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RESEARCH ARTICLE

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Describing whisker morphology of the Carnivora

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Abstract

One of the largest ecological transitions in carnivoran evolution was the shift from terrestrial to aquatic lifestyles, which has driven morphological diversity in skulls and other skeletal structures. In this paper, we investigate the association between those lifestyles and whisker morphology. However, comparing whisker morphology over a range of species is challenging since the number of whiskers and their positions on the mystacial pads vary between species. Also, each whisker will be at a different stage of growth and may have incurred damage due to wear and tear. Identifying a way to easily capture whisker morphology in a small number of whisker samples would be beneficial. Here, we describe individual and species variation in whisker morphology from two-dimensional scans in red fox, European otter and grey seal. A comparison of long, caudal whiskers shows inter-species differences most clearly. We go on to describe global whisker shape in 24 species of carnivorans, using linear approximations of curvature and taper, as well as traditional morphometric methods. We also qualitatively examine surface texture, or the presence of scales, using scanning electron micrographs. We show that gross whisker shape is highly conserved, with whisker curvature and taper obeying simple linear relationships with length. However, measures of whisker base radius, length, and maybe even curvature, can vary between species and substrate preferences. Specifically, the aquatic species in our sample have thicker, shorter whiskers that are smoother, with less scales present than those of terrestrial species. We suggest that these thicker whiskers may be stiffer and able to maintain their shape and position during underwater sensing, but being stiffer may also increase wear.

KEYWORDS
aquatic, curvature, mechanoreception, touch, vibrissae

1 | INTRODUCTION

The order Carnivora is an ecologically and taxonomically diverse group of mammals. An aquatic lifestyle has evolved a number of times in the Carnivora, including in the pinnipeds (seals, sea lions and walruses), as well as in semi-aquatic mustelids (otters and minks) (Botton-Divet et al., 2017) and ursids (polar bears) (Slater et al., 2010). An aquatic lifestyle has driven morphological diversity in the Carnivora, especially in skeletal structures, such as skulls (Botton-Divet et al., 2017; Goswami et al., 2011; Jones et al., 2015; Radinsky, 1981; Slater et al., 2010; Van Valkenburgh, 2007). Indeed, an aquatic liftesyle has important implications for feeding, locomotion and sensing the environment (Botton-Divet et al., 2017; Van Valkenburgh, 2007).

Whiskers are the primary tactile organs in many mammals (Grant & Arkley, 2015). The geometric properties of a whisker can be characterised by its length, curvature, taper, cross-sectional shape and torsion. It has been observed that aquatic mammals, especially pinnipeds, can have diverse whiskers, varying in size, length, number and shape (Dougill et al., 2020; Ginter et al., 2009; Ginter et al., 2012). In the case of phocid seal whiskers, the cross sections undulate (Ginter et al., 2012; Hanke et al., 2010; Starostin et al., 2022), a feature thought to reduce signal-to-noise ratios in flowing water (Hanke et al., 2010), allowing the seals to distinguish prey movement from flowing water. Whiskers in aquatic mammals are also thought to be more sensitive than those of terrestrial species (Hyvärinen, 1989; Marshall et al., 2006; Mattson & Marshall, 2016; Reep et al., 2001; Stephens et al., 1973), perhaps indicating a greater reliance on tactile sensing, rather than vision, in the dark underwater environments (Grant & Arkley, 2015). Since an aquatic lifestyle is evidently an important determinant for various physiological and anatomical adaptations in the Carnivora, we ask the question, is such change evident in the morphology and function of whiskers? Therefore, we compare the whisker morphology of a selection of terrestrial, semi-aquatic and aquatic species of Carnivora.

While whisker shape and function differ between species, the difficulty in comparing whisker shape quantitatively means that comparative whisker morphology has yet to be explored in depth. Other studies have previously approximated whisker shape. Some have used quadratic curves (Quist et al., 2011; Towal et al., 2011), although these cannot replicate inflections in whisker curvature that have been observed. Cubic splines can be used to capture inflections (Bagdasarian et al., 2013; Belli et al., 2018), although it is then difficult to compare between quadratic and cubic models. Elliptic Fourier harmonic coefficients (Ginter et al., 2012) or Bezier curves (Campagner et al., 2018; Gillespie et al., 2019; Hewitt et al., 2016; Petersen et al., 2020) can capture whisker outlines well for comparative analysis. For example, using Elliptic Fourier harmonic coefficients and morphometric analysis, Ginter et al. (2012) described whisker shape in pinnipeds (Ginter et al., 2012) and showed that the method was sensitive enough to group undulating phocid whiskers, smooth phocid whiskers and smooth otariid whiskers. However, while these methods present visual information, they do not offer a description that captures the essential reference features of whisker form, for example, length, curvature and taper, in a manner that is relatively easy to interpret for comparative analysis, and which is necessary for mechanical models (i.e., where a descriptive equation is preferable). We have recently shown that the whisker shape of many mammalian species can be accurately described by Euler Spiral models of curvature and linear models of taper (Dougill et al., 2020; Starostin et al., 2020), which offer a succinct way to compare between species. In 19 species of mammals, we demonstrated that aquatic and semi-aquatic mammals tended to have shorter, thicker and stiffer whiskers than terrestrial mammals (Dougill et al., 2020). However, this needs to be investigated in more species to ensure that these patterns hold true across a broad range of mammalian species.

Despite Dougill et al.'s (2020) analysis method being a succinct way to compare whiskers between species, the process of plucking, labelling, scanning and analysing every single whisker can be time-consuming (taking around one day per specimen) and also carries its own inherent variability. For example, whiskers cannot be compared like-for-like, since they vary in number and position on a species' mystacial pad (Ahl, 1986). Furthermore, individual whiskers can be missing, at different stages of growth or damaged (Dehnhardt & Hanke, 2018; McHuron et al., 2019), so it is rare to capture all the whiskers on a mystacial pad. In addition, availability of samples may drastically increase if only one or two whiskers were needed for comparison, as they could be extracted from museum skin samples or even from live animals. In this article, we make recommendations for studying whisker morphology comparatively. We first describe whisker morphology in red fox (*Vulpes vulpes*), Eurasian otter (*Lutra lutra*) and grey seal (*Halichoerus grypus*), comparing within and between species differences. We go on to describe whisker shape in a further 21 species of Carnivora from a variety of different samples, including skin collections, perfused examples and even live specimens. Fourteen of these species have never had their whiskers described before. We will describe shape using our approximations of curvature and taper (Dougill et al., 2020; Starostin et al., 2020) as well as traditional morphometric methods. We will explore the differences between whisker morphology of terrestrial and aquatic species, and, in agreement with Dougill et al. (2020), we hypothesise that aquatic Carnivora species will have shorter, thicker whiskers than terrestrial species.

2 | MATERIALS AND METHODS

2.1 | Sample preparation

For the first part of this study, five individual specimens of red fox (*V. vulpes*; all unregistered with the museum), Eurasian otter (*L. lutra*; museum IDs: GH40.22, GH80.22, 3x unregistered) and grey seal (*H. grypus*; museum IDs: GH72.22, GH65.22, SR53.22, SS 423/2017, 1x unregistered) were donated by National Museums Scotland. These species are representative of terrestrial, semi-aquatic and aquatic Carnivora species, respectively. All specimens were stored frozen at the museum, and once they were defrosted, the mystacial pads were

dissected from the specimens and stored in 10% formalin until further processing. After a few weeks in formalin, the most intact whisker pad was selected from each specimen. The selected pad was plucked, and each whisker was labelled in terms of whisker row (with the most dorsal whisker being labelled row A) and column (with the most caudal whisker being labelled column 1). Some whiskers showed signs of damage, which could include split or cut ends that likely occurred during the animal's life, but also included whiskers being bent during tweezer plucking. Any whiskers with signs of damage were rejected from the study. This was judged by investigating the physical specimens and scans, especially the whisker shaft to ensure a smooth shape (without any kinks), and the whisker tip to ensure a curved ending (e.g., see Figure 5), with no abrupt blunt end, or splitting. This included removing 18 red fox, 11 Eurasian otter and four grey seal whiskers. All work in this study was approved by the local ethics committee at Manchester Metropolitan University.

2.2 | Whisker shape approximations

523 whiskers were scanned from five adult individuals of red fox (*V. vulpes*), Eurasian otter (*L. lutra*) and grey seal (*H. grypus*), ranging from 16 to 41 whisker per individual. There has been no evidence for age or sex differences in whisker morphology studies, so whiskers were assumed to be sexually monomorphic throughout (also in agreement with the findings of Ginter et al. (2012) and Grant et al. (2018)). Scanning was undertaken using an Epson V600 photo-scanner (Epson, Tokyo, Japan) to gather 2-dimensional whisker shape parameters of curvature, length, width and taper. Image resolutions were between 2 and 8 microns, depending on the overall whisker size, to capture the full length from base to tip. Whiskers were laid on to the scanner and were found to lie flat in their unstressed state, though in some cases there was some out-of-plane deformation towards the distal end, which was flattened as the scanner lid was put down. While red fox and Eurasian otter whiskers had circular cross-sections, grey seal whiskers are oval in cross-section, but lay flat so that the widest axis lay in the image plane. Whisker shapes were extracted from 469 of the scanned images and processed as previously described in (Starostin et al., 2020) and (Dougill et al., 2020), using 100 key points. A full explanation of this process can be found in the supplementary online material (SM2) in (Dougill et al., 2020) (https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fjmor.21246&file=jmor21246-sup-0001-Supinfo.docx). In summary, curvature, κ, of whisker centrelines were approximated by a linear function of their arc length, s, with two coefficients A and B:

$$\kappa(s) = As + B. \tag{1}$$

Coefficient B gives the curvature at the base, whilst coefficient A denotes the rate of change in curvature from the whisker base towards the tip. Thus, if A and B are equal to zero then the whisker is straight and if A equals zero, but B does not equal zero, then the whisker is in the form of a circular arc.

When normalised, these planar model curves can be mapped onto an Euler spiral with their position on the spiral being indicative of the change in curvature from whisker base to tip. Whiskers that exhibit increasing curvature towards the tip are positioned in the first quadrant (positive x and y in Figure 3a); those with decreasing curvature from base to tip are in the third quadrant (negative x and y in Figure 3a), whilst those with a change in curvature direction (an inflection) span the axis origin (around 0, 0 in Figure 3a). By this method, whiskers from any species can be compared regardless of true size. Absolute whisker length was extracted during the curve-fitting procedure. By normalising whisker lengths against species body lengths taken from the literature, the adjusted whisker length allows for interspecies comparison.

Whisker taper was modelled using the same 100 key points. It was approximated as a linear function of whisker arc length, s, such that the half-width, w, defined as the distance between the whisker edge and its centreline, is modelled as a linear function:

$$\text{half} - \text{width}(s) = \omega_0 + \omega_1 s. \tag{2}$$

Coefficient ω_1 represents the taper gradient of the whisker (a negative value indicates a whisker, that is, thinner at the tip than the base). Coefficient ω_0 is the normalised whisker radius (half-width) at the base, termed normalised whisker base radius. Model curves for the two edges of each whisker were parameterised by the four coefficients A, B, ω_0 and ω_1 (normalised for whisker length = 1). They were fitted to whisker coordinate data using a nonlinear fit procedure in Maple (Waterloo Maple, Ontario), minimising the sum of squared distances between the whisker edge coordinates and the model curves (Dougill et al., 2020); the quality of the approximation is estimated by residual standard deviation (RSD).

2.3 | Establishing whisker measurement recommendations

Measures of curvature (A and B), whisker length and width/taper (ω_0 and ω_1) were used in the analysis of our whisker shape approximations. To reduce the dimensionality of the data, a PCA was conducted on these measures. The effect of species (red fox, Eurasian otter and grey seal) and individual (5 of each species) was investigated using multivariate analysis of variances (ANOVAs), and effect sizes (partial-eta squared) were reported. A whisker position allocation (using row and column position) was assigned to each whisker and normalised such that the most rostral and dorsal whisker of that individual had a zero score for row and column, and the most caudal and ventral whisker had a one score for row and column. Adjusted row and column position, and whisker length, were correlated to PC1 and PC2 to investigate which whiskers captured the most differences, and stepwise regression models were also constructed to make recommendations about whisker selection for further studies. The coefficient of variation (standard deviation divided by the mean) was calculated for each species and individual mean values of PC1 and PC2.

Following recommendations from the first part of this study, a further 21 adult species were then studied, with a total of 45 whiskers scanned. 18 species were obtained from spirit, frozen and skin specimens at National Museums Scotland (Table 1). Long, caudal, shed whiskers from a live domestic cat, _Felis catus_, and a perfused domestic ferret, _Mustela furo_, from the University of Nottingham laboratories were also donated to the study. Each species was coded for their general substrate preferences: Terrestrial, Aquatic and Semi-aquatic (Table 1). Two of the longest, caudal whiskers were plucked from each specimen (one specimen per species, including selected specimens of red fox (_V. vulpes_), Eurasian otter (_L. lutra_) and grey seal (_H. grypus_) used in the first part of this study); apart from the domestic cat, which were shed whiskers from a live animal. Frozen and perfused specimens had their mystacial pads dissected, fixed in 4% paraformaldehyde for a minimum of 24 h and stained in 1% Lugol's solution for 48 h before individual whiskers were plucked from the pad. This enabled the whiskers to be imaged clearly during scanning. Whiskers from shed and skin specimens were not stained, and were just plucked directly from the specimen, since the skin specimens had to be tested non-destructively for the museum. Any whiskers with signs of damage were rejected from the study, which included removing one of the whiskers from aardwolf (_Proteles cristatus_). Similarly, only one whisker was plucked from the California sea lion (_Zalophus californianus_) specimen, since the other whiskers were tightly attached and might have caused tearing of the skin.

TABLE 1 Specimen details.

Species	Common name	Family	Source	Specimen ID	Substrate Preference	Whiskers plucked
Arctocephalus pusillus	South African fur seal	Otariidae	Skin	Unreg.	Aquatic	B1, C1
Zalophus californianus	California sea lion	Otariidae	Skin	Z.2003.41.2	Aquatic	E1
Cystophora cristata	Hooded seal	Phocidae	Skin	Z.1914.107	Aquatic	B1, C1
Halichoerus grypus	Grey seal	Phocidae	Frozen	SS 423/2017	Aquatic	E1, F1
Pusa hispida	Ringed seal	Phocidae	Skin	Z.1928.31	Aquatic	D1, E1
Phoca vitulina	Harbour seal	Phocidae	Frozen	M324/18	Aquatic	E1, F1
Leptonychotes weddellii	Weddell's seal	Phocidae	Skin	Z.1905.167.8	Aquatic	C1, D1
Mirounga angustirostris	Northern elephant seal	Phocidae	Skin	Z.1908.170	Aquatic	C1, C2
Lutra lutra	Eurasian otter	Mustelidae	Frozen	Unreg.	Semi-Aquatic	H1, I1
Neogale vison	American mink	Mustelidae	Spirit	Unreg.	Semi-Aquatic	D1, E1
Mustela nivalis	Weasel	Mustelidae	Spirit	Unreg.	Terrestrial	D1, E1
Mustela furo	Domestic ferret	Mustelidae	Perfused	n/a	Terrestrial	D1, E1
Mustela erminea	Stoat	Mustelidae	Spirit	Unreg.	Terrestrial	E1, F1
Vulpes vulpes	Red fox	Canidae	Frozen	Unreg.	Terrestrial	I1, J1
Felis catus	Domestic cat	Felidae	Live	n/a	Terrestrial	Unknown
Panthera Leo leo	Lion	Felidae	Skin	Z.1924.128	Terrestrial	B1, C1
Panthera pardus	Leopard	Felidae	Skin	Unreg.	Terrestrial	B1, C1
Panthera onca	Jaguar	Felidae	Skin	Z.2004.208.1	Terrestrial	C1, D1
Panthera tigris altaica	Siberian tiger	Felidae	Skin	Z.2005.114.2	Terrestrial	C1, D1
Arctictis binturong	Binturong	Viverridae	Skin	Unreg. Thrigby	Terrestrial	C1, D1
Suricata suricatta	Meerkat	Herpestidae	Frozen	GH 49.19 986	Terrestrial	D1, E1
Hyaena hyaena	Striped hyaena	Hyaenidae	Skin	Z.2004.45	Terrestrial	C1, C2
Parahyaena brunnea	Brown hyaena	Hyaenidae	Skin	Unreg. How101192	Terrestrial	A1, D1
Proteles cristatus	Aardwolf	Hyaenidae	Skin	Unreg. PH37/98	Terrestrial	C1, D1

Note: One specimen of each species was obtained from National Museums Scotland's frozen, spirit and skins collections. Two whiskers, where possible, were plucked from each specimen. Otherwise, whiskers were donated from a live domestic cat that had shed its larger caudal whiskers, and from a perfused (paraformaldehyde) laboratory ferret. Species substrate preferences were obtained from the literature. Specimen ID is the museum log number, unregistered (Unreg.) or not applicable (n/a). Whiskers plucked were allocated grid positions with a letter for row (where A is most dorsal), and a number for column (where 1 is most caudal); although these are rough estimates as it can be challenging to estimate grid orientation, and whiskers can be missing from the pad, especially in the skin collection.

Whiskers were scanned and analysed using the procedure above, to extract measures of curvature (A, B), length (mm) and width/taper (ω_0, ω_1). Unlike the other groups, pinniped whiskers are oval in cross-section, but tended to lay flat so that the widest axis lay in the image plane. Since the size of the animals drastically varied between our sample (i.e. from weasel, *M. nivalis*, to elephant seal, *Mirounga angustirostris*), whisker length was normalised to body length (without tail), to give the metric adjusted whisker length. Many of our species, and especially the pinnipeds, show large sexual dimorphism. Therefore, we investigated the effect of body length estimates (male and female) on the adjusted whisker length (Figure S1). We observed that the different adjustments with body length did not significantly alter any of our statistical interpretations (i.e., when we investigate the association of different whisker metrics with substrate preference; Figure S1). Therefore, we chose to use an average (middle) value of body length for each species for the adjusted whisker length metric. The metric, ω_0, was already normalised to whisker length via the earlier calculation processes. The presence of undulations on the whisker shaft was also noted as present or absent from the scanned images. However, since these undulations are only found in phocids, they were not included in any further statistical analyses.

2.4 | Presence of whisker scales

Following 2D scanning, each of the 45 whiskers were imaged in a Field Emission Scanning Electron Microscope (Supra 40VP scanning electron microscope, Carl Zeiss Ltd.). Whiskers did not need to be coated in gold as they provided clear images without sputter coating. Images were taken at three scanning locations: The tip, a point one-third along the shaft from the tip, and a point two-thirds along the shaft from the tip at seven different magnifications (at 250, 500, 1000, 2500, 5000, 10,000 and 20,000 times magnification). The presence and absence of scales was recorded at each scanning location and quantified on a scale from 0 to 3. A maximum score of 3 indicates that scales were present at each location, whereas zero would be no scales present at all. Scales showed signs of wear from the tip first in all our specimens, so that a score of 1 in all instances indicated that scales were present at a point two-thirds from the tip (near the base), and a score of 2 indicated that scales were only absent at the tip. A pairwise Spearman's Rank correlation was conducted to examine correlations between the presence of scales and other whisker measures (A, B, adjusted whisker length, ω_0 and ω_1).

2.5 | Whisker morphometrics

Whisker shape was also approximated using morphometric analysis, by adopting the R Package Momocs (Bonhomme et al., 2014). The whisker outlines of the scanned images were imported into Momocs. All whiskers were aligned using a full generalised Procrustes alignment using three landmark points: The leading and trailing edge base points (above the papilla section) and one tip point. Outlines were aligned, primarily from the tip point, and were confirmed manually by eye. Outline x and y points were then approximated using elliptical Fourier transforms with 12 harmonics. A principal component analysis was then conducted on the approximated outlines, termed PCA^M, and a pairwise Spearman's Rank correlation was conducted to examine associations between the principal components and the whisker measures (A, B, adjusted whisker length, ω_0, ω_1 and scale presence).

2.6 | Statistical analysis

Species means were used for all analyses. A table of species mean results can be found in Table S1. Measures of curvature (A and B), adjusted whisker length, width/taper (ω_0 and ω_1) and scale presence were used in the analysis of our whisker shape approximations. To reduce the dimensionality of the data, a PCA was conducted on the normalised version of these measures, termed PCA^A. All these measures, alongside the morphometric principal component analysis, were compared between substrate preferences (terrestrial, semi-aquatic and aquatic species), using multivariate ANOVAs with a significance level of $p < .05$. PCA loadings were used to examine associations between the principal components and the whisker measures.

To account for the nonindependence of data points due to phylogenetic relationships, statistical tests were also conducted using phylogenetic comparative methods. A carnivoran consensus tree and posterior distribution of 10,000 trees was downloaded from the 10kTrees website (https://10ktrees.nunn-lab.org/). Maximum likelihood ancestral state conditions for whisker parameters and their 95% confidence intervals were calculated on the consensus tree, using the 'fastAnc' function of the R package 'phytools' (Revell, 2012). The strength of phylogenetic signal present in whisker parameters across the 10,000 trees was calculated as Pagel's lambda (λ), using the 'phylosig' function of phytools. A lambda (λ) value of 1 means all the difference in morphology has accumulated directly in proportion to the phylogenetic distance between the species along the branch with true Brownian motion. Therefore, a strong phylogenetic signal, indicating that the trait is evolving by Brownian motion, is indicated by a λ-value close to 1 and a *p*-value < .05. Potential differences in whisker parameters (adjusted length, A, B, ω_0, ω_1 and scale presence) between substrate groups were tested on species means using a phylogenetic multivariate analysis of variance (MANOVA) using the 'aov.phylo' function of the R package 'geiger', with a Wilks test statistic (Harmon et al., 2008). This technique first calculates a MANOVA test statistic, and obtains a null distribution of the test statistic by simulating new sets of dependent variables on the phylogenetic tree (under a Brownian motion model; Harmon et al., 2008). Tests were run with 1000 simulations and were conducted across the 10,000 trees, to incorporate uncertainty in tree topology, and mean test statistics were calculated. A phylogenetically corrected PCA was conducted using the 'phyl.pca' function of phytools, using a

correlation matrix to account for the varying scales of the input data. This technique is a PCA-like ordination that takes into account the phylogenetic nonindependence between species (Revell, 2009). The major axes of this PCA space are not the major axes of shape variation, as per an ordinary PCA, but the major axes of the non-phylogenetic residual variation once phylogenetic covariation has been removed (Polly et al., 2013).

3 | RESULTS

3.1 | Species and individual differences in whisker morphology

Linear models of curvature and taper were good fits in all cases, with low RSD between model curves and scanned whisker shapes (mean: 0.23% of whisker length SD: 0.23%). Significant between-species effects were observed in all whisker metrics (Table 2), with the aquatic grey seal having larger values of A and ω_0 than the red fox and Eurasian otter, the terrestrial red fox having larger values of B and ω_1 than grey seal and Eurasian otter, and the Eurasian otter having the shortest whiskers overall.

TABLE 2 Multivariate ANOVA results for species and individual differences.

	F	p	Effect size ($\eta^2 p$)	Post hoc
Species	**F(2,46)**			
PC1	23.62	<.001	.186	F > S,O
PC2	53.10	<.001	.092	F > S > O
A	22.97	<.001	.090	S > F,O
B	16.32	<.001	.065	F > S,O
Length	25.40	<.001	.098	O < S,F
ω_0	97.87	<.001	.296	S > O > F
ω_1	40.36	<.001	.148	F > O > S
Individual	**F(14,454)**			
PC1	6.65	<.001	.17	-
PC2	13.07	<.001	.29	-
A	5.77	<.001	.15	-
B	8.12	<.001	.20	-
Length	9.19	<.001	.22	-
ω_0	19.35	<.001	.37	-
ω_1	8.63	<.001	.21	-

Note: Effect size is partial-eta squared, where $\eta^2 p$ of = .01 indicates a small effect, $\eta^2 p$ = .06 indicates a medium effect and $\eta^2 p$ = .14 indicates a large effect. Tukey pairwise comparisons are presented for the species (F = red fox, S = grey seal, O = Eurasian Otter), but it is not possible to succinctly summarise here results for the 15 individuals (-), although there were no significant differences between individuals of the same species.
Abbreviation: ANOVA, analysis of variance.

Principal component analyses of the metrics A and B (curvature), whisker length, and ω_0 and ω_1 (width/taper) explained the variance of the whisker morphology data for red fox, Eurasian otter and grey seal by 42.0% (PC1), 35.5% (PC2) and 13.0% (PC3). Principal component loadings (loadings ≥ 0.5) indicated that PC1 was mainly explained by whisker length (0.50) and width/taper (ω_0: −0.52, ω_1: 0.58), PC2 by whisker curvature (A: −0.61, B: 0.65) and PC3 by length (0.86) (PCA loadings and eigenvalues can be seen in Table S2a). Focussing now on the main principal components (PC1 and PC2), we can see that these vary by species (Figure 1a, Table 2). A multivariate ANOVA shows that species had a large effect ($\eta^2 p$ = .186) on PC1, with red fox having significantly larger PC1 values than both Eurasian otter and grey seal (Figure 1a, Table 2). Species also had a moderate effect on PC2 ($\eta^2 p$ = .092), with red fox having significantly larger PC2 values than Eurasian otters, which also had significantly larger values than grey seal (Figure 1a, Table 2).

There were also individual effects too, with individuals having large, significant effects ($\eta^2 p$ > .14, Table 2) on both PC1 and PC2 (Figure 1c,e). However, no individual had significantly different values of PC1 or PC2 (or any other measured metric) from another individual of the same species. Only between-species differences could be observed within the individuals. Indeed, looking at the scatterplots in Figures 1b,d,f, it can be seen that no one individual stands out as having particularly different whisker PC values from any others of the same species. Furthermore, when the coefficients of variation were calculated, inter-species differences were much larger (PC1 = 5.81, PC2 = 16.29), than any individual differences for each species, including red fox (PC1 = 0.29, PC2 = 0.76), Eurasian otter (PC1 = −3.54, PC2 = 0.88) or grey seal (PC1 = −1.61, PC2 = −0.82).

PC1 was significantly correlated to whisker length and whisker column position, with longer and more caudal whiskers (lower column numbers) having higher PC1 values (Table 3), suggesting that more caudal whiskers are both longer and thicker. In agreement, a stepwise linear regression significantly included both whisker length and column position into a model to predict PC1 (adjusted r^2 = .525, p < .001), and excluded row position. PC2 was significantly correlated to row position (Table 3), with more dorsal whiskers (lower row letters) having higher values of PC2. Similarly, a stepwise linear regression significantly included only row position into a model to predict PC2 (adjusted r^2 = .021, p = .001), and excluded both whisker length and column position; however, the adjusted r^2 values are much lower for this model.

3.2 | Summary and recommendations for whisker selection

Significant between-species differences can be observed (Table 2) between red fox, Eurasian otter and grey seal, especially at high values of PC1 and PC2 (Figure 1a within the dotted box in the top, right quadrant of the plot). There are also differences between individual specimens, although individuals of the same species tended to be more similar to each other than to individuals of other species
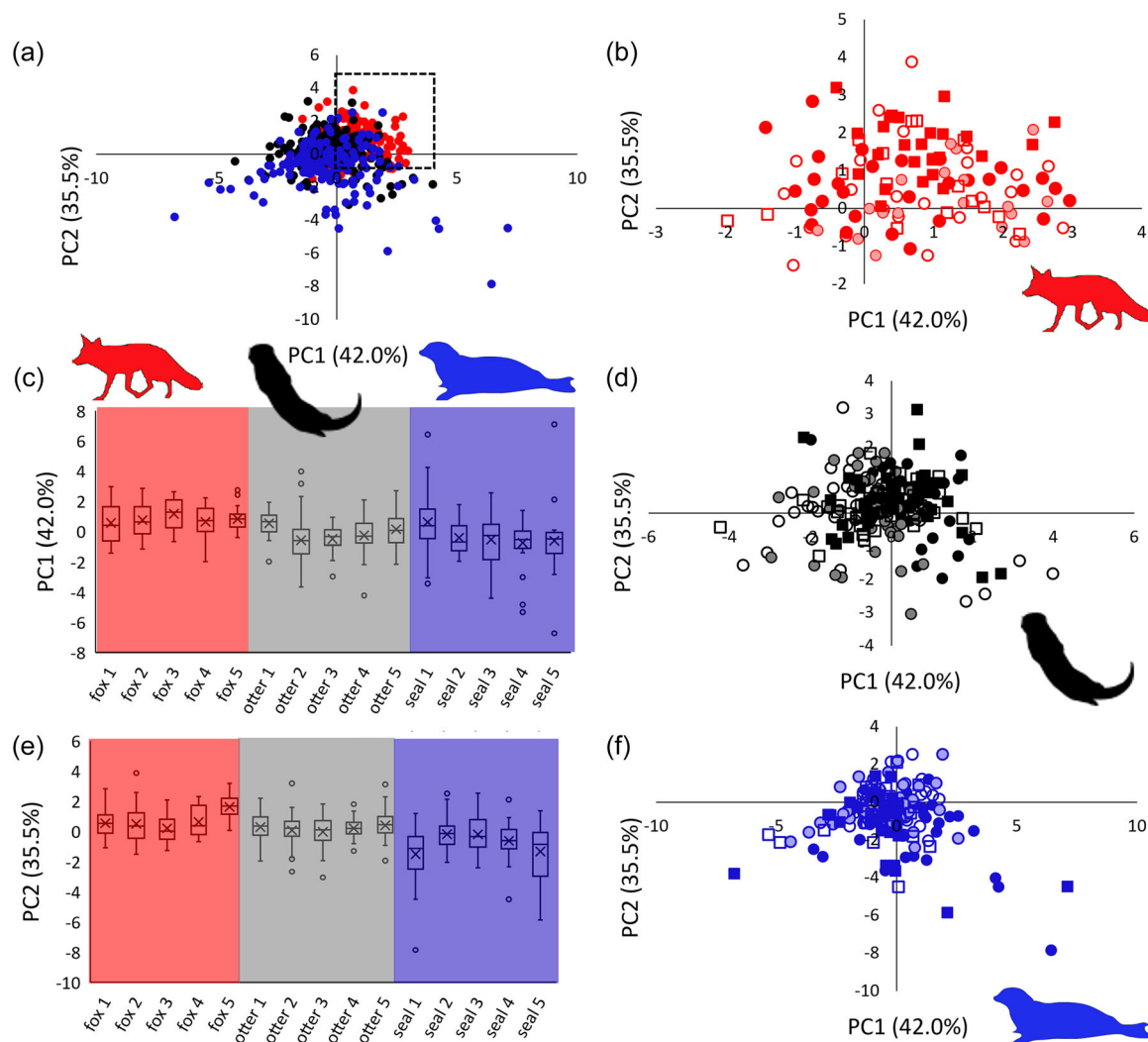
FIGURE 1 Principal component analysis (PCA) of whisker morphology metrics in red fox (*Vulpes vulpes*, in red), Eurasian otter (*Lutra lutra*, in black) and grey seal (*Halichoerus grypus*, in blue). (a) shows a scatterplot of PC1 and PC2 for all species. There are between-species differences in both PC1 and PC2, which are most clear within the dotted box, in the top, right-hand quadrant of the plot. Individual differences for each species can be seen in panels (b), (d) and (f). Box plots of PC1 and PC2 per individual can be seen in panels (c) and (e), respectively. Different individuals are indicated by different markers on the plot (colours and shapes).

TABLE 3 Spearman's correlation test of whisker length and position (column and row), with PCA1 and 2.

n = 469	PC1		PC2	
	r	p	r	p
Length	.719	<.001	.027	.559
Adjusted Column number	−.423	<.001	−.052	.264
Adjusted row number	−.053	.256	−.154	.001

(Figure 1b,d,f), with no significant differences in any measured metric. However, there is variation in whiskers in individuals and we suggest that this is mainly due to whiskers varying over a pad, that is, increasing in length and width towards the most caudal whiskers. Given the larger prediction of variance of the data of PC1 (42.0%) and the good regression model prediction of whisker length and column

position (Adjusted r^2 = .525), we would recommend that the longest, caudal whiskers should be selected for further comparisons, especially to investigate terrestrial, semi-aquatic and aquatic differences, such as we do here. This agrees with our inspections, by eye, of the shapes of the whiskers, that the long, caudal whiskers appear to vary more between species than the shorter rostral whiskers (see examples in Figure 2). When we calculate the coefficient of variation on a long, caudal whisker selected from each individual, inter-species differences were also larger (PC = 0.92, PC2 = −2.85) than any individual differences for each species, including red fox (PC1 = 0.51, PC2 = −2.79), Eurasian otter (PC1 = 0.52, PC2 = 0.55) or grey seal (PC1 = 0.72, PC2 = −0.95). If it is challenging to judge which is the longest of the most caudal whiskers, then we would recommend selection of a more dorsal whisker, which may perhaps give higher values of PC2 as well. Given our findings here, we will go on to apply this suggestion by plucking only two long, caudal whiskers from a
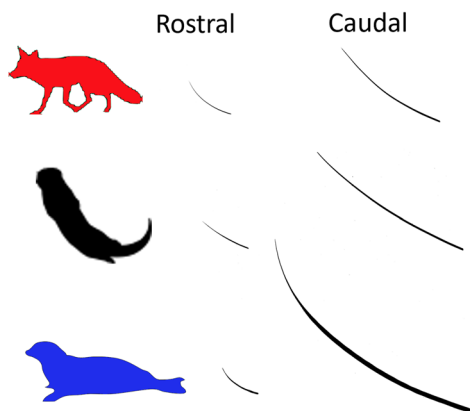
FIGURE 2 Examples of rostral and caudal whiskers from red fox (*Vulpes vulpes*, in red), Eurasian otter (*Lutra lutra*, in black) and grey seal (*Halichoerus grypus*, in blue). Lengths to scale.

broader range of species to further investigate whisker morphology variation in the Carnivora.

3.3 | Whisker base radius, length and scale presence differs between substrate preferences

Henceforth, only the longest, caudal whiskers were analysed in 24 species of Carnivora, usually corresponding approximately to whisker positions B1, C1, D1 or E1 (Table 2). Linear models of curvature and taper were a good fit in all cases, with low RSD between model curves and scanned whisker shapes (mean: 0.28% of whisker length SD: 0.24%). These linear relationships reveal that whiskers map well on to an Euler spiral (Figure 3a, Table S1), indicating that whiskers can either linearly increase or decrease in curvature along their length, or are s-shaped. Figure 4 presents a heatmap summary of the measured whisker metrics, plotted next to a phylogenetic tree, where hot colours correspond to high values, and white corresponds to low values. A highly significant phylogenetic signal was detected in adjusted whisker length (λ = .97, p = .002) and whisker base radius ω_0 (λ = .97, p < .001), suggesting that more closely related species had more similar values for these metrics (Figure 4). No signal was detected in curvature values A (λ = .31, p = .05) or B (λ = .41, p = .08), nor taper gradient ω_1 (λ = .15, p = .55) or scale presence (λ = .30, p = .22), suggesting that more related species did not have more similar values for these metrics (Figure 4). A phylogenetically uncorrected MANOVA found overall significant differences in whisker measures between substrate preferences ($F(2,21)$ = 2.55, p = .016). However, when the MANOVA was repeated whilst accounting for phylogenetic nonindependence of the data points, the MANOVA was not significant ($F(2,21)$ = 3.87, Wilks' lambda = .17, p = .67). This is likely due to the closely related nature of our sample groups, where all aquatic species were pinnipeds, and all semi-aquatic species were mustelids.

Nevertheless, we continued to conduct subsequent univariate ANOVAs on the phylogenetically uncorrected data, and found

the Euler spiral approximations of whisker curvature A and B did not significantly vary between species' substrate preferences (Figure 3c,e, Table 4), nor did the degree of whisker taper (ω_1) (Figure 3d, Table 4). However normalised whisker base radius, ω_0 (Figure 3b, Table 4) ($F(2,21)$ = 16.041, p < .001) and adjusted whisker length (Figure 3f, Table 4; $F(2,21)$ = 5.451, p = .0124) significantly differed between substrate preferences. Figures 3 and 4 both show that aquatic species had shorter whiskers that were thicker at the base, compared to terrestrial species, with semi-aquatic species being somewhat intermediary.

The presence of scales also significantly varied with substrate preferences. On the whole, there were fewer or absent scales in aquatic species compared to terrestrial species (Table 4, Figure 4), i.e. compare Figure 5b (elephant seal) to Figure 5d (ferret) and 5e (jaguar). However, there were exceptions to this observation, since the aquatic harbour seal, *Phoca vitulina*, had scales along the full length of its whiskers (Figure 4, Figure 5a), and the terrestrial red fox, *V. vulpes*, did not have scales on its whiskers (Figure 5f). When species had intermediate scores for scale presence, scales were present at the point two-thirds along the length, but absent from the tip, or the tip and a point one-third along the length (i.e. in American mink, Figure 5c); perhaps indicating that scales are removed from the tip first, and then along the length of the whisker. Whisker scale presence was significantly correlated to adjusted whisker length (Spearman's Rank: r = .494, p = .014) and normalised whisker base radius, ω_0 (r = −.461, p = .023), suggesting that long, slim whiskers had more scales than short, thick whiskers.

3.4 | Principal components analysis using shape approximations and morphometric approaches

A PCA was conducted on the species mean whisker shape approximation measures (curvature (A and B), adjusted whisker length, width/taper (ω_0 and ω_1) and scale presence), termed PCA^A. PCA^{A}1 accounted for 42.6% of the variation in the data, and PCA^{A}2 accounted for 32.3%. PCA^{A}1 was positively loaded (with loadings ≥0.5) with normalised whisker base radius, ω_0, and negatively loaded with adjusted whisker length and taper gradient, ω_1 (Table 4). This meant that short, wide, highly tapered whiskers have higher values of PCA^{A}1, and long, slim, less tapered whiskers have lower values of PCA^{A}1 (Figure 6a). PCA^{A}2 was positively loaded (loadings ≥ 0.5) with curvature value A and negatively loaded with curvature value B. PCA^{A}1 and PCA^{A}2 both significantly varied with substrate preference (Table 4), such that aquatic species had higher values of both PCA^{A}1 and PCA^{A}2 compared to terrestrial species, occupying the top right quadrant of Figure 6a (PCA loadings and eigenvalues can also be seen in Table S2b). This suggests that, overall, aquatic species tended to have whiskers that were wider, shorter and more tapered compared to those of terrestrial mammals (agreeing with our findings in Figure 3).

We conducted the above analysis using a phylogenetic PCA (which investigates non-phylogenetic residual variation once
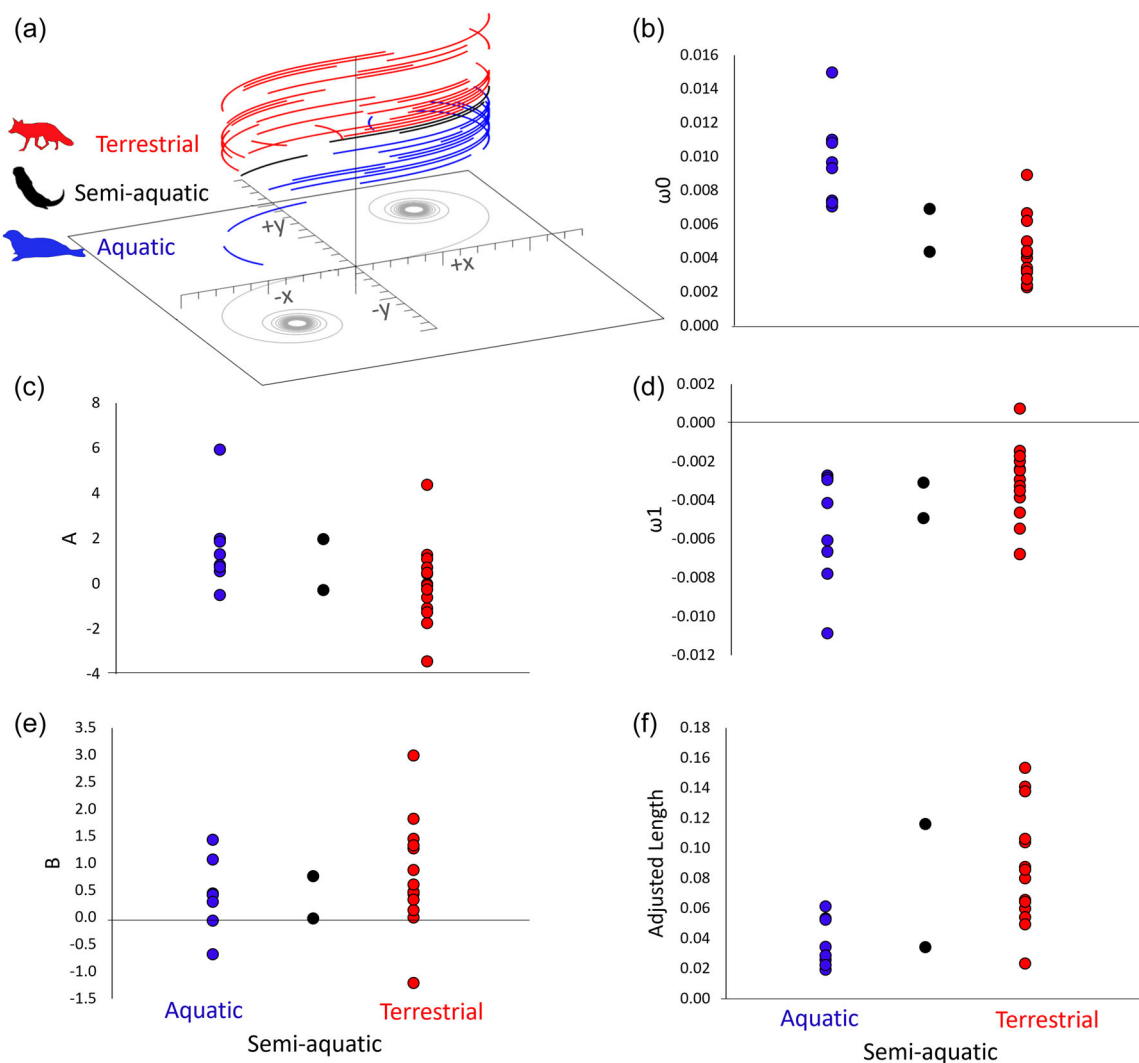
FIGURE 3 Whisker metrics. In all species, whisker curvature mapped well on to an Euler spiral. Panel a shows shapes of individual whiskers (1–2 per species) mapped on to the Euler spiral. (b–f) Show the distribution of whisker metrics, where each point corresponds to the mean for a particular species, with species grouped by aquatic, semi-aquatic and terrestrial locomotion types. Whisker curvature (A and B) did not vary significantly between aquatic and terrestrial species (c and d) ($p > .05$). Whisker width/taper (ω_0 and ω_1, b and d) and adjusted whisker length (f); ω_0 and adjusted whisker length significantly varied between aquatic and terrestrial species ($p < .05$). A, B, ω_0 and ω_1 are all normalised to a whisker length of 1, whereas adjusted (whisker) length is normalised to a mean body length value of the species. Red corresponds to terrestrial species, black to semi-aquatic species and blue to aquatic species. A, B, ω_0 adjusted (whisker) length are all proportions, and ω_1 is dimensionless.

phylogenetic covariation has been removed), and found that PCA^{A}1 accounted for 43.0% of the variation in the data, and PCA^{A}2 accounted for 26.8% of the variation in the data. PCA^{A}1 scores were not significantly different between substrate preferences (Table 4, $F(2,21) = 9.14$, $p = .09$). However, substrate preferences differed significantly on the basis on PCA^{A}2 scores ($F(2,21) = 3.35$, $p < .001$), which were positively loaded (loadings ≥ 0.5) with ω_0 and curvature value A, and negatively loaded with adjusted whisker length and curvature value B (Table 4, PCA loadings and eigenvalues can be seen in Table S2c, and a Phylomorphospace plot of PCA^A results in Figure S2). It is worth noting that in phylogenetic PCA, scores are correlated to each other (unlike standard PCA) and the axes are also different to standard PCA axes (Polly et al., 2013). Therefore, we would not necessarily expect the uncorrected and corrected PCA

results to match. However, it is encouraging that both ω_0 and adjusted whisker length were significantly affected by substrate preference in both analyses.

A PCA was also conducted on morphometric approximations of whisker shape, termed PCA^M. PCA^{M}1 accounted for 43.5% of variation and PCA^{M}2 accounted for 21.1% of variation. Substrate preference did not have a significant effect on PCA^{M}1 or PCA^{M}2 (Table 4, Figure 6b). PCA^{M}1 was positively correlated to curvature value A (Spearman's Rank: $r = .742$, $p < .001$; Figure 6b shows that high values of PCA^{M}1 were visually associated with large changes in curvature, and low values of PCA^{M}1 with small changes in curvature. PCA^{M}2 had a small negative correlation with the width value ω_0 (Spearman's Rank: $r = −.441$, $p = .031$), indicating that lower values of PCA^{M}2 were associated with whiskers that were thicker at the base.
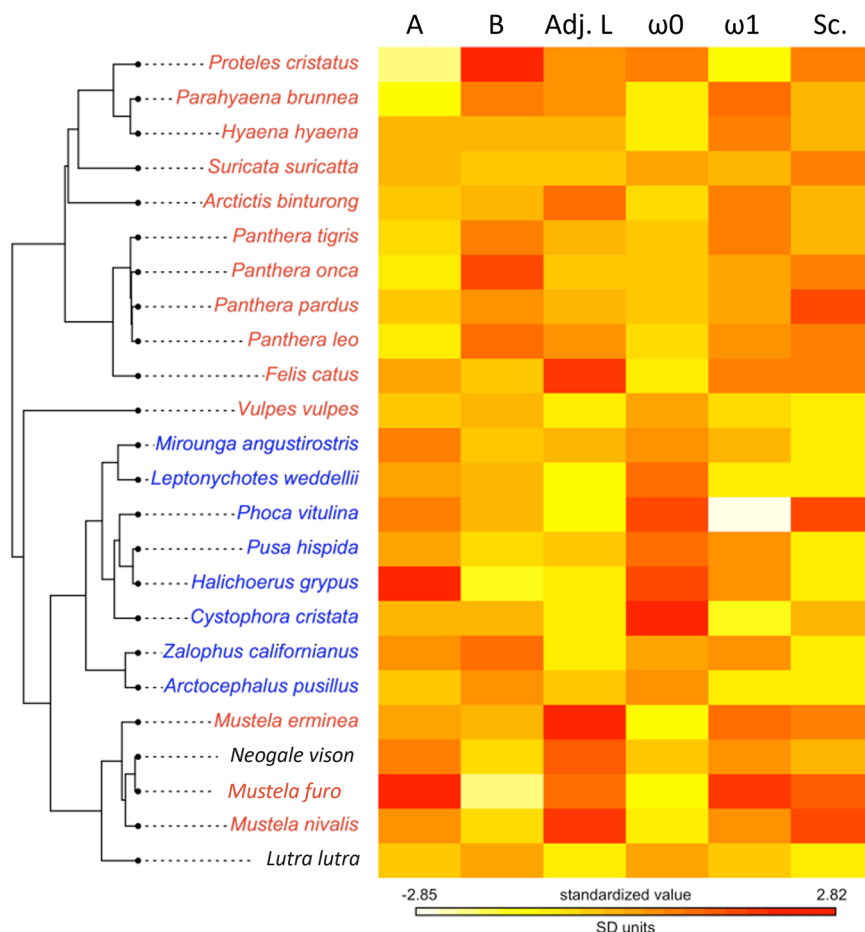
FIGURE 4 Phylogeny of the carnivorans in this study and heatmap of whisker metrics. Substrate preference is indicated as terrestrial in red, aquatic in blue and semi-aquatic in black. Values in each column of the heatmap have been standardised to have the same mean and variance, to facilitate the use of a shared colourmap. Adj. L is adjusted length, and Sc. is the presence of scales.

TABLE 4 Statistical analysis.

Sp	ω_0	ω_1	Adj. Length	A	B	Sc	PCA1[A]	PCA2[A]	PCA1[M]	PCA2[M]
Substrate effect (MANOVA): $F(2,21)$ = 2.55, p = 0.016 (phylogenetically corrected p = 0.67)										
Subsequent univariate ANOVAs:										
$F(2,21)$=	16.041	3.175	5.451	1.974	0.556	5.354	9.136	3.348	2.756	1.773
p=	<.001	.062	.012	.164	.582	.013	.001	.055	.087	.182
P(phylo)=	0.175	0.594	0.423	0.726	0.916	0.427	0.092	<0.001	n.a	n.a.
PCA loadings:										
PCA1[A]	0.556	−0.517	−0.549	−0.085	0.171	−0.291				
PCA2[A]	0.183	0.125	−0.126	0.679	−0.653	−0.217				
phylPCA loadings:										
PCA1[A]	−0.756	0.838	0.665	0.607	−0.683	0.172				
PCA2[A]	0.487	−0.045	−0.585	0.712	−0.622	−0.366				

Note: Classical statistical results (without phylogenetic corrections) are reported alongside phylogenetic comparisons ('phyl'), to explore the effect of substrate preference (terrestrial, semi-aquatic, aquatic) on whisker measures of curvature (A and B), adjusted whisker length, width/taper (ω_0 and ω_1) and scale presence, as well as principal components approximated from whisker shape analysis (PCA$_A$) and morphometric analysis (PCA$_M$). Principal component loadings are also reported for PCA$_A$.
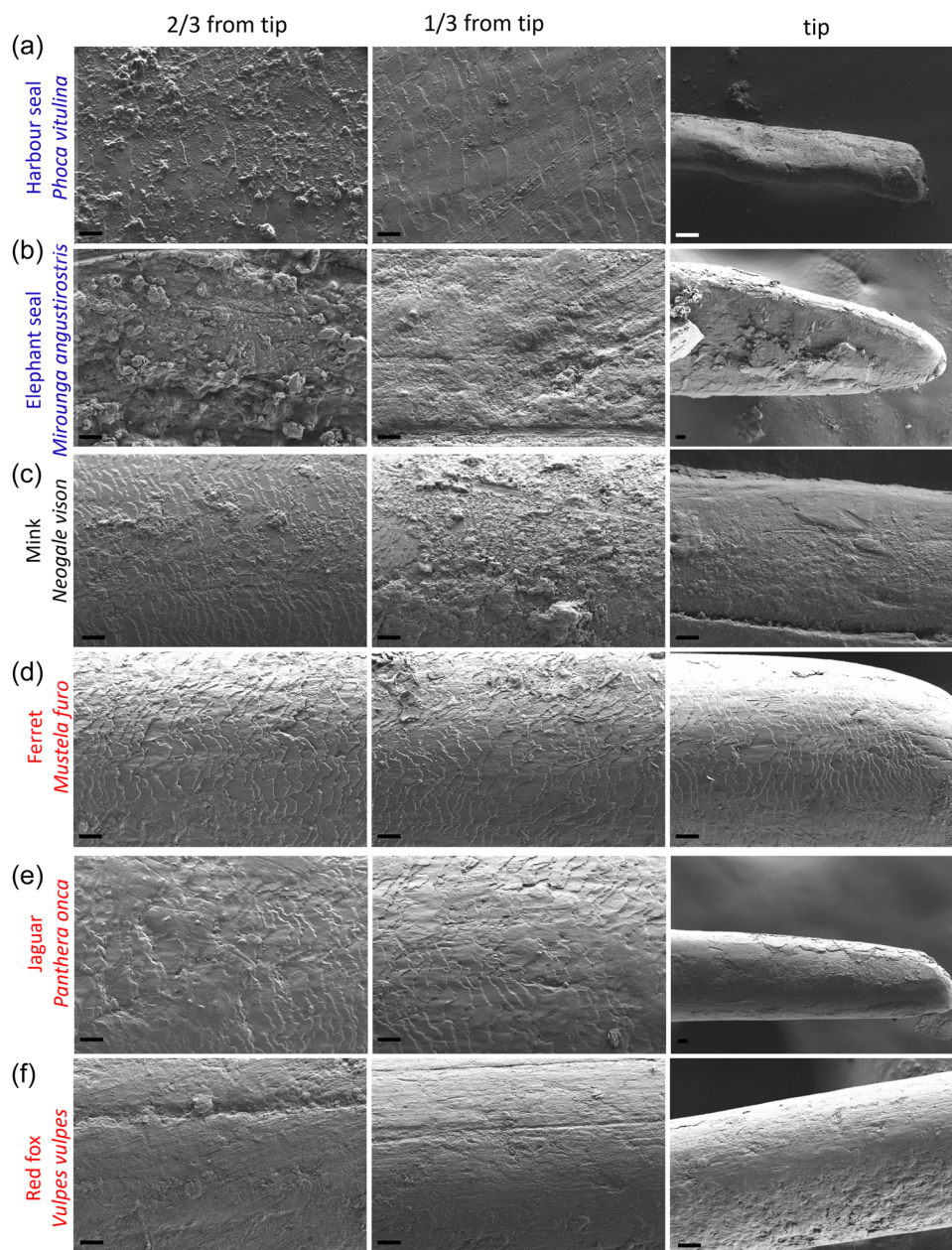
FIGURE 5 Example whisker SEM images from the whisker tip, 1/3 from tip and 2/3 from tip, in 6 example species. Most images taken at 250,000x magnification, although tip images in panel b and e were taken at 500,000x magnification. Scale bars correspond to 10 μm. Blue text corresponds to aquatic species, black to semi-aquatic and red to terrestrial. Whiskers from *Mirounga angustirostris* and *Panthera onca* were from museum skin specimens, whereas all other specimens were frozen.

4 | DISCUSSION

This study represents the largest description and analysis of Carnivora whiskers. We examined 25 species, with 14 species having their whisker shapes described for the first time, including South African fur seal (*Arctocephalus pusillus*), hooded seal (*Cystophora cristata*), Weddell's seal (*Leptonychotes weddellii*), Northern elephant seal (*Mirounga angustirostris*), domestic cat (*Felis catus*), lion (*Panthera Leo leo*), leopard (*Panthera pardus*), jaguar (*Panthera onca*), Siberian tiger (*Panthera tigris altaica*), binturong (*Arctictis binturong*), meerkat (*Suricata suricatta*), striped hyaena (*Hyaena hyaena*), brown hyaena (*Parahyaena brunnea*) and aardwolf (*Proteles*

cristatus). We can see that whisker curvature and taper in these species of carnivorans obey simple linear relationships with length. However, specific measures of whisker base radius, length and curvature vary between species and substrate groupings.

4.1 | Whisker selection

We observed large and significant differences in whisker morphology PC values between species and individuals of red fox, Eurasian otter and grey seal (Figure 1). However, individual differences only really
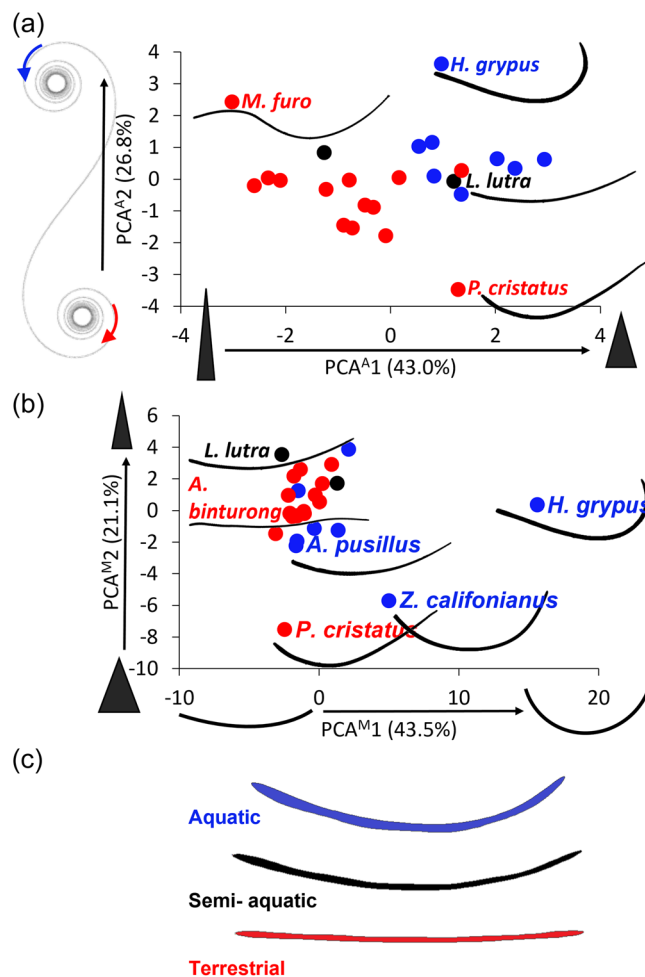
FIGURE 6 Summary measures of whisker data. (a) PCA^{A}1 accounted for 43.0% of the variation in the data and PCA^{A}2 accounted for 26.8%, and both significantly differed between terrestrial and aquatic species; (b) PCA^{M}1 accounted for 43.5% of variation and PCA^{M}2 accounted for 21.1% of variation, and both did not differ between terrestrial, semi-aquatic and aquatic species; (c) mean whisker shapes approximated by r package Momocs. These are normalised for whisker length, but show here a wide, highly-tapered aquatic whisker, and a slim, less tapered terrestrial whisker, with the semi-aquatic whisker being somewhat intermediate. PCA^A was constructed from our whisker shape approximation measures (curvature (A and B), adjusted whisker length, width/taper (ω_0 and ω_1) and scale presence, whereas PCA^M was constructed from morphometric outline analysis.

captured species differences, and PC1 and PC2 values largely overlapped between individuals of the same species (Figure 1). Variation in whisker morphology seemed to correlate with the position of the whisker on the mystacial pad. The more rostral whiskers tend to be shorter and thinner (i.e., with lower values of PC1, see also Figure 2) than more caudal whiskers. Since species-specific differences were more apparent at higher values of PC1 (Figure 1a), we recommended to compare the longest caudal whiskers between species. We propose that inter-species morphological differences manifest more readily with respect to the longer

caudal whiskers. This observation also became apparent as we plucked and handled the whiskers (Figure 2). However, if the aim is to find an average or representative whisker, then using the mean shapes in Figure 6c from morphometric analyses, or median whiskers from the PCA results might be a better approach, although all whiskers will need to be plucked and scanned to establish such a mean or median value. Plucking all whiskers is sometimes just not possible, especially when using precious museum specimens or live animals. Indeed, we show that selecting long caudal whiskers for measuring can be carried out using a variety of specimens, and provides results that are consistent with our previous morphological analyses (Dougill et al., 2020). Previous studies have also only compared only a few whiskers. For example, Milne et al. (2022) investigated whisker morphology in pinnipeds using only the two longest whiskers Williams & Kramer (2010) measured three of the longest whiskers from a skin collection to describe whisker morphology in 11 species of Carnivora and Rodentia. Indeed, in neuroscience studies often only one whisker is used for neural recordings (Lefort et al., 2009; O'Connor, Peron, Huber, & Svoboda, 2010) or mechanical measurements (Pammer et al., 2013).

4.2 | Whisker diversity in the Carnivora

In agreement with Dougill et al. (2020), we show that in the Carnivora, whisker shape varied with lifestyle (Figures 3, 6, and Table 4). Specifically, that aquatic species have whiskers that are relatively short in length and thick at the base compared to terrestrial species, with semi-aquatic species being somewhat intermediate. These findings were also supported by principal component analysis (PCA^{A}1 and PCA^{M}2). Dougill et al. (2020) suggested that thicker whiskers are stiffer and better able to maintain their shape and position during underwater sensing. However, it might be that the keratin of whiskers becomes soft during exposure to water in aquatic mammals; therefore, this additional thickness may allow aquatic whiskers to have the same stiffness underwater as terrestrial whiskers on land. However, thus far, there is no evidence for this. When Ginter-Summarell et al. (2015) tested the stiffness (EI) of dry and wet Pinniped whiskers, there were no significant differences between them (Ginter Summarell et al., 2015). Therefore, it is likely that aquatic mammal whiskers are, indeed, stiffer, although more stringent material testing could explore this in more detail. Dougill et al. (2020) showed that the whisker taper gradient (ω_1) was also significantly higher in aquatic species. Our data did not show specifically show this (Figure 3d), although the p-value was relatively low ($p = .06$, Table 4) for the effect of substrate on ω_1, and values of PCA^{A}1 (which was associated with ω_1) was significantly affected by substrate (Figure 6a); this result may become more apparent with larger sample sizes.

Our principal component analysis also suggests that curvature might vary somewhat between aquatic and terrestrial species. Although curvature values A and B are not significantly affected by substrate preferences (Figure 1b,c, Table 4), PCA^{A}2 was significantly

affected and was associated with these curvature values (Figure 6, Table 4). Figure 6a shows that most aquatic and semi-aquatic species have whiskers that increase in curvature along the whisker length and may also be more curved overall. We generated mean whisker shapes for aquatic, semi-aquatic and terrestrial species from our morphometric analysis, and they also captured our observed variations in whisker shape (Figure 6c). However, curvature might be a more variable measure. It may be affected by the degree of dehydration and freezing of the sample during preservation, and in the live animal can be actively controlled during whisker torsion (Knutsen et al., 2008). Therefore, we feel that perhaps whisker length, width and taper are more robust measures of shape in dead specimens. Furthermore, whisker scales are also not likely to be affected by preservation techniques, since we observed the presence of scales in both preserved museum skins and frozen specimens (Figure 5).

Phylogenetic statistics did not support our classical statistical results and did not show a difference between aquatic and terrestrial species (Table 4). This is likely due to substrate preference not being independent of phylogenetic relationships (all aquatic species are pinnipeds and all semi-aquatic species are mustelids; Figure 4). Therefore, phylogenetic statistics considerably reduced our effective sample size. Phylogenetic associations were found in adjusted whisker length and normalised whisker base radius (Figure 4), indicating that more closely related species have similar whisker lengths and widths, whilst no phylogenetic signal was detected in curvature parameters. The estimation of parameters, such as lambda (a measure of phylogenetic signal), has been shown to be extremely sensitive to such small sample sizes (Freckleton et al., 2002). In common with most phylogenetic comparative studies (Garamszegi & Møller, 2010), we also cannot account for any potential *intraspecific* variation in whisker morphology, caused by phenotypic plasticity, seasonality, population differences and measurement errors within a species (Ives et al., 2007). Repeating this study with more species, and incorporating intraspecific variation and parametric bootstrapping, could explore phylogenetically corrected associations better, as well as provide the opportunity to trace and date the ancestral state of the aquatic whisker morphotype—perhaps even identifying the first pinniped species with short, thick whiskers. Furthermore, while these findings may apply to aquatic Carnivora species, it is unlikely to apply to aquatic mammals more generally, since other aquatic mammals, such as cetaceans and sirenians, have rather extreme adaptations, with cetaceans having reduced or even a lack of whiskers, and sirenians having over a thousand small whiskers (Grant & Goss, 2022).

4.3 | Whisker scales and wear

The presence of scales on the cuticle surface of whiskers differed significantly between aquatic and terrestrial species, since aquatic species tended to have fewer scales than terrestrial species. (Table 4). Since there was no instance where scales were present at the tip but not the base, we propose that all whiskers might have scales initially and then, owing to wear, scales are increasingly reduced from the tip

to the base. Whisker wear has previously been documented in pinnipeds. For example, abrasion of whiskers has been noted throughout the year for harbour seals (*Phoca vitulina*; Dehnhardt et al., 2014), resulting in split and broken whiskers, and even wearing down of whisker undulations over time. McHuron et al. (2016) observed broken and worn whiskers in phocid spotted seals (*Phoca largha*) and less wear in otariid California sea lions (*Zalophus californianus*). They suggested that since otariid whiskers are smooth in profile and grow continuously, abrasion or breakage would not necessarily diminish the effectiveness of the whisker as a sensory structure (McHuron et al., 2016). However, if wear reduces both scales and undulations in phocids, this is likely to have implications for both tactile and hydrodynamic sensing. It is unknown if having smoother whiskers has any benefits to sensing, although the presence of scales on a whisker is likely to increase stick-slip interactions as a whisker moves over a surface, and will alter the mechanical signals received by the follicle (Lottem & Azouz, 2009; Zuo, Perkon, & Diamond, 2011). Mechanical and fluid dynamics models may be useful for gaining a better understanding of the role of scales along the whisker.

Wear may occur due to extended whisker contact during tactile exploration, or face rubbing on environmental objects and surfaces, as well as on the self and conspecifics. Face rubbing has been observed in otariids (Kuhn & Frey, 2012), felids (Wemmer & Scow, 1977) and canids (Fox & Cohen, 1977). If most carnivorans engage in face rubbing, we may expect their whisker wear to be similar. However, whisker scale scores varied, and were correlated with whisker length and normalised whisker base radius, ω_0, which suggests that long, slim whiskers have more scales than short, thick whiskers. That may be because the contact forces that arise when a whisker is dragged across a rough surface are lessened in the case of a more flexible whisker. Indeed, the semi-aquatic Eurasian otter (*L. lutra*) and terrestrial red fox (*V. vulpes*) both have whisker width, taper, curvature and length measurements similar to those of aquatic pinnipeds (approximated by PCA^A1 and PCA^A2, Figure 6), and both these species have no scales on their whiskers. Therefore, scale wear may be a function of environmental factors and the mechanical properties of a whisker, rather than any specific lifestyle adaptations or phylogenetic relationships (Figure 4). The whisker cuticle, where these scales are found, has a higher modulus of elasticity (Adineh et al., 2015) and Young's modulus (Quist et al., 2011), compared to the interior cortex and medulla regions of the whisker. Therefore, increased wear on the surface might affect the material properties and hence the deformation of the whisker during contact.

4.4 | Pinniped whisker shape and stiffness

Whereas most mammalian whiskers have circular cross-sections, those of phocids and otariids are better described by an ellipse (Ginter et al. 2009, 2012). This means that Pinniped whiskers are stiffer in the direction where the whiskers are thicker (along the major axis) (Ginter Summarell et al., 2015). Our scanning identified the largest width (major axis) of pinniped whiskers. However, the

radius of each axis should be taken in to account in pinnipeds before making too many inferences about stiffness, especially as the difference between the minor and major axis can be really quite variable between pinniped species (Ginter Summarell et al., 2015). Another factor that will influence whisker shape and stiffness is the presence of undulations in some phocid species (Table S1). The size of the undulations varies according to the individual whiskers, their position on the whisker, the age of the whisker, wear and the species (Dehnhardt et al., 2014; Ginter et al., 2012; Starostin et al., 2022). A previous morphometric analysis of whisker shape found that undulating phocid whiskers, smooth phocid whiskers and smooth otariid whiskers were grouped to be similar in shape (Ginter et al., 2012). Our analyses do not show such a distinction, as all aquatic pinniped species were clustered quite close together in both our principal component analyses (Figure 6). While we suggest here that aquatic mammal whiskers are stiffer than those of terrestrial mammals, for pinnipeds, more complex material testing (especially on wet and dry whiskers) and a three-dimensional approach is needed to fully compare whisker stiffness and shape between these species.

AUTHOR CONTRIBUTIONS

Gary Dougill: Conceptualisation; investigation; writing—original draft; methodology; writing—review & editing; data curation; visualization. **Charlotte A. Brassey**: Conceptualization; formal analysis; writing—original draft; writing—review & editing. **Eugene L. Starostin**: Methodology; validation; visualization; formal analysis; data curation; writing—review & editing. **Hayley Andrews**: Writing—review & editing; formal analysis; data curation. **Andrew Kitchener**: Resources; writing—review & editing; data curation. **Gert H. M. van der Heijden**: Conceptualization; funding acquisition; formal analysis; supervision. **Victor G. A. Goss**: Conceptualization; investigation; funding acquisition; writing—original draft; writing—review & editing; formal analysis; supervision; resources; project administration. **Robyn A. Grant**: Conceptualization; investigation; funding acquisition; writing—original draft; methodology; validation; visualization; writing—review & editing; formal analysis; project administration; data curation; supervision; resources.

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CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article Summary data is all provided in the tables in the manuscript and supplementary material.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.