



# Swimmers, Diggers, Climbers and More, a Study of Integration Across the Mustelids' Locomotor Apparatus (Carnivora: Mustelidae)

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## Abstract

Phenotypic integration, defined as the coordinated co-variation of parts of an organism can be an important constraint on phenotypic diversification. Functional factors, by having an heterogeneous impact across the animal body, may reinforce the integration of some parts while causing a perturbation of the integration among other parts. The integration across the locomotor apparatus should thus reflect to a certain extent the locomotor ecology of the animal. Using the mustelids as study group, we track changes in the patterns of co-variation in species belonging to four different locomotor ecologies (terrestrial, semi-arboreal, semi-fossorial, and semi-aquatic). Our results highlight the strong overall integration in mustelid long bones. The main shape changes associated with co-variations between skeletal elements are the bone robustness and proportions of the epiphyses. The pattern of co-variation is, however, only slightly impacted by allometry. Changes in co-variation between species mostly scale with phylogenetic divergence time, except for the (*Mustela putorius*, *M. eversmanni*, *M. lutreola*) clade which, despite a short divergence time, presents strong differences in co-variation. Co-variation patterns differ between locomotor ecologies, but few of these variations match the hypothesis of a reduction of integration due to functional specialization. This may reflect our lack of knowledge on the functional modules in species with locomotor ecologies that differ from terrestrial locomotion rather than invalidate our a priori hypotheses.

**Keywords** Mustelidae · Long bones · Integration · Locomotor ecology · Geometric morphometrics

## Introduction

The locomotor apparatus is an integrated structure (Hallgrímsson et al. 2002; Young and Hallgrímsson 2005; Martín-Serra et al. 2014). Its function and performance are determined not only by the shape and performance of its parts but above all by how the parts articulate and work together (Polly 2007, 2008). The degrees of freedom, stability, and

load bearing capabilities of an articulation are determined by the shape of the two (or more) bones that constitute this articulation as well as by the shape and properties of the ligaments, tendons and muscles surrounding the articulation. Bone shape is influenced by functional, developmental, and architectural constraints as well as phylogeny (Gould and Lewontin 1979; Cubo 2004). Some of these constraints impact the skeleton as a whole or only part of it, and may induce coordinated variation in these parts (phenotypic integration; Hallgrímsson et al. 2002, 2009; Young and Hallgrímsson 2005; Mitteroecker and Bookstein 2007; Goswami et al. 2014). Thus, the integration across body structures is usually explored through co-variation and correlation approaches (Adams and Collyer 2016; Mitteroecker and Bookstein 2007, 2008). Other constraints produce local effects or affect differentially the skeletal parts and drive a greater phenotypic co-variation within parts (then referred as module) than between the parts. This can be due to the functional specialization of a structure (Young and Hallgrímsson 2005; Arias-Martorell et al. 2014; Goswami et al. 2014). This led Young and Hallgrímsson (2005) to predict that the

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co-variation between limbs or serially homologous elements should decrease with limb functional specialization while the within-limb co-variation may at the same time become stronger. The functional analogy among fore and hind limb is said to be separated from the between-limb serial homology in mammals (Gasc 2001; Fischer et al. 2002; Schmidt and Fischer 2009). This hypothesis is supported by the analysis of locomotion in small mammals (Fischer et al. 2002). The species examined in Fischer et al.'s (2002) study show a role of spinal flexion during locomotion. This role in animals with a long body and short legs like mustelids is even greater (Williams 1983a; Williams et al. 2002) and could possibly be a driver of changes in the function of the limb segments. Moreover, in mustelids, the contribution of the scapula to total limb length can be as little as 15% when it goes up to 35% in Lagomorpha and Bovidae (Schmidt and Fischer 2009). Nevertheless the clavicle is reduced or even lost in mustelids, which allows greater degree of freedom in the mustelids' scapula (Fisher 1942; Howard 1973; Leach 1977; Holmes 1980). Previous studies have explored patterns of integration between the limb long bones across taxa with different ecologies, but most focused on terrestrial species with a particular attention toward cursoriality (Martín-Serra et al. 2014).

In this context we use the mustelids as model group and investigate changes in co-variation among the limb bones within groups that differ in their locomotor ecology. Even if most species of mustelids present a vast locomotor repertoire and are able to move in diverse environments, many of them show some degree of locomotor specialization (Schutz and Guralnick 2007; Fabre et al. 2015a, b; Botton-Divet et al. 2016). The locomotor diversity in mustelids includes semi-aquatic (otters and minks), semi-fossorial (e.g. badgers), semi-arboreal (martens, tayra), and terrestrial (e.g. weasels, stoats, polecats) species (Nowak 2005; Larivière and Jennings 2009; Hunter and Barrett 2011). These specializations led to different bone shapes that reflect the constraints of locomotor ecology, body mass, and the phylogenetic background of the species (Heinrich and Biknevicius 1998; Schutz and Guralnick 2007; Fabre et al. 2013a, b, 2015a, b; Botton-Divet et al. 2016). Using Young and Hallgrímsson's (2005) principle as assumption, we use the phenotypic integration in mustelids to determine the degree and location of functional specializations in the limb skeleton. Thus locomotor specialists should be qualified by decreased co-variation between serially homologous elements and a greater intra-limb co-variation. Here, we describe the patterns of co-variation observed in long bones of mustelids with different locomotor ecologies. Then we discuss these changes under the initial assumption of a higher inter-limb modularity induced by a higher degree of specialization. Finally we investigate the roles of phylogeny and size in the observed differences in the patterns of co-variation.

## Materials and Methods

### Material

Twenty-one species of Mustelidae were selected to encompass all major locomotor ecologies (terrestrial, semi-aquatic, semi-fossorial and semi-arboreal) and a large amount of the size variability present in this group. Specimens used are housed in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN); the National Museum of Natural History, Washington (NMNH); the Museum of comparative Zoology, Harvard (MCZ); the University of Alaska Museum, Fairbanks (UAM); the Museum für Naturkunde, Berlin (MFN); the Naturhistorisches Museum Basel (MHNBS); the Staatliches Museum für Naturkunde Stuttgart (SMNS) and the Royal Museum for Central Africa Tervuren (RMCA). Species and their ecologies are given in Table 1, see S1 for details on specimens. We studied the humerus, ulna, radius, femur, tibia, and fibula from the left side in all specimens when available; otherwise right bones were selected and digitized bones were mirrored prior to analysis. Specimens identified as juveniles on the basis of the incomplete fusion of the epiphyses, showing abnormal bone

**Table 1** Species used in this study and their locomotor ecology based on (Larivière and Jennings 2009; Nowak 2005)

Sub-families	Genus	Species	Locomotor category
Lutrinae	<i>Aonyx</i>	<i>capensis</i>	AQ
Lutrinae	<i>Enhydra</i>	<i>lutris</i>	AQ
Lutrinae	<i>Lontra</i>	<i>canadensis</i>	AQ
Lutrinae	<i>Lontra</i>	<i>felina</i>	AQ
Lutrinae	<i>Lutra</i>	<i>lutra</i>	AQ
Lutrinae	<i>Pteronura</i>	<i>brasiliensis</i>	AQ
Mustelinae	<i>Mustela</i>	<i>eversmannii</i>	T
Mustelinae	<i>Mustela</i>	<i>frenata</i>	T
Mustelinae	<i>Mustela</i>	<i>lutreola</i>	T
Mustelinae	<i>Mustela</i>	<i>putorius</i>	T
Mustelinae	<i>Neovison</i>	<i>vison</i>	T
Martinae	<i>Martes</i>	<i>foina</i>	SA
Martinae	<i>Martes</i>	<i>martes</i>	SA
Martinae	<i>Gulo</i>	<i>gulo</i>	T
Mellivorinae	<i>Mellivora</i>	<i>capensis</i>	SF
Melinae	<i>Meles</i>	<i>meles</i>	SF
Galictinae	<i>Galictis</i>	<i>vittata</i>	T
Galictinae	<i>Ictonyx</i>	<i>striatus</i>	SF
Galictinae	<i>Poecilogale</i>	<i>albinucha</i>	T
Galictinae	<i>Vormela</i>	<i>peregusna</i>	T
Helictidinae	<i>Melogale</i>	<i>moschata</i>	SF

AQ semi-aquatic, T terrestrial, SA semi-arboreal, SF semi-fossorial

accretion or uncommon articular surface wear were excluded from the analysis. Seven specimens were presenting a supra-trochlear foramen on the humerus open to various extents (RMCA 3589, RMCA 26529, MNHN 1996-2439, MNHN 1995-3150, MNHN 1934-107, USNM 396644, USNM 255528). As no foramen was widely open and because other specimens from the same species were not presenting such an open foramen, we hypothesized that neither the medial coronoid process of the ulna nor the anconeus process were inserting through that foramina and therefore estimated its functional role was weak in mustelids. Thus, choice was made to arbitrarily fill these foramina by a plane and bones were then included in the analysis with all others.

## Methods

Bones were digitized in three dimensions using a white light fringe Breuckmann 3D surface scanner (StereosCAN<sup>3D</sup>, five megapixels resolution). Bone shape was quantified using a set of anatomical landmarks and semi-landmarks sliding on curves and surfaces as described in Botton-Divet et al. (2016). Anatomical landmarks and curves were digitized on bone digital models using IDAV Landmark software (Wiley et al. 2005). The templates defined for each bone are given in Supplementary Data 1. Following Gunz and Mitteroecker (2013), a semi-automatic point placement strategy was used to place surface sliding semi-landmarks on the digitized bone surfaces, as implemented in the ‘placePatch’ function (Schlager 2016). Semi-landmarks on surfaces and curves were slid in order to minimize the bending energy of a thin plate spline (TPS) between each specimen and a common reference. A first TPS relaxation was run using the template as common reference and next three iterative relaxations were computed using the Procrustes consensus of the previous step as a reference.

Next, all specimens were superimposed using a Generalized Procrustes Analysis (GPA; Gower 1975; Rohlf and Slice 1990) to place them in a common space, extract centroid size, and allow homologous comparisons. The GPA was performed using the ‘procSym’ function (Schlager 2016).

To investigate the patterns of co-variation between bones we used Two-Blocks Partial Least Square (2bPLS) analyses implemented in Morpho (‘pls2B’, Schlager 2016). This method extracts the major axes of co-variation between two datasets and allows to visualize the position of each specimen relative to these axes as well as the changes in shape associated to the main axes of co-variation. To visualize the shape deformations associated with the first PLS axes, the ‘plsCoVar’ function was used and shape computed at for one standard deviation on each side of each axis (Schlager 2016). Coordinates computed for each side of the axis were then used to compute a TPS deformation of the bone template.

As phylogeny constrains shape evolution and its co-variation, we used a pruned version of Slater et al.’s (2012) phylogeny in the Phylogenetic Two Blocks Partial Least Square analysis (ϕ2bPLS) function ‘phylo.integration’ implemented in ‘geomorph’ (Adams and Otárola-Castillo 2013), which takes the phylogeny into consideration.

To visualize the differences in the integration pattern depending on the ecology of the species, we computed the covariance ratio (CR; Adams 2016). Most studies use the RV coefficient (Escoufier 1973) to quantify integration among morphometric data (Klingenberg 2009; Goswami and Polly 2010; Fabre et al. 2014; Martín-Serra et al. 2014). As a homogeneous sample size is critical to compare RV coefficients, we here used the CR proposed by Adams (CR; Adams 2016) which is not sensitive to sample size nor to its dimensionality.

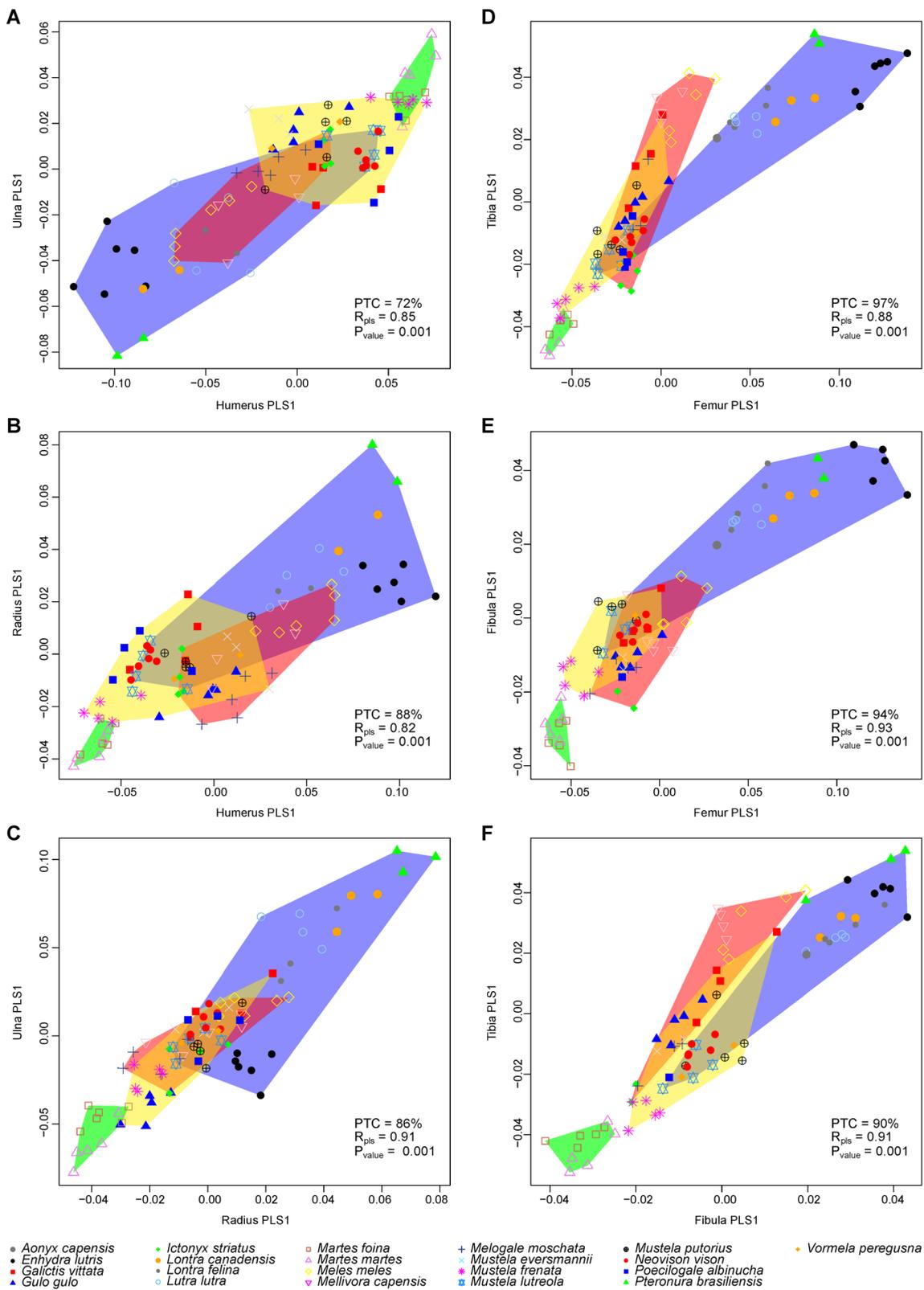
As size has been shown to be a determinant factor in the evolution of long bone shape in mustelids (Fabre et al. 2013a; Botton-Divet et al. 2016) we assessed the effect of allometries on our analyses by regressing the shape against the centroid size of the bones and computing allometry-free shapes from the residuals. Next the CR were computed on the allometry-free shapes.

To investigate the changes in the limb segment proportions, the linear lengths of stylopod (humerus and femur) and zeugopod (radius and tibia) bones of fore- and hindlimbs were measured. To do so we extracted the distance between two landmarks of the humerus (landmark 5–25), radius (landmark 1–10), femur (landmark 9–23), and tibia (landmark 14–16; Supplementary Material 1). Then we computed the ratio of the radius length over the humerus length and the ratio of the tibia length over the femur length (log<sub>10</sub> of linear dimensions).

All analyses were performed in the R environment (R Core Team 2014).

## Results

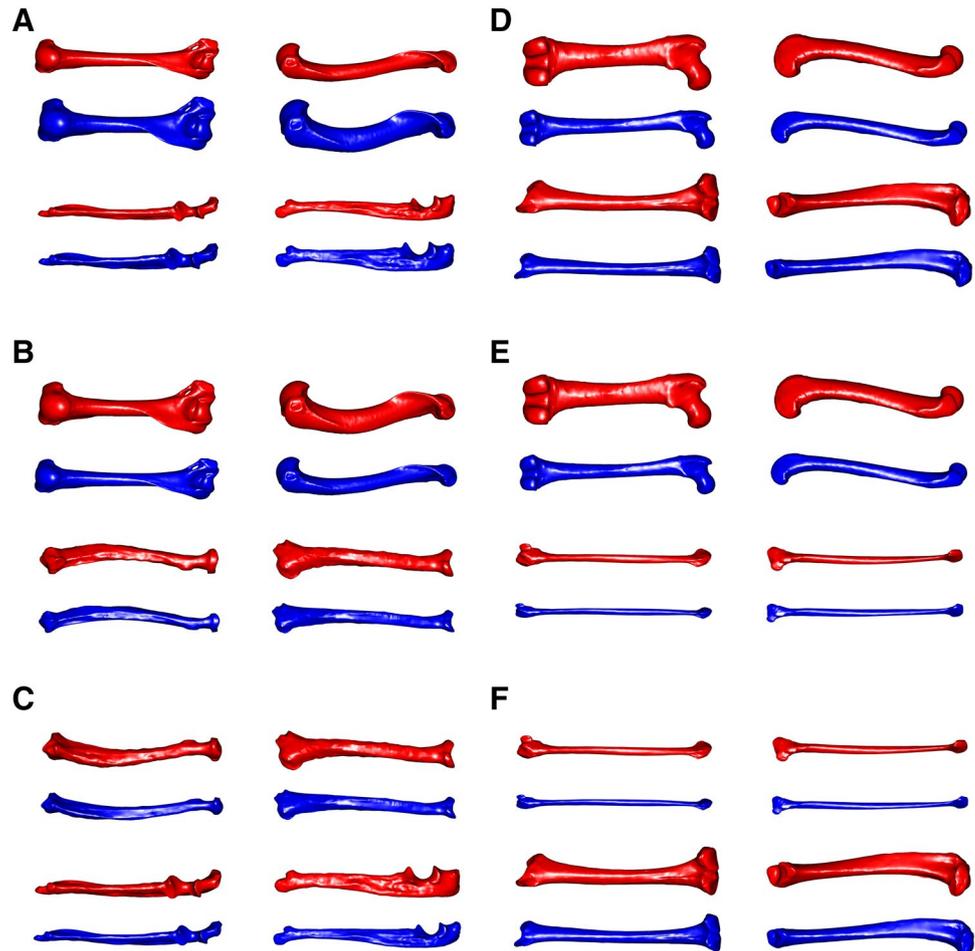
All first axes of the 2bPLS are significant (Figs. 1, 2). The rPLS (PLS correlation coefficient) are over 0.82 in all tested couples and reach up to 0.93 for the femur–fibula PLS. The first PLS axes gather between 72% (Fig. 1a) and 97% (Fig. 1d) of the total co-variation between the couples of bones. The first PLS axis of all intra- and inter-limb PLS analyses are bordered by martens (negative values except for the humerus–ulna PLS Fig. 1a) on one side and otters on the other side. The two martens present very similar co-variation patterns for all the couples of bones examined (Figs. 1, 2). *Gulo gulo* (wolverine) tends to pool with martens for the ulna–radius PLS. *Mustela frenata* (long-tailed weasel) tends to present covariance patterns close to those of the martens for most couples of bones examined and overlaps with



**Fig. 1** Plot of the first Partial Least Square axis of: **A** humerus and ulna; **B** humerus and radius; **C** radius and ulna; **D** femur and tibia; **E** femur and fibula; **F** fibula and tibia. *PTC* proportion of total covari-

ance. *Rpls* PLS correlation coefficient. Blue area: semi-aquatic; red area: semi-fossorial; orange area: terrestrial; green area: semi-arboreal. (Color figure online)

**Fig. 2** Shape deformations associated with the first PLS axes presented in Fig. 1. In blue: shape associated with negative side of the axis. In red: shape associated with the positive side of the axis. **A** humerus–ulna; **B** humerus–radius; **C** radius–fibula; **D** femur–tibia; **E** femur–fibula; **F** fibula–tibia. (Color figure online)

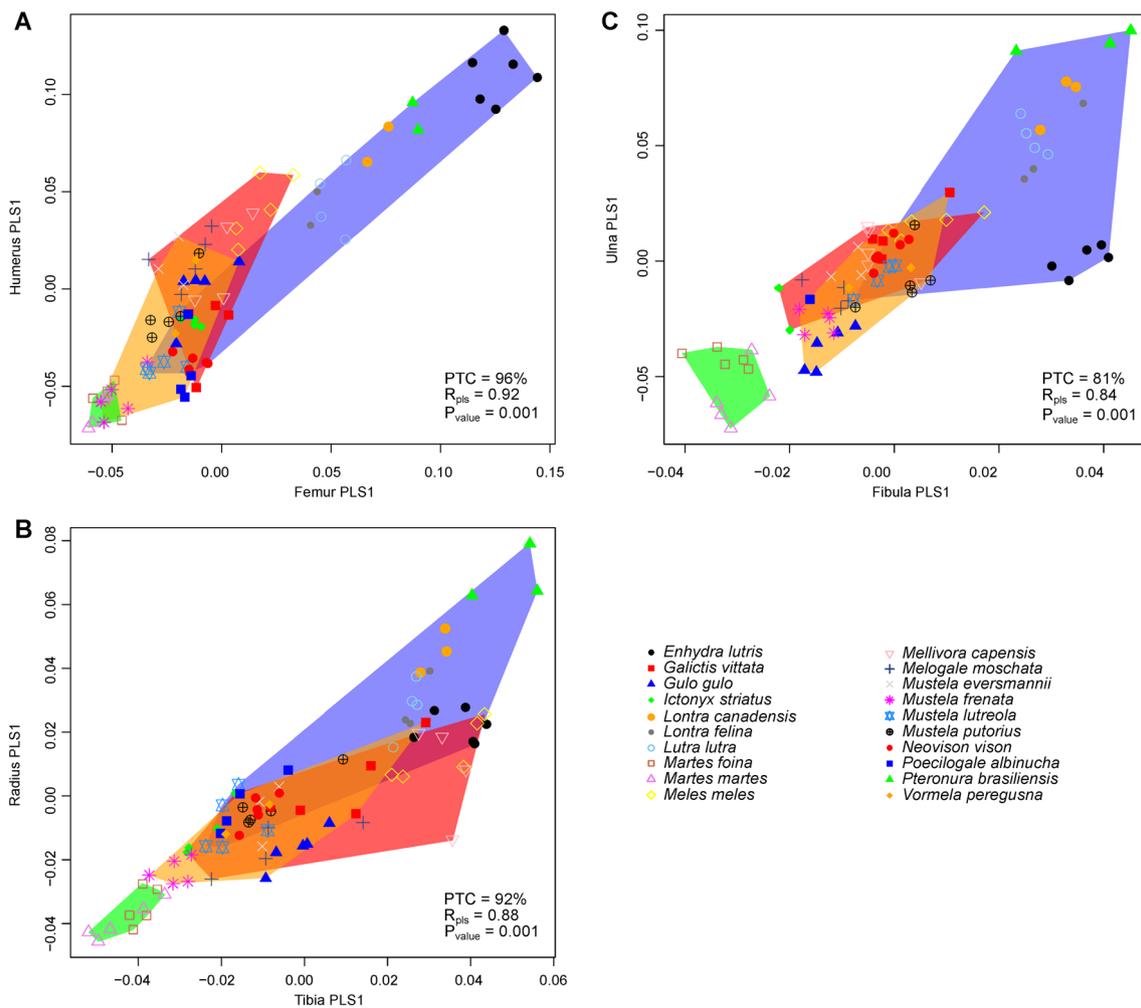


martens for humerus–ulna, humerus–radius and femur–tibia (Figs. 1a, b, d, 3a). The otters tend to separate from the other species in within- as well as between-limbs PLS except for the humerus (Figs. 1, 3). *Melogale moschata* (Chinese ferret badger) tends to separate from the common slope of the humerus–radius PLS (Fig. 1b). *Enhydra lutris* (sea-otter) presents very distinctive ulna–radius PLS scores (Fig. 1c). This species separates clearly from the other otters as well as from the global covariance slope. Large sized semi-fossorial species (*Meles meles* and *Mellivora capensis*; Old world badger and Honey badger) show PLS scores that tend to be similar with the ones of the otters except for the ulna–radius and ulna–fibula PLS (Fig. 1b, c). Otters separate from the common slope in femur tibia PLS (Fig. 1d) and they tend to follow a different slope.

Specimens on the negative side of the first PLS axis of the humerus–ulna PLS tend to show more robust humeri with larger epiphyses and a straighter diaphysis than specimens on the positive side (Fig. 2). Specimens on the negative side of the axis present an ulna that is more expanded on the palmar side with a larger olecranon process and a coronoid process extending more cranially. Specimens

on the positive side of the humerus–radius PLS present a more robust humerus and radius with larger epiphyses. The humerus shows a large capitulum and trochlea and a more curved diaphysis. Articular surfaces of the radius are larger, particularly the distal ulna–radial articulation. Specimens on the positive side of the axis of the ulna–radius PLS present more robust bones. The ulna is more expanded on the palmar side, the olecranon process is larger and more square shaped, and the coronoid process extends more both cranially and distally. The radius is more curved and articular surfaces are wider. Specimens on the positive side of all first axes of PLS analyses between hind limb bones present more robust bones with larger epiphyses. The tibia of specimens with high scores present an antero–posterior curvature of the diaphysis around its middle. The fibula shows larger epiphyses particularly the distal one that presents a lateral malleolus that extends more laterally.

The first PLS axes between serially homologous bones (Fig. 3) are all significant and present high correlation value (from 0.84 to 0.92) and gather between 81 and 96% of the covariance between humerus and femur, radius and tibia, and ulna and fibula. They are bordered by martens



**Fig. 3** Plot of the first Partial Least Square axis of: **A** humerus and femur; **B** radius and tibia; **C** ulna and fibula. *PTC* proportion of total covariance. *R<sub>pls</sub>* PLS correlation coefficient. Blue area: semi-aquatic;

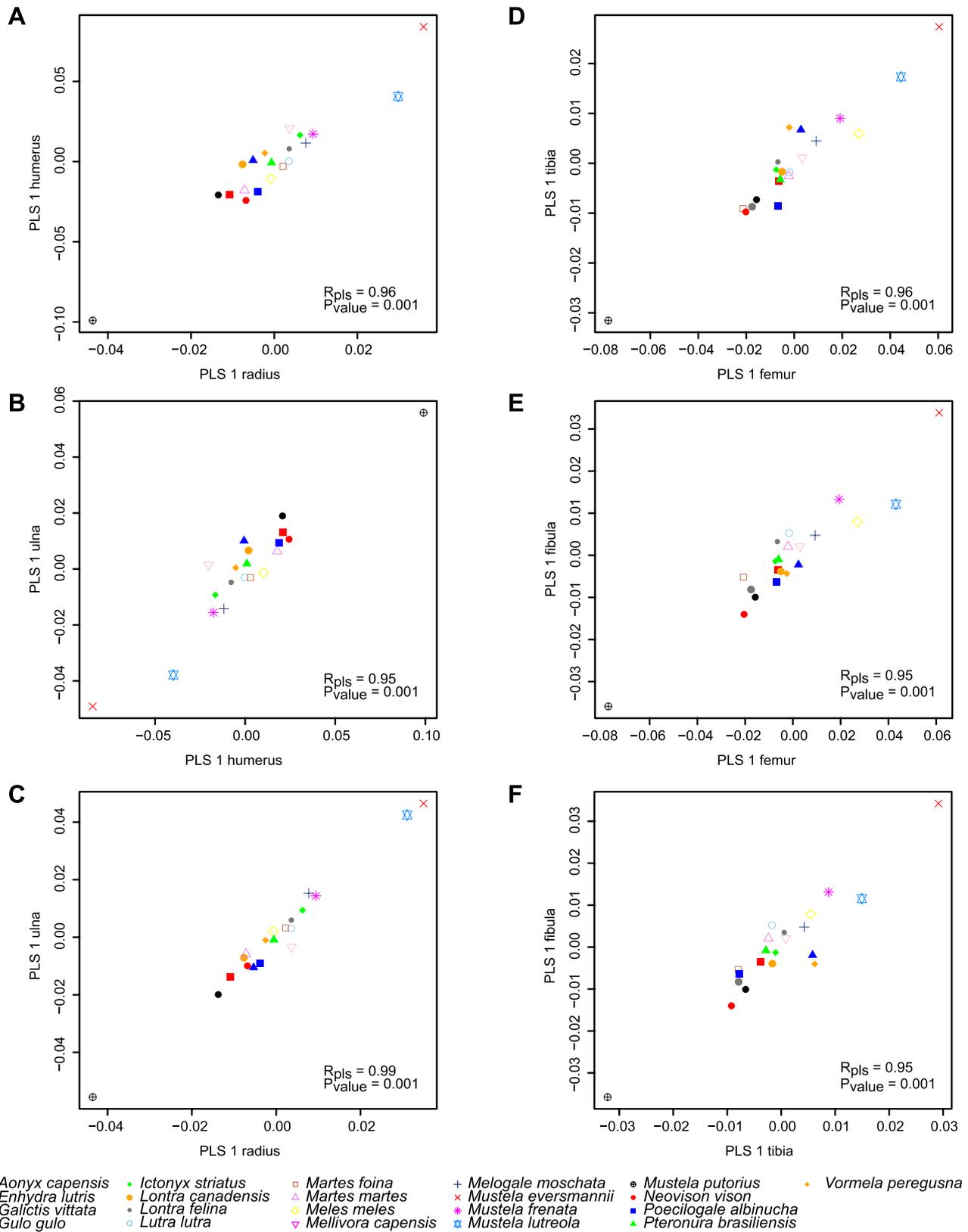
red area: semi-fossorial; orange area: terrestrial; green area: semi-arboreal. (Color figure online)

on the negative side and otters on the positive one. *E. lutris* separates from other otters for the radius–tibia and ulna–fibula PLS. For the first axis of the radius–tibia PLS, *E. lutris* tends to pool with large sized semi-fossorial species.

Partial Least Square analyses on shapes corrected for allometry are rather similar to those on shapes including allometries. The amount of co-variation explained by the first axes is comprised between 74 and 95% of the total co-variation and correlations range from 0.76 to 0.92. All axes are significant. A few differences can nevertheless be noted. *G. gulo* tends to pool with the martens at one extreme of the PLS axes. *M. frenata* groups with the other Mustelinae in the center of the plot. *M. moschata* separates from the main axis for the humerus–radius PLS. Otters present a slope that diverges from the one of the other species for the femur–tibia PLS.

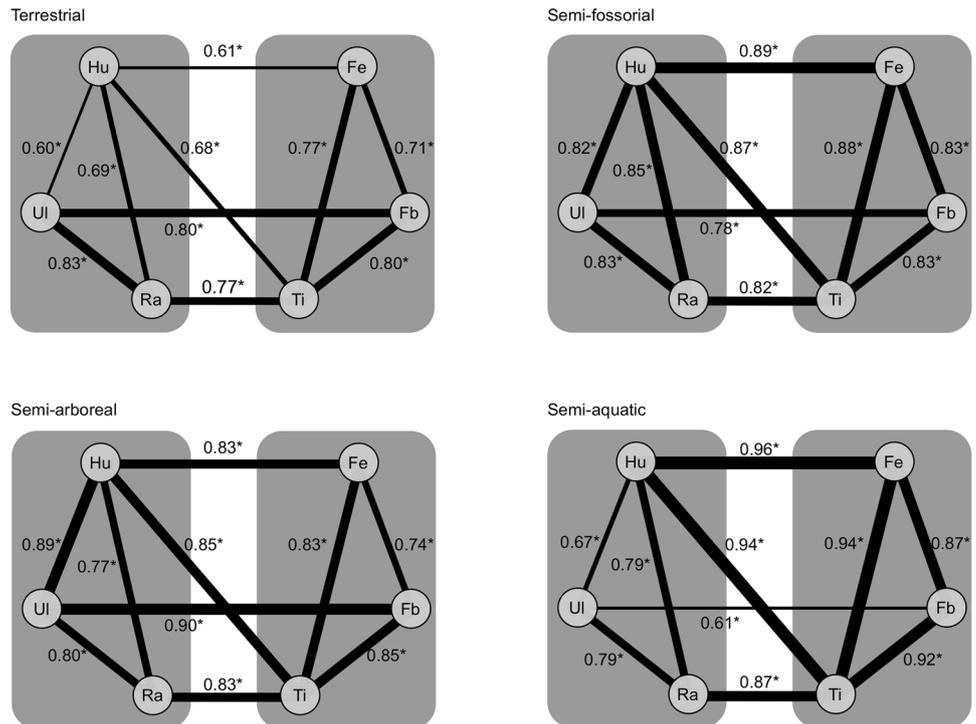
The first axes of the phylogenetic PLS are significant for all couples of bones tested (Fig. 4). The rPLS ranges between 0.95 and 0.99. All phylogenetic PLS axes are bordered by *Mustela putorius* on one side and *Mustela eversmannii* on the other side. *Mustela lutreola* tends to move closer to *M. eversmannii* in  $\varphi 2b$ PLS particularly for forelimb bones and the tibia–femur (Fig. 4a–d).

The pattern observed in terrestrial species shows a relatively weak integration as highlighted by the CR values ranging from 0.60 to 0.83. The integration is stronger in the hind limb and between the ulna and radius (from 0.71 to 0.80 and 0.83 respectively; Fig. 5). Semi-arboreal species show a high and homogeneous integration pattern with CR values ranging from 0.77 to 0.90. Similarly, semi-fossorial species show a strong integration pattern with values ranging between 0.78 and 0.89. The CR between bones are homogeneous across all couples of bones tested. Semi-aquatic species present a rather



**Fig. 4** Plot of the first phylogenetic Partial Least Square axis of: **A** humerus and femur; **B** radius and tibia; **C** ulna and fibula; **D** femur and tibia; **E** femur and fibula; **F** tibia and fibula.  $R_{pls}$  PLS correlation coefficient. (Color figure online)

**Fig. 5** Co-variation pattern per ecology, CR for in limb and serially homologous bones. *Hu* humerus; *Ul* ulna; *Ra* radius; *Fe* femur; *Fb* fibula; *Ti* tibia. Asterisk: significant (p value <0.05) based on 1000 iteration test



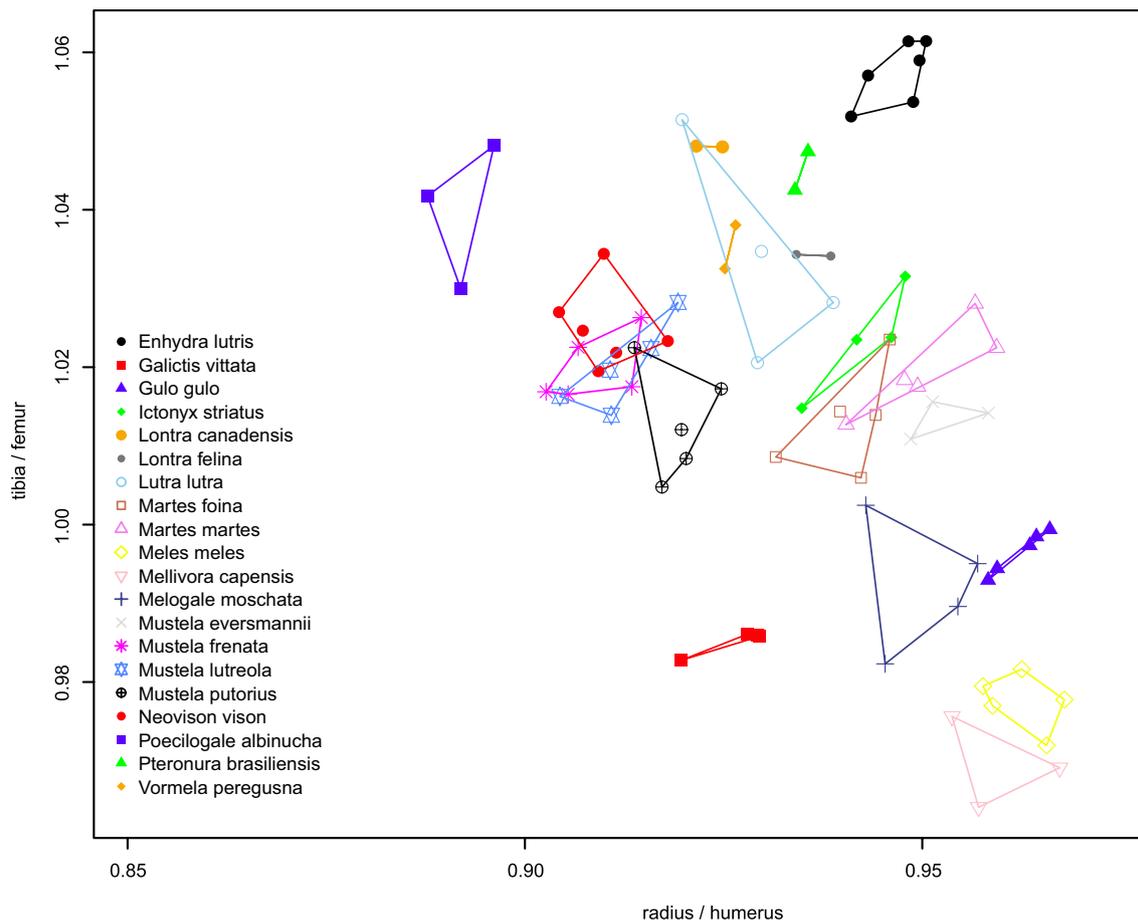
unbalanced integration pattern. The co-variation in the forelimb is relatively weak, ranging from 0.67 to 0.79, yet the hind limb presents a strong integration with CR values between 0.87 and 0.94. The inter-limb pattern shows a strong co-variation between the humerus and femur (0.96) and radius and tibia (0.87). In contrast, the co-variation between the ulna and fibula is weak (0.61). The co-variation between the humerus and tibia (functional equivalent) ranges from 0.68 for terrestrial species to 0.94 for semi-aquatic ones.

Otters present relatively short femora (Fig. 6). Large semi-fossorial species (*G. gulo* and *M. capensis*) have the lowest ratio for the hind limb and the highest one for the forelimb, with similar values between hind- and forelimbs. Small semi-fossorial species (*M. moschata* and *Ictonyx striatus*) present high forelimb ratios but lower hind limb ratios compared to large semi-fossorial species. The Mustelinae are all pooled together with medium values for the hind limb and low forelimb values except for *M. evermannii* (higher forelimb values). Martens present average scores for hind limb ratios and relatively high forelimb ratios. *G. gulo* presents lower hind limb ratios and higher forelimb ratios than its relatives, the martens.

## Discussion

The co-variation between the bones of the hind limb is stronger (over 90% of the variance explained) than for the forelimb (<90%; Fig. 1). This suggests that the co-variation

is less variable in the hind limb compared to the forelimb across our study group. This is in accordance with what was demonstrated for a larger sample of quadrupedal mammals by Schmidt and Fischer (2009). The percentage of common covariance explained by the first PLS axes are also high between radius and tibia and humerus and femur (respectively 92 and 96%; Fig. 3), suggesting that serially homologous bones are strongly integrated, potentially due to developmental processes or to a similar evolutionary response to an increase in body mass (allometries). However, allometries seem to play only a small role in shaping the co-variation in this group as PLS on data corrected for allometry show patterns similar to those observed for non-corrected data (respectively Figs. 1, 7). The main differences are observed within sub-families. Indeed *G. gulo* tends to group with its sister taxon of smaller size (martens) when co-variations are corrected for allometry and *M. frenata* pools with the other Mustelinae species (Koepfli et al. 2008; Sato et al. 2003; Slater et al. 2012). When compared to the semi-arboreal martens and despite of its terrestrial habits, *G. gulo* divergences are mainly driven by its larger size. On the opposite, *M. frenata* differs from other Mustelinae by its smaller size but retains similar co-variations. Shape changes associated with the main co-variation axes are mainly related to bone robustness, in accordance with previous studies (Martín-Serra et al. 2014). However, the multivariate regression used here does not allow identification of group specific (clade or locomotor category) allometric trends if these occur in a different direction from the common allometric



**Fig. 6** Limb segment proportions. Zeugopod length over stylopod length (log<sub>10</sub>) ratio for hind- and fore-limb. (Color figure online)

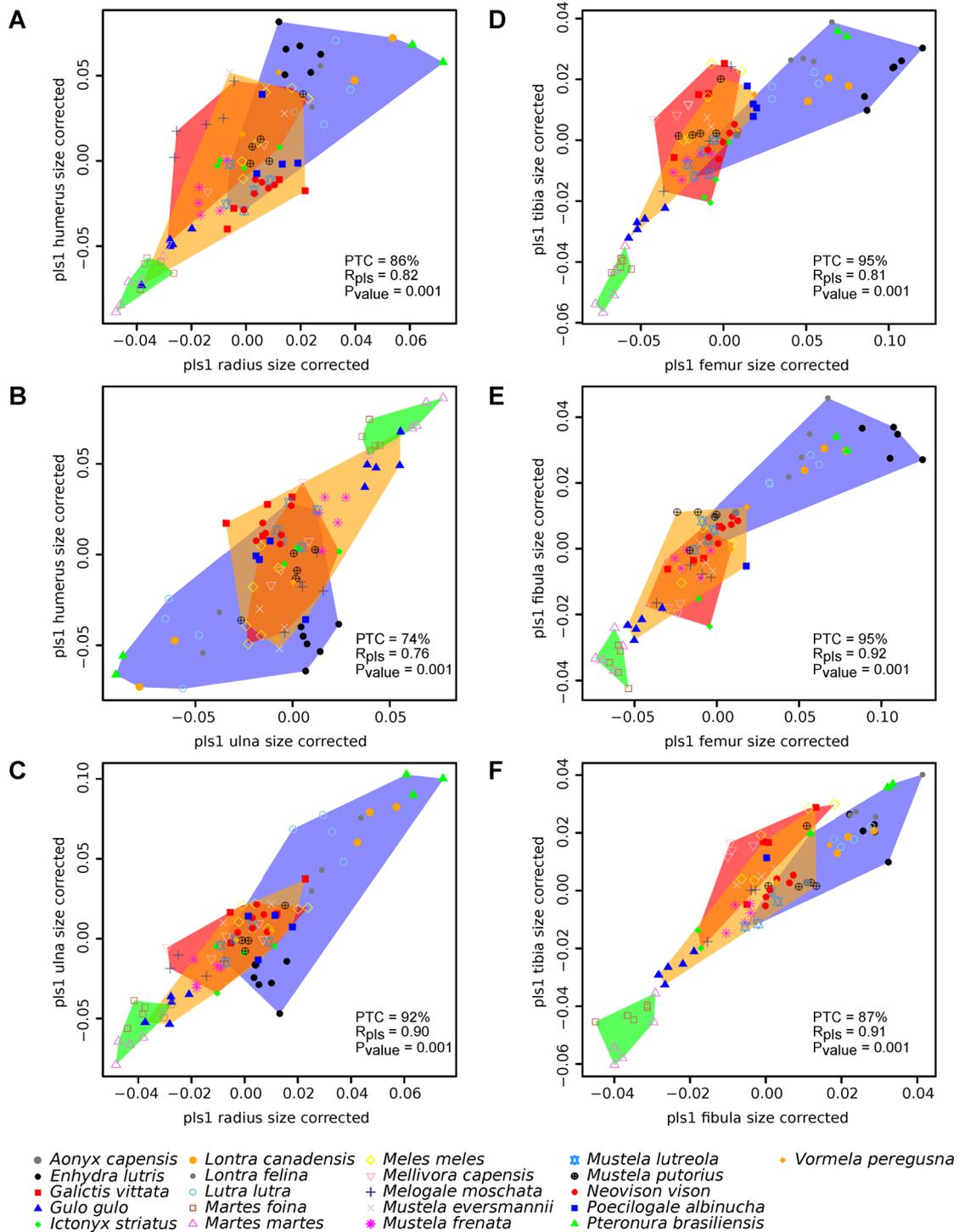
trend. Considering that allometries might differ depending on ecologies (Bou et al. 1987), that changes in allometries might affect bones differentially (Casinos et al. 1993), and that clades might have distinct allometric trends (Sansalone et al. 2017), the role of allometry shifts in shaping the co-variation pattern remains to be studied in mustelids.

The lower overall co-variation observed among terrestrial species may result from the large diversity among terrestrial mustelids. Terrestrial mustelids use a wide variety of locomotor behaviors and are not strictly restricted to one type of locomotion (Fabre et al. 2015a). Indeed as all terrestrial species are pooled in a single category, the presence of various distinct co-variation patterns could lead to a low overall co-variation pattern. This hypothesis is corroborated by the diversity of limb segment ratios obtained for terrestrial species (Fig. 6). The terrestrial category includes large sized animals (e.g. *G. gulo*; Pasitschniak-Art and Larivière 1995) as well as small ones that are able to enter burrows in search for food (e.g. *M. frenata*; Reid and Helgen 2008). Nevertheless the CR computed on allometry-free shape is similar to the CR on raw shapes, except for the radius–tibia which reaches about 0.90 (Fig. 8). Therefore, in terrestrial

mustelids, the differences in co-variation appear related to allometry in the distal part of the limbs only.

The shape of the femur and tibia in otters does not co-vary the same way as in other mustelids (Fig. 1d). When testing the co-variation among otters alone, the first PLS axis is significant (PTC = 75%, rPLS = 0.96  $P = .001$ ) suggesting that otters display a common slope that diverges from that of the other species. The shift in this axis remains when data are corrected for the allometric effect (Fig. 7d) suggesting that the shift in co-variation is not induced by a shift in allometries but rather a functional shift that potentially occurred in the common ancestor of otters, likely induced by the extensive aquatic locomotion that characterizes this group.

The sea otter *E. lutris* diverges from the other otters in its forelimb co-variations (Fig. 1). Moreover this species presents a co-variation between the ulnar and radial shape that separates itself from the axis of co-variation common to all other species (Fig. 1c). When correcting for the effect of size on shape (allometry), this difference remains and is also visible in the co-variation between the humerus and ulna. Therefore the divergence in the forearm co-variation

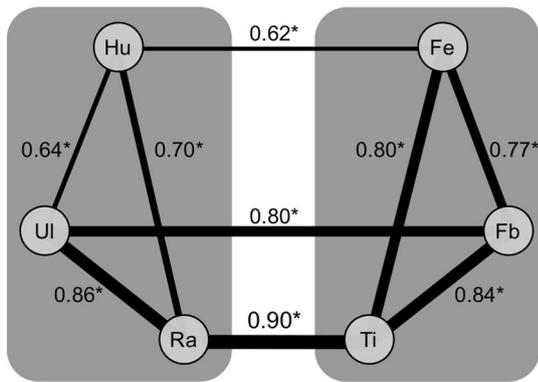


**Fig. 7** Plot of the first Partial Least Square axis on allometry free shapes: **A** humerus and ulna; **B** humerus and radius; **C** radius and ulna; **D** femur and tibia; **E** femur and fibula; **F** fibula and tibia. *PTC*

proportion of total covariance. *Rpls* PLS correlation coefficient. Blue area: semi-aquatic; red area: semi-fossorial; orange area: terrestrial; green area: semi-arboreal. (Color figure online)

of this species is not due to the large size of this species. In the sea otter, the forearm is not involved in aquatic locomotion (Bodkin 2001) and terrestrial locomotion is rare and not efficient (Estes 1980). However, the forepaw is involved in

precise manipulation for food gathering and processing as well as grooming to an extent that is unique among otters (Riedman and Estes 1990; Fujii et al. 2015). Moreover, relative short stylopod observed in both the fore- and hind limb



**Fig. 8** CR values for data corrected for allometry for terrestrial species

of otters is thought to be an adaptation to drag-based swimming. Indeed the reduction of the first segment of the limb reduces the drag induced by the recovery phase and therefore might improve swimming efficiency (Stein 1988).

The strong integration between the hind limb bones in this group suggest a strong functional constraint acting on the limb as a whole, potentially due to a specialization of the hind limb to aquatic locomotion. Indeed all otters use their hind-limbs for aquatic locomotion (Duplaix 1980; Fish 1994; Larivière 1998, 2001; Bodkin 2001; Larivière and Jennings 2009), sometimes in combination with other sources of thrust generation (forepaw, body and/or tail undulation) depending on the species and the swimming velocity. Still the high CR value between humerus and femur does not support the hypothesis of a distinct specialization between fore and hind limb in these species. Only minks swim using four-limb paddling, though dominated by the forelimb (Williams 1983b; Lodé 1999). The ulna presents the lowest CR. This lower integration of the ulna suggests, in accordance with the hypotheses of Young and Hallgrímsson (2005), a higher specialization of the ulna in semi-aquatic mustelids. This specialization of the ulna is likely to be in the development of the manipulative behavior observed in otters (Bodkin 2001; Larivière 2001).

Alternatively the lower integration of the ulna may result from the reduced functional constraints on the forelimb as a whole given that this limb is likely under selection for mobility rather than force transfer in opposition with terrestrial species. However co-variation remains high between serially homologous (humerus and femur, radius and tibia) and between functional equivalent during terrestrial locomotion (humerus and tibia).

The divergence time between *M. putorius*, *M. eversmannii* and *M. lutreola* is rather short (<2 My; Slater et al. 2012), but the morphological divergence between these species is marked. The differences in shape co-variation among long bones between these three species are not particularly

noticeable (Fig. 1), but when taking into consideration the phylogeny the divergence becomes particularly striking (Fig. 4). Additionally the relative proportions of the different segments of the limbs diverge strongly in *M. eversmannii* (Fig. 6) as compared to in the two other species. This suggest that the rate of divergence in this group is greater than in any other group of species. *M. putorius* and *M. eversmannii* are said to be very similar (Larivière and Jennings 2009) with a very low genetic divergence between the species (Sato et al. 2003; Koepfli et al. 2008). We thus suggest that a strong functional shift has occurred between *M. putorius* and *M. eversmannii*. This divergence in functional constraints may come from the fact that these two species occur in distinct habitats and regions. *M. putorius* lives in open forests and meadows and is regularly found close to water. This species occurs across Western Europe and north of a line from the Black Sea to the West of the Urals, except in the north of Scandinavia. *M. eversmannii* inhabits dry open grassland (steppe) along the Eurasian continent from Eastern Europe to China (Larivière and Jennings 2009). Nonetheless the functional consequences cannot be evaluated here. This higher ratio may result from both a reduction of the stylopod or an elongation of the zeugopod. As it was debated for the otters, a reduction of the stylopod is said to be an adaptation to reduce drag forces during the recovery phase of drag-based swimming (Stein 1988). The elongation of the distal parts of the legs are said to provide greater stride length and to be an adaptation to terrestrial locomotion (Polly 2007). As *M. eversmannii* is the species that frequent the driest environment in the (*M. putorius*, *M. eversmannii* and *M. lutreola*) clade, the hypothesis of an optimization of terrestrial locomotion efficiency is more likely although this requires further investigations with a larger number of specimens and the actual size and-or mass of the specimens for scaling.

Semi-fossorial species should display lower co-variations between the forelimb and the hind limb as postulated by the functional specialization hypothesis (Young and Hallgrímsson 2005). Indeed the forelimb is used for digging and several anatomical features were described as adaptations of the forelimb to fossoriality in mustelids (Van Valkenburgh 1987; Samuels et al. 2013; Fabre et al. 2015a, b). The role of the hind limb during digging is supposed to be minor and no morphological patterns were highlighted as it was done for rodents (Samuels and Van Valkenburgh 2008). Moreover the analysis of the limb segment proportions (Fig. 6) shows that semi-fossorial species have a short forearm relative to the humerus, suggesting smaller out-levers required for powerful digging. However, semi-fossorial species show high and homogeneous CR values in the limbs as well as between limbs suggesting that specialization does not per se involve a rupture of the patterns of integration among serially homologous elements.

Under Young and Hallgrímsson's (2005) assertion the integration between serially homologous bones should decrease when integration between functional analogs (humerus and tibia) increases. On the opposite, terrestrial species show relatively low CR between humerus and femur (serial homologue) as well as between humerus and tibia (functional analogs). Moreover other ecologies show high CR between humerus and femur and between humerus and tibia. Nevertheless, the functional equivalences between bones were defined on the basis of terrestrial locomotion (Fischer et al. 2002; Gasc 2001), and this equivalence may differ during digging, climbing, and aquatic locomotion. Moreover all mustelids species do not use the same gaits during both terrestrial and aquatic locomotion (Tarasoff et al. 1972; Williams 1983a, b; Lodé 1999; Williams et al. 2002).

The drastic changes in the co-variation pattern observed in otters and in the sea otter suggest that the development of aquatic habits goes hand in hand with a great modification of the integration pattern of limb long bones in these species. This must be put in parallel with the case of the most aquatic mammals, such as cetaceans or sirenians. Indeed these groups present a highly modified morphology with a regression of the hind limb and pelvic girdle and a modification of the axial skeleton (Adam 2002; Thewissen et al. 2006; Buchholtz 2007). The change of locomotor environment between land and water leads to a redistribution of locomotor needs across the body, with drastic functional rearrangements, allowing the specialization to new function such as item manipulation (sea otter) or the reduction of the number of limbs (cetaceans and sirenians). In this context the evolution of the axial skeleton of otters remains understudied when considering the light it could bring to the understanding of the development of aquatic habits in numerous taxa (Thewissen and Fish 1997; Buchholtz 2007; Amson et al. 2015).

## Conclusion

The co-variations are strong in mustelid long bones, likely due to developmental processes. Our results suggest that size has only a small impact on the co-variations in this group except at a sub-family level, where the most extreme sized members (*M. frenata* and *G. gulo*) of two sub-families (Mustelinae and Martinae respectively) show a pattern of co-variation that is more similar to that of their relatives when allometries are taken into account. Changes in co-variation between bones are strongly linked with the divergence time between species, except for the (*M. putorius*, *M. eversmanni*, *M. lutreola*) clade where drastic changes occurred within a short time period. While a decrease in integration between serially homologous bones was hypothesized as a response

to functional specialization, this was observed only in the ulna of the semi-aquatic group. The main co-variation axes in all couples of bones tested are associated with bone robustness. Bone robustness varies homogeneously across the mustelid skeleton, whatever its ecology.

When hypothesizing that the reduction of the co-variation between serially homologous bones results from specialization, our results suggest more specialization in terrestrial (particularly the humerus) forms and in the forearm of semi-aquatic mustelids. If the specialization of the ulna in semi-aquatic mustelids might be expected considering the manipulative abilities of these species, evolutionary sources of the forelimb specialization in terrestrial mustelids remain unclear.

Otters present a divergent co-variation pattern in the hind limb that we hypothesize to be linked with the functional constraints of aquatic locomotion (higher density and viscosity). The ulna presents low co-variations in semi-aquatic mustelids, likely due to a specialization to manipulation in otters in general and in *E. lutris* more specifically. Future work should experimentally identify the functional modules in the locomotor apparatus of mustelids for other functions than terrestrial locomotion. Such study would benefit from a wider sampling, across the phylogeny.

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## Compliance with Ethical Standards

**Conflict of interest** Authors declare that they have no conflict of interest.

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