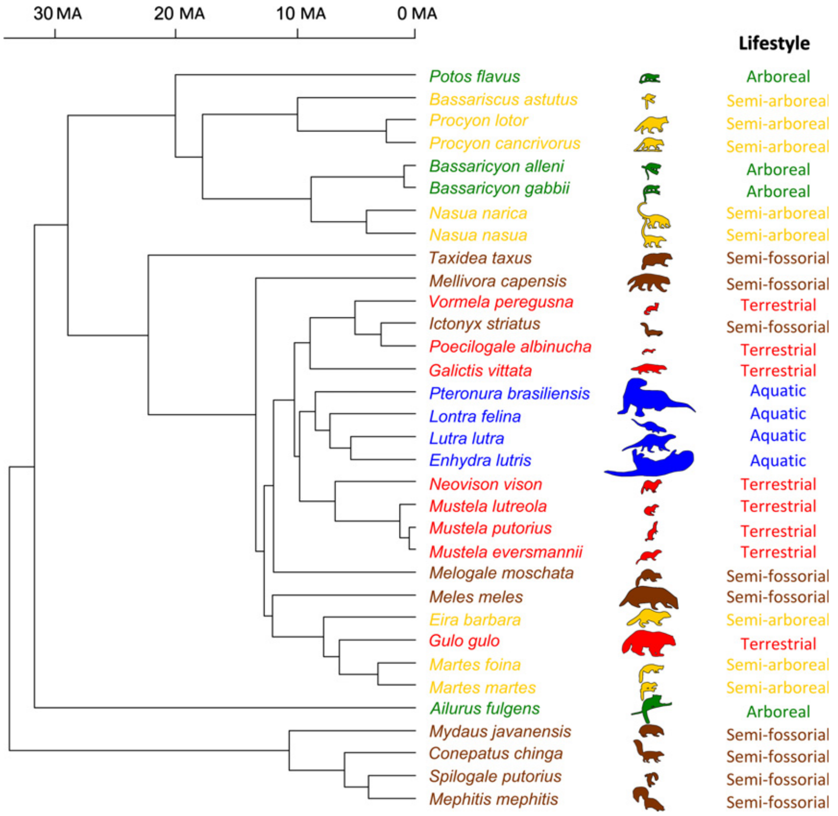

Fig. 1 The phylogenetic relationships of the musteloid species used in this study, derived from Slater et al. (2012). The time scale is in millions of years. Outlines used as symbols scaled relatively to body size and colors indicate as follow: green for arboreal; yellow for semi-arboreal; red for terrestrial; brown for semi-fossorial; and blue for aquatic species.

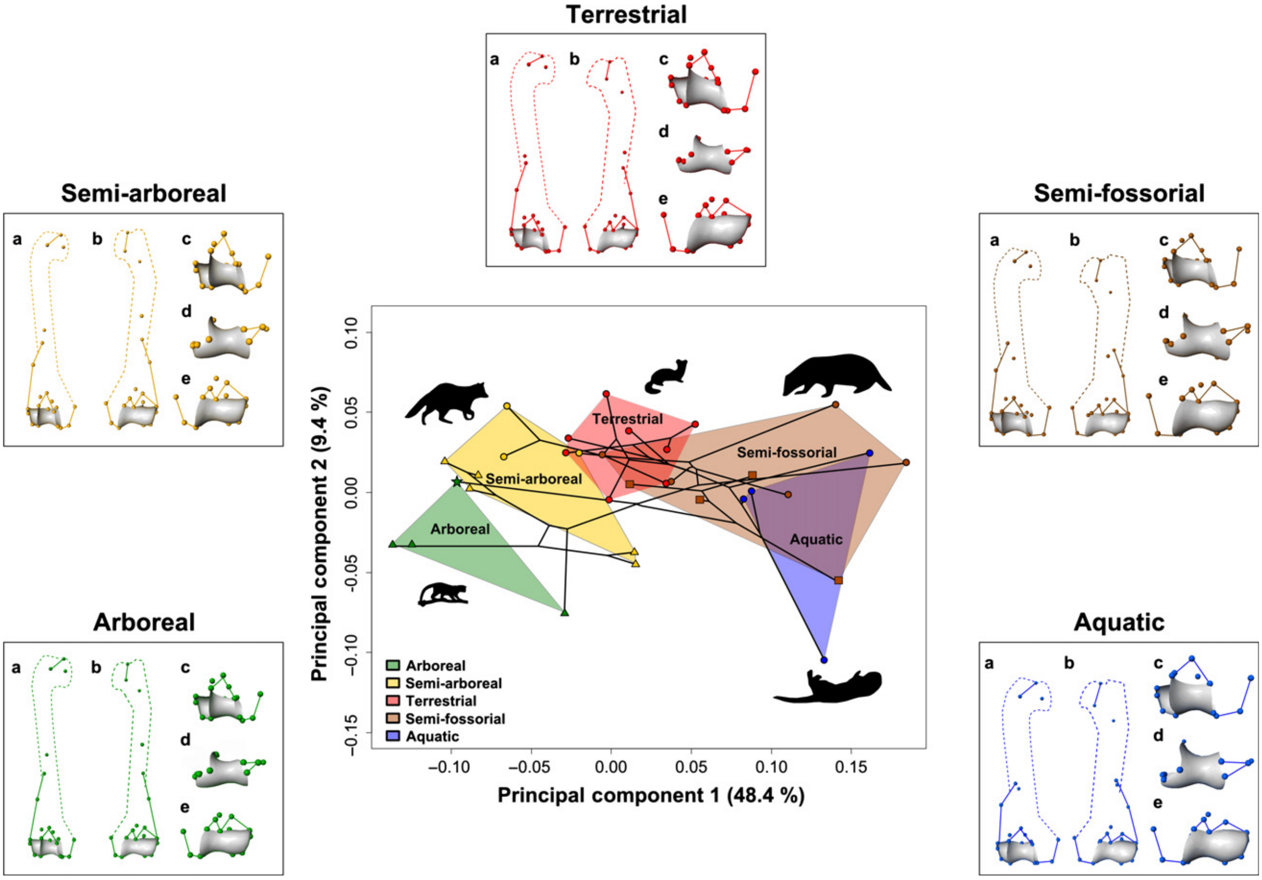

Fig. 2 Results of the PCAs performed on the morphometric data of the humerus and their associated mean shape for each lifestyle. The phylogeny (Slater et al. 2012) is plotted in the shape space. Symbols are as follows: green polygon indicates arboreal species; yellow polygon indicates semi-arboreal species; red polygon indicates terrestrial species; brown polygon indicates semi-fossorial species; blue polygon indicates aquatic species. The family of each species is represented by a circle for mustelids, a triangle for procyonids, a square for mephitids and a star for ailurids. Mean shape of humerus of arboreal species (green), semi-arboreal species (orange), terrestrial species (red), aquatic species (blue) and semi-fossorial species (brown): a, posterior view; b, anterior view; c, close up of the distal articulation in posterior view; d, close up of the distal articulation in distal view; e, close up of the distal articulation in anterior view. Dots and gray surfaces represent landmarks; lines represent real links between landmarks; dashed-lines represent a schematic representation of the bone.

et al. (2013a; Tables 1 and S1): arboreal; semi-arboreal; terrestrial; aquatic; and semi-fossorial. Note, however, that while animals were classified as belonging to a single lifestyle, they may occasionally also show other locomotor behaviors. For example, most musteloids will swim when needed, and many will dig in the leaf-litter and top soil to find food.

A traditional ANCOVA was performed on the first four PCs of the humerus, the first three PCs of the ulna and the first two PCs of the radius. Next, phylogenetic ANCOVAS (Garland et al. 1993) testing for differences in the shape of the forelimb long bones between species with different lifestyles while taking into account body mass and phylogeny were performed. Simulations were performed using the PDAP package. Brownian motion was used as the model for evolutionary change, and 1000 simulations were ran using the PDSIMUL and PDANOVA routines in the PDAP package (Garland et al. 1993) to create an empirical null distribution against which the *F*-value from the original data could be compared. Differences among categories were considered significant if the original *P*-value was higher than the *P*95-value derived from the empirical, simulated distribution.

Results

Geometric morphometrics

PCA

The first four PCs of the humerus account for 69.9% of the variance. The overall distribution defined by the scatter plot of the first two axes (Fig. 2) tends to separate arboreal and semi-arboreal species from aquatic and semi-fossorial ones, whereas terrestrial species tend to fall in the middle of the morphospace. While there is an overlap between the aquatic and semi-fossorial species, arboreal species tend to be separated from the semi-arboreal species. The distribution of the species on the first and third axes (Fig. S2) is similar to the previous one, except that this time, semi-fossorial and aquatic species tend to be differentiated whereas arboreal and semi-arboreal overlap. The distribution of the species on the second and third axes as well as second and

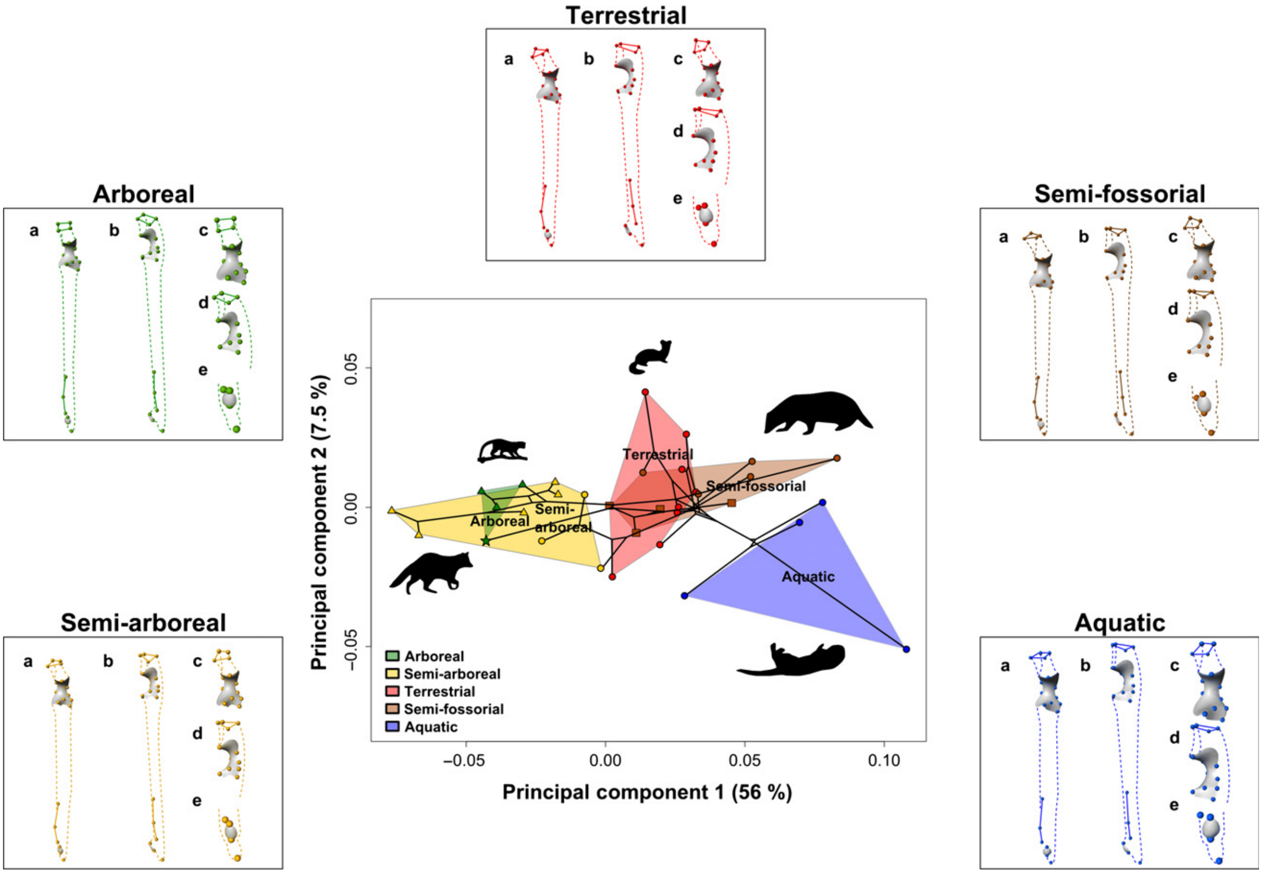

Fig. 3 Results of the PCAs performed on the morphometric data of the ulna and their associated mean shape for each lifestyle. The phylogeny phylogeny (Slater et al. 2012) is plotted in the shape space. Symbols are as follows: green polygon indicates arboreal species; yellow polygon indicates semi-arboreal species; red polygon indicates terrestrial species; brown polygon indicates semi-fossorial species; blue polygon indicates aquatic species. The family of each species is represented by a dot for mustelids, a triangle for procyonids, a square for mephitids and a star for ailurids. Mean shape of ulna of arboreal species (green), semi-arboreal species (orange), terrestrial species (red), aquatic species (blue) and semi-fossorial species (brown): a, anterior view; b, lateral view; c, close up of the proximal articulation in anterior view; d, close up of the proximal articulation in lateral view; e, close up of the distal articulation in anterior view. Dots and gray surfaces represent landmarks; lines represent real links between landmarks; dashed-lines represent a schematic representation of the bone.

fourth axes, and also third and fourth axes does not display any particular pattern (Fig. S2).

The first three axes of the ulna account for 70% of the overall shape variation. The morphospace, as defined by the scatter plot of the first and second axes (Fig. 3), also tends to distinguish the semi-arboreal and arboreal species falling in one part of the morphospace from the semi-fossorial and aquatic species falling in the other part of morphospace. In contrast to the PCA of the humerus, there is a clear separation of the aquatic and semi-fossorial species. In the middle of the morphospace, there is an overlap between the terrestrial and semi-fossorial species. The distribution of the species as defined by the first and third axes (Fig. S3) is quite similar to the previous one, except that semi-fossorial species have the largest distribution and tend to overlap with the terrestrial and aquatic species. The scatter plot of the second and third axes tends to separate some aquatic species from all other species (Fig. S3).

The first two PCs of the radius accounted for 73.5% of the total shape variation. The overall distribution of taxa on the first and second axes (Fig. 4) is quite similar to that for the humerus and ulna, with overlapping arboreal and semi-arboreal species that tend to be separated from the semi-fossorial and aquatic species, which fall in the opposite part of the morphospace. Once again, there is an overlap between terrestrial and semi-fossorial species.

Mean conformations associated to each lifestyle

The long bones associated with terrestrial, semi-fossorial and aquatic species are robust in comparison to those of arboreal and semi-arboreal species (Figs 2–4 and S5–S7). The distal articulation of the humerus (Figs 2 and S5) in the terrestrial, semi-fossorial and aquatic species is relatively broad in comparison to that of the arboreal and semi-arboreal species. Furthermore, the terrestrial, semi-fossorial and aquatic species show a relatively broad trochlea. The capitulum of the arboreal, semi-arboreal and aquatic species is rel-

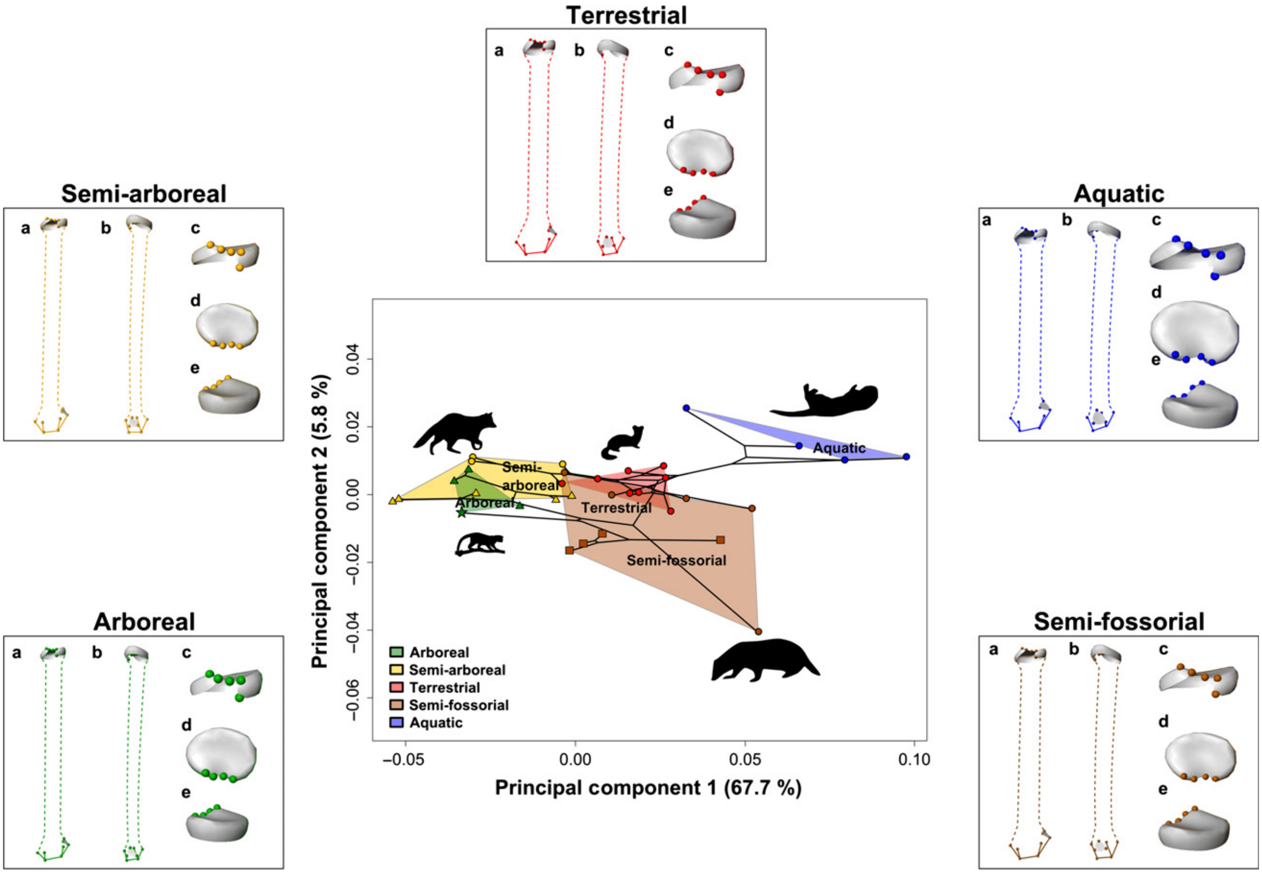

Fig. 4 Results of the PCAs performed on the morphometric data of the radius and their associated mean shape for each lifestyle. The phylogeny phylogeny (Slater et al. 2012) is plotted in the shape space. Symbols are as follows: green polygon indicates arboreal species; yellow polygon indicates semi-arboreal species; red polygon indicates terrestrial species; brown polygon indicates semi-fossorial species; blue polygon indicates aquatic species. The family of each species is represented by a dot for mustelids, a triangle for procyonids, a square for mephitids and a star for ailurids. Mean shape of radius of arboreal species (green), semi-arboreal species (orange), terrestrial species (red), aquatic species (blue) and semi-fossorial species (brown): a, anterior view; b, lateral view; c, close up of the proximal articulation in anterior view; d, proximal view; e, close up of the proximal articulation in posterior view. Dots and gray surfaces represent landmarks; lines represent real links between landmarks; dashed-lines represent a schematic representation of the bone.

Table 1 Definitions of lifestyle categories used in this study.

Lifestyle	Definition
Terrestrial	Species that spend the most part of their time on the ground, but occasionally climb, swim or dig
Semi-arboreal	Species that spend both time in trees and on the ground without a clear preference for either
Arboreal	Species that spend the majority of their time in trees
Aquatic	Species that spend the most of their time in water to forage, escape, disperse
Semi-fossorial	Species that spend the majority of time on the ground, but regularly dig burrows or dig to find food

atively large in comparison to that of the terrestrial and semi-fossorial species. The medial epicondyle of the aquatic and semi-fossorial species is relatively well developed. The lateral epicondylar crest of the semi-fossorial and aquatic species is also relatively well developed. The olecranon fossa of the arboreal, semi-arboreal and terrestrial species is relatively higher and asymmetric in comparison to that of the semi-fossorial and aquatic species. The radial fossa of the semi-fossorial, terrestrial, semi-arboreal and arboreal species is relatively higher than that in the aquatic taxa, where it is relatively low. Finally, the most distal part of the deltopectoral crest is relatively low, and extends further in semi-fossorial and aquatic species.

The olecranon process of the ulna (Figs 3 and S6) of the arboreal, semi-arboreal and terrestrial species is relatively short in comparison to those of the aquatic and semi-fossorial species. The radial notch of the semi-arboreal, terrestrial and semi-fossorial species displays a relatively small surface area in comparison to that of the arboreal and aquatic species, which show a broad articulation with the radial head. Furthermore, the radial notch of arboreal and semi-arboreal species is laterally oriented. The trochlear notch of the arboreal, semi-arboreal and terrestrial species is small and narrow in comparison to that of the semi-fossorial and aquatic species. The distal radial notch is antero-distally oriented for the arboreal and semi-arboreal species in comparison to the other kinds of lifestyles, where it is more anteriorly oriented. The olecranon process is particularly straight among arboreal taxa, while it is medially deflected in musteloids with other lifestyles, especially in semi-fossorial species.

The radial head (Figs 4 and S7) of the arboreal and semi-arboreal species is round in comparison to that of the terrestrial, semi-fossorial and aquatic species where it is oval. The antero-medial part of the proximal surface contacting the radial notch of the ulna of the aquatic and semi-fossorial species is relatively thin in comparison to other groups. The distal ulnar notch of the arboreal species is relatively larger than that of species with other lifestyles. The distal epiphysis of the semi-fossorial, terrestrial and aquatic species is relatively large with a prominent medial styloid process, whereas those of the arboreal and semi-arboreal display the opposite morphology.

Phylogenetic signal

The results of the multivariate *K*-statistic calculated on the shape data are significant for each long bone of the forelimb (humerus: $K_{\text{mult}} = 0.35$, $P = 0.01$; ulna: $K_{\text{mult}} = 0.48$, $P = 0.01$; radius: $K_{\text{mult}} = 0.5$, $P = 0.001$). The *K*-statistics and their associated significance levels calculated separately for each forelimb long bone are presented in Table 2, and are very similar to those reported in a previous study (Fabre et al. 2013a). These results show significant phylogenetic signal in the shape of the long bones and highlight the importance of performing phylogenetic corrections to the data.

Table 2 Results of *K*-statistics and their associated *P*-value.

	K	*P*-value
Humerus		
PC1	0.65	**0.0009**
PC2	0.47	**0.003**
PC3	0.24	0.25
PC4	0.27	0.089
Ulna		
PC1	**1.12**	**0.0009**
PC2	0.24	0.225
PC3	0.81	**0.0009**
Radius		
PC1	0.87	**0.0009**
PC2	0.93	**0.0009**

K-statistics and their associated *P*-value calculated for the first four principal shape components of the humerus, the first three principal shape components of the ulna, and the first two principal shape components of the radius.PCs showing significant phylogenetic signal ($\alpha < 0.05$) are indicated in bold.

ANCOVAs and phylogenetic ANCOVAs

The results of the ANCOVAs and phylogenetic ANCOVAs (Table 3) on the aspects of forelimb shape described by the first PC of the humerus are highly significant (humerus PC1: ANCOVA, $F_{4,27} = 19.42$, $P < 0.001$; phylogenetic ANCOVA, $F_{4,27} = 8.7$, $P = 0.002$). However, results are not significant for the second, third and fourth PCs.

The results of the ANCOVAs and phylogenetic ANCOVAs performed on the first three PCs of the ulna (Table 3) are highly significant for the first shape axis (ulna PC1: ANCOVA, $F_{4,27} = 18.21$, $P < 0.001$; phylogenetic ANCOVA, $F_{4,27} = 7.24$, $P = 0.002$), revealing that there is a significant difference in ulnar shape between species with different lifestyles. The results are, however, not significant for the other PCs describing the ulnar shape.

Table 3 Results of the ANCOVAs (*F* and *P*-value) and phylogenetic ANCOVAs (Phyl *F* and Phyl *P*-value).

	ANCOVA		Phylogenetic ANCOVA	
	$F_{4,27}$	*P*-value	Phyl $F_{4,27}$	Phyl *P*-value
Humerus				
PC1	**19.42**	**< 0.001**	**7.67**	**< 0.001**
PC2	2.07	0.11	7.45	0.58
PC3	1.19	0.34	7.44	0.75
PC4	1.32	0.29	7.17	0.74
Ulna				
PC1	**18.21**	**< 0.001**	**7.24**	**< 0.001**
PC2	0.95	0.45	7.9	0.82
PC3	1.79	0.16	7.38	0.59
Radius				
PC1	**17.39**	**< 0.001**	**7.75**	**< 0.001**
PC2	**6.67**	**< 0.001**	7.73	0.078

Both analyses calculated for the first four PCs of the humerus, the first three components of the ulna, and the first two components of the radius.PC showing significant differences between lifestyles ($\alpha < 0.05$) are indicated in bold.

The ANCOVAs and phylogenetic ANCOVAs performed on the first two PCs of the radius shape (Table 3) are significant for the first PC (radius PC1: ANCOVA, $F_{4,27} = 17.39$, $P < 0.001$; phylogenetic ANCOVA, $F_{4,27} = 7.75$, $P = 0.001$). The result of the ANCOVA performed on the second PC of the radius is also significant (radius PC2: ANCOVA, $F_{4,27} = 6.67$, $P = 0.001$), whereas it is not significant when phylogeny is taken into account.

Discussion

Musteloid carnivorans are an incredibly diverse group of mammals (Ewer, 1973; Nowak, 2005; Sato & Wolsan Minami, 2009; Wilson & Mittermeier, 2009; Eizirik et al. 2010; Hunter & Barrett, 2011; Sato et al. 2012). They display wide variation in body mass, yet do not change limb posture (Heinrich & Biknevicius, 1998; Fabre et al. 2013a). Moreover, these animals occupy an array of habitats more diverse than any other clade of carnivorans, making them especially suited for ecomorphological analyses. The results of our analyses on forelimb long-bone shape show that there are significant differences between species with different lifestyles, even when taking into account the effects of body mass and phylogeny. These results suggest that forelimb shape is adaptive and evolves, at least partly, in response to the constraints imposed by the different lifestyles. In a previous study, the influence of body mass on the forelimb shape in musteloids was explored (Fabre et al. 2013a) and showed that body mass evolution affects the evolution of forelimb shape, although not equally for all elements. Indeed, Fabre et al. (2013a) showed that whereas 42% of the first PCs describing humerus shape are explained by variation in body mass, only 7.6% of the variation in radial shape and 5.3% of the variation in ulnar shape was explained by variation in body mass. Moreover, the current analyses confirm previously published data (Fabre et al. 2013a) and suggest that there is a significant phylogenetic signal in the shape data. This is not too surprising as, for example, all the aquatic species included in the study belong to a single clade (Lutrinae). Thus, shared ancestry, body mass and locomotor habitat are all important factors in explaining variation in limb shape across species.

The PCAs performed on the shape of the humerus show a gradient from arboreal and semi-arboreal species over terrestrial species to aquatic and semi-fossorial species (Figs 2 and S2). Arboreal and semi-arboreal species tend to be separated, whereas semi-fossorial and aquatic species show distinct overlap. The PCAs performed for the ulna (Figs 3 and S3) and radius (Figs 4 and S4) show similar results and display a gradient with, on one side of the morphospace the arboreal species, which are subsumed by the morphospace of the semi-arboreal ones, and at the other side the morphospace of the aquatic species, which tend to be separated from the overlapping terrestrial and semi-fossorial species. The current results, moreover, show that each bone has its own ecological/functional signal. For example, the signal provided by the shape of the humerus tends to separate arboreal and semi-arboreal species (Figs 2 and S2), whereas the analyses on the shape of the ulna and radius (Figs 3, 4, S3 and S4) tend to separate the aquatic from the semi-fossorial species.

In this study, the mean shape for arboreal species is characterized by a long and gracile humerus, with a gracile articulation with a broad capitulum (Figs 2 and S5). These results are consistent with previous studies that show that there is an increase in the elongation of the humerus in arboreal species, as this may facilitate tree climbing (Cartmill, 1985; Heinrich & Biknevicius, 1998; Argot, 2001; Sargis, 2002; Samuels & Van Valkenburgh, 2008; Samuels et al. 2013). The broad capitulum is related to an increase in joint mobility, thus increasing the range of motion of the forearm, which is important for climbing (MacLeod & Rose, 1993; Argot, 2001; Szalay & Sargis, 2001; Candela & Picasso, 2008; Flores & Díaz, 2009; Ercoli et al. 2012; Fabre et al. 2013b; Samuels et al. 2013). The mean shape of semi-arboreal species is similar to that of arboreal ones with some intermediate features (Figs 2 and S5), consistent with previous results (Samuels et al. 2013). Nevertheless, the semi-arboreal species tend to show a lateral epicondylar crest, which is more strongly developed than that of the arboreal species. This feature is difficult to interpret functionally and may be related to differences in muscular development in the different groups (Schutz & Guralnick, 2007; Ercoli et al. 2014). Unfortunately, no quantitative data exist that would allow us to test this hypothesis. As some semi-arboreal species such as *Nasua* are known to occasionally dig (Braddy, 2003), this may potentially explain the greater development of the lateral epicondylar crest.

The mean shapes of the ulna and radius of arboreal species are also rather gracile (Figs 3, 4, S6 and S7). The ulna also possesses a broad articulation surface with the radial head. Furthermore, the radial head of the radius is round and, together with the broad capitulum of the humerus, this facilitates the rotation of the radius on the ulna and the capitulum of the humerus, thus increasing the range of motion of the forearm, which has been suggested to be important when moving in an arboreal environment (MacLeod & Rose, 1993; Argot, 2001; Szalay & Sargis, 2001; Flores & Díaz, 2009; Fabre et al. 2013b). The trochlear notch on the ulna is small and narrow, thus conferring stability at the elbow joint. This is important to avoid elbow dislocation during arboreal locomotion. Furthermore, the olecranon process of the ulna is short, which allows a full extension of the elbow (Samuels & Van Valkenburgh, 2008). The radial notch is also oriented laterally in arboreal species such as the kinkajou (*Potos flavus*) and the red panda (*Ailurus fulgens*). This has been interpreted in previous studies as increasing the degree of pronation and supination of the forelimb, thus allowing a wider range of rotation at the elbow (Hildebrand, 1988; Andersson, 2003, 2004b; Peigné et al. 2008; Ercoli et al. 2012).

These results are consistent with previous studies that showed that arboreal species have elongate and gracile limbs with an articulation allowing a greater mobility of the limb (Cartmill, 1985; Argot, 2001; Sargis, 2002; Samuels & Van Valkenburgh, 2008). The semi-arboreal species display a morphology that is close to that of arboreal species allowing mobility of the forelimb. They also show gracile long bones, an ulna with a short olecranon process and a radial head that is round.

The mean shape of the humerus in semi-fossorial and aquatic species is similar (Figs 2 and S5). Both groups display a short and robust humerus, with a well-developed shaft, a large distal articulation, a large lateral epicondylar crest, a deltopectoral crest that extends distally and a broad medial entepicondyle (Fig. 5; Table 4). These results show that for both lifestyles, adaptations that involve a morphology allowing the generation of large forces in order to move in an environment imposing high resistance to motion are needed. The short and the robust humerus allows for a reduction of the out-lever and an increase in the in-lever of the humeral retractors. Moreover, robust bones with a broad surface area for the attachment of the powerful musculature that controls the movements of the forearm during scratch-digging in semi-fossorial (Hildebrand, 1985, 1988; Hildebrand & Goslow, 2001; Samuels & Van Valkenburgh, 2008; Elissamburu & De Santis, 2011; Moore et al. 2013; Samuels et al. 2013; Ercoli et al. 2014; Rose et al. 2014), or paddling and changing of direction in water for aquatic species (Tarasoff et al. 1972; Fish, 1994; Samuels & Van Valkenburgh, 2008; Samuels et al. 2013) are observed. The extended deltopectoral crest of the humerus of the American badger (*Taxidea taxus*) and the sea otter (*Enhydra lutris*) results in a distal insertion of the pectoralis profundus muscles on the shaft (Fig. 5; Table 4), allowing both stabilization of the shoulder and a powerful flexion, retraction and rotation of the arm, which is also an important feature during digging and/or swimming behavior (Savage, 1957; Gambaryan & Karapetjan, 1961; Tarasoff et al. 1972; English, 1976; Fish, 1994; Hildebrand & Goslow, 2001; Samuels & Van Valkenburgh, 2008; Elissamburu & De Santis, 2011; Moore et al. 2013; Samuels et al. 2013; Ercoli et al. 2014; Rose et al. 2014). Furthermore, the medial epicondyle is also well developed in aquatic and semi-fossorial species (Figs 2 and S5). The medial epicondyle is the origin of the wrist and digital flexors (the flexor carpi radialis and ulnaris, the flexor digitorum longus, and the flexor palmaris longus; Fig. 5; Table 4), and should thus play an important role during digging behavior. Yet, explicit functional tests of the

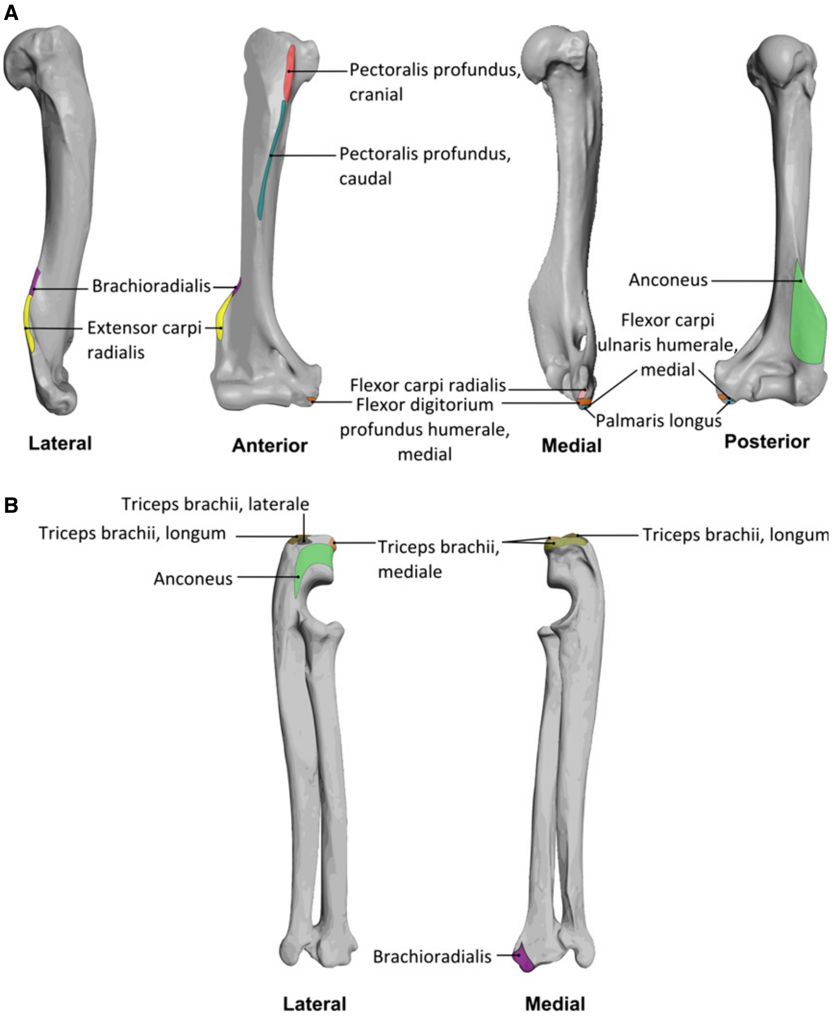

Fig. 5 Schematic figure illustrating the origins and insertions of muscles discussed in the manuscript. (A) Humerus. (B) Radius and ulna (modified from Leach, 1977; Ercoli et al. 2014). Definition of the muscle origins, insertions and their suggested action can be found in Table 4.

Table 4 General origin and insertion of the muscles depicted in Fig. 5 and their hypothesized action (modified after Leach, 1977; Moore et al. 2013; Ercoli et al. 2014).

Muscle	Origin	Insertion	Hypothesized action
Triceps brachii, longum	Axillary border of the scapula	Posteromedial aspect of olecranon of ulna	Elbow extension, shoulder flexion, forelimb retraction
Triceps brachii, mediale	Mid-axillary border of scapula	Proximal aspect of olecranon of ulna	Elbow extension, shoulder flexion, forelimb retraction
Triceps brachii, laterale	Deltoid ridge, greater tubercle of humerus	Lateral posterodorsal aspect of olecranon of ulna	Elbow extension
Anconeus	Lateral epicondyle, supracondyloid ridge of humerus	Dorsolateral aspect of olecranon of ulna, distal margin of trochlear notch	Elbow extension
Brachioradialis	Dorsal aspect of lateral epicondyle of humerus	Styloid process of radius	Elbow flexion
Extensor carpi radialis	Lateral epicondyle of humerus	Distal ends of metacarpals	Wrist and digits extension
Pectoralis profundus, cranial	Cranial area of the sternebra (third; sometimes extends until fifth)	Craniolateral side of the cranial portion of the deltopectoral crest of the humerus	Stabilization of the shoulder; flexion, retraction and rotation of the forelimb
Pectoralis profundus, caudal	Caudal half of the third sternebra; from the distal region of the costal cartilage of the 10th and 11th ridge	Craniolateral side of the middle portion of the deltopectoral crest of the humerus	Stabilization of the shoulder; flexion, retraction and rotation of the forelimb
Flexor carpi radialis	Medial epicondyle of the humerus	Carpal and metacarpal bones	Carpal flexion
Flexor carpi ulnaris humerale, mediale	Medial epicondyle of the humerus	Pisiform bone	Carpal flexion
Flexor digitorium profundus humerale, medial	Medial epicondyle of the humerus	Distal phalanges of digits	Digital flexion
Palmaris longus	Medial epicondyle of the humerus	Palmar fascia connected indirectly with radial sesamoid and accessory carpal bone	Assist in carpal flexion

role of these muscles in the different behaviors are currently lacking. The large lateral epicondylar crest likely serves as a greater muscular attachments area for the flexors, extensors and supinators of the elbow in addition to the digital extensors (e.g. m. extensor carpi radialis; the m. brachioradialis; Fig. 5; Table 4; Evans, 1993; Argot, 2001; Szalay & Sargis, 2001; Ercoli et al. 2012). Aquatic species such as otters (*Pteronura brasiliensis*, *Lutra lutra*, *Enhydra lutris* and *Lontra felina*) tend to display a shorter lateral epicondylar crest, which is maybe related to different muscular attachment. A shorter and strong lateral epicondylar crest was already observed in aquatic species (Lutrinae) by Ercoli et al. (2012), and was suggested to improve antebrachial supination abilities and allow a higher extension of the forelimb during swimming (Schutz & Guralnick, 2007; Ercoli et al. 2012). This feature has also been suggested to be related to manipulation during climbing (Lemelin, 1999; Argot, 2004; Ercoli et al. 2012). Furthermore, differences can also be shown in the shape of the distal articulation that is somewhat different between aquatic and semi-fossorial species. Indeed, aquatic species display a broad capitulum in comparison to semi-fossorial ones. These observations (broad capitulum and short and strong lateral epicondylar crest) can potentially be explained by the fact that the aquatic species in the current sample are otters. Otters are known to be able to perform complex grasping behaviors that involve pronation–supination movements for which a broad capitulum may be advantageous. Furthermore, we showed in a previous study (Fabre et al. 2013b) that species with grasping behaviors display a broad capitulum allowing an increase of rotation and mobility of the forearm, which is important during food manipulation. Interestingly, this feature is also present in species able to climb (arboreal and semi-arboreal species).

Semi-fossorial species display a radius and an ulna that are robust (Figs 3, 4, S6 and S7). The olecranon process of the ulna is long and well developed, thus providing a mechanical advantage of the forelimb musculature by giving a high out-force during scratch digging (Hildebrand, 1982; Hildebrand & Goslow, 2001; Schutz & Guralnick, 2007; Samuels & Van Valkenburgh, 2008; Hopkins & Davis, 2009; Moore et al. 2013; Samuels et al. 2013; Rose et al. 2014). The olecranon process is the area of insertion of the main elbow extensor, the triceps brachii (Fig. 5; Table 4). In previous studies, elongated olecranon processes in semi-fossorial species have been suggested to provide a considerably greater and advantageous moment arm at the elbow joint (Hildebrand, 1982; Iwaniuk et al. 1999; Hildebrand & Goslow, 2001; Ercoli et al. 2012; Moore et al. 2013). The medial orientation of the olecranon process in the current sample including the American badger (*Taxidea taxus*) and the Sunda stink badger (*Mydaus javanensis*) can potentially be explained by the ability of the triceps brachii to apply high extensor torques at the elbow joint and a high outforce to the soil (Moore et al. 2013; Fig. 5; Table 4). The radial head is oval-shaped, which likely restricts the overall mobility of the forelimb. All of these features are consistent with the results observed in previous studies on semi-fossorial mammals (Hildebrand, 1985, 1988; Samuels & Van Valkenburgh, 2008; Elissamburu & De Santis, 2011; Ercoli et al. 2014; Rose et al. 2014). As previously noted, mean shapes of terrestrial species for the ulna and radius are intermediate between those of semi-arboreal species and semi-fossorial species. The intermediate shape of terrestrial species is not surprising, and highlights at least partly the artificial nature of the lifestyle groups used here as well as more generalized lifestyle. The recurrent overlap of the terrestrial species with other kinds of lifestyle in the morphospace (humerus, ulna and radius) and the intermediate shape show the difficulty to define discrete groups when describing an essentially continuous feature such as lifestyle or locomotor behavior (Carrano, 1999; Ercoli et al. 2012).

Aquatic species also show a robust ulna and radius (Figs 3, 4, S5 and S6). Their ulnae display a long and robust olecranon process, which provides a broad area of insertion for muscles such as the triceps brachii muscles (Fig. 5; Table 4), allowing for powerful and precise extension of the elbow (Fisher, 1942; Ercoli et al. 2014).). Similarly, this feature is well developed in the ulna of semi-fossorial species. This feature may also be related to the anconeus muscle (Fig. 5; Table 4), which is principally a forelimb extensor (Tarasoff et al. 1972; Fisher et al. 2009; Ercoli et al. 2014) and rotator, and which is also important when the otter turns in the water or manipulates its food, especially because otters mainly use their forelimb during swimming (Macalister, 1873; Tarasoff et al. 1972; Holmes, 1980; Peigné et al. 2008; Ercoli et al. 2014). Furthermore, the locomotor medium of aquatic species more strongly constrains movement than that of semi-fossorial species. Indeed, whereas aquatic species need to both extend and flex their arms against the resistance of the medium, semi-fossorial species are only faced with this issue during limb flexion. The current results are consistent with previous studies of aquatic mammals and confirm that, when compared with other kinds of lifestyle, their bones are more robust with more strongly developed muscular attachments (Samuels et al. 2013). Nevertheless, it is important to note that not all otters use their forelimbs for swimming, and that the morphology of their forelimb can also be constrained by the fact that they are all closely related (Fish, 1994).

In summary, the long bones of the forelimb of arboreal and semi-arboreal species display a morphology that tends to increases mobility, thus providing a greater range of movement, which is important for species moving in complex, three-dimensional environments like trees. Indeed, moving in branches or a trunk likely implies constraints that are very different from those of animals moving through homogeneous, predictable media. Indeed, the arboreal medium is discontinuous, unstable, and is made of substrates of different widths and orientations (Cartmill, 1985). In contrast, terrestrial and semi-fossorial species display a pattern of form that tends to restrict movement and rather allows stabilization of the elbow joint. This morphology is also efficient in load bearing and may help dissipate the load of the anterior part of the animal transferred to the front limbs during locomotion. Finally, aquatic species seem to display a forelimb morphology that both stabilizes the elbow joint yet allows powerful rotation of the forelimb (Savage, 1957; Gambaryan & Karapetjan, 1961; English, 1976; Williams, 1983; Fish, 1994; Ercoli et al. 2014). This difference of morphology between aquatic and semi-fossorial species can potentially be explained by the fact that during aquatic locomotion (Tarasoff et al. 1972; Fish, 1994), movements operate in different planes compared with the scratch-digging in semi-fossorial species, where the forelimb operates mainly in the sagittal plane (Moore et al. 2013). However, these results also highlight a convergence of the shape of the long bones of the forelimb due to functional demands imposed by the environment. Aquatic and semi-fossorial species are subject to a similar mechanical locomotor environment with a high resistance, resulting in similar selective pressures and similar phenotypes.

Concluding remarks

To conclude, the current results show that musteloids living in environments with similar selective pressures (i.e. the functional constraints imposed by the medium through which they move) tend to evolve similar phenotypes resulting in similarities in forelimb shape in animals as diverse as otters and badgers. Furthermore, our results show that the shape of the long bones of the forelimb is influenced by lifestyle, body mass and phylogeny. Arboreal and semi-arboreal species such as the ringtail (*Bassariscus astutus*), the bushy tailed olingo (*Bassaricyon gabbii*) and the red panda (*Ailurus fulgens*) display forelimb long bones that are elongated and gracile with articulations allowing a high degree of movement. Terrestrial species display an intermediate morphology between arboreal species on the one hand, and semi-fossorial and aquatic species in the other hand. This is consistent with their more generalist lifestyle. Semi-fossorial and aquatic species display generally similar morphologies with long bones that are short and robust and improving stability and load transfer, due to the similar mechanical constraints imposed by the locomotor environment. Nevertheless, differences are found at the level of the articulation, which may be related to the development of a high level of grasping ability in aquatic species. However, similarities in the morphology of the articulation of the elbow (broad and rounded capitulum, and rounded radial head) can be observed in species able to grasp (aquatic and arboreal/semi-arboreal species). Interestingly, this study shows the importance of investigating the forelimb as a whole, as each bone has its own functional signal. Analysing all of the elements together provides better insights into the relation between morphology and ecology. Quantitative data on locomotion (e.g. kinetic and kinematic data, the proportion of time spend on different supports), complemented by quantitative data on muscular anatomy, are needed in order to provide a better interpretation of the evolution of forelimb morphology in the context of locomotion.

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Author contributions

A-CF conceived the paper, performed the data acquisition and analysis, and drafted the manuscript. RC helped conceive the paper and helped with data analysis. AG and SP helped design the study, and all authors contributed to the writing of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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*These references are cited in Supporting Information.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Landmarks used in our analyses to quantify shape variation on the forelimb bones.

Fig. S2. Results of the PCAs performed on the morphometric data of the humerus.

Fig. S3. Results of the PCAs performed on the morphometric data of the ulna.

Fig. S4. Results of the PCAs performed on the morphometric data of the radius: scatter plot illustrating the position of different species on the first two PCs.

Fig. S5. Mean shape of humerus of arboreal species (green), semi-arboreal species (orange), terrestrial species (red), aquatic species (blue) and semi-fossorial species (brown): (A) posterior view; (B) anterior view; (C) close up of the distal articulation in posterior view; (D) close up of the distal articulation in distal view; (E) close up of the distal articulation in anterior view.

Fig. S6. Mean shape of ulna of arboreal species (green), semi-arboreal species (orange), terrestrial species (red), aquatic species (blue) and semi-fossorial species (brown): (A) anterior view; (B) lateral view; (C) close up of the proximal articulation in anterior view; (D) close up of the proximal articulation in lateral view; (E) close up of the distal articulation in anterior view.

Fig. S7. Mean shape of radius of arboreal species (green), semi-arboreal species (orange), terrestrial species (red), aquatic species (blue) and semi-fossorial species (brown): (A) anterior view; (B) lateral view; (C) close up of the proximal articulation in anterior view; (D) proximal view; (E) close up of the proximal articulation in posterior view.

Table S1. Details of specimens used in analyses with species name, common name, family, number of individuals included (*N*), lifestyle (A = arboreal; SF = Semi-fossorial; SA = semi-arboreal; AQ = aquatic; T = terrestrial) and average body mass (kg).

Table S2. Specimens used in the analyses.

Table S3. Definition of the landmarks of the humerus used for geometric morphometric analyses.

Table S4. Definition of the landmarks of the ulna used for geometric morphometrics analyses.

Table S5. Definition of the landmarks of the radius used for geometric morphometrics analyses.