

Cranial shape variation in mink: Separating two highly similar species

Eloy Gálvez-López¹  | Brandon Kilbourne² | Philip G. Cox¹

¹PalaeoHub, Department of Archaeology, University of York, York, United Kingdom

²Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

Correspondence

Eloy Gálvez-López, PalaeoHub, Department of Archaeology, University of York, Wentworth Way, YO10 5DD Heslington, York, United Kingdom.
Email: eloy.galvezlopez@york.ac.uk

Funding information

European Union's Horizon 2020 research and innovation program, Grant/Award Number: 835736

Abstract

European and American minks (*Mustela lutreola* and *Neovison vison*, respectively) are very similar in their ecology, behavior, and morphology. However, the American mink is a generalist predator and seems to adapt better to anthropized environments, allowing it to outcompete the European mink in areas where it has been introduced, threatening the survival of the native species. To assess whether morphological differences may be contributing to the success of the American mink relative to the European mink, we analyzed shape variation in the cranium of both species using 3D geometric morphometrics. A set of 38 landmarks and 107 semilandmarks was used to study shape variation between and within species, and to assess how differences in size factored into that variation. Sexual dimorphism in both size and shape was also studied. Significant differences between species were found in cranial shape, but not in size. Relative to American mink, European mink have a shorter facial region with a rounder forehead and wider orbits, a longer neurocranium with less developed crests and processes, and an antero-medially placed tympanic bullae with an anteriorly expanded cranial border. Within species, size-related sexual dimorphism is highly significant, but sexual dimorphism in shape is only significant in American mink, not in European mink. Additionally, two trends common to both species were discovered, one related to allometric changes and another to sexual size dimorphism. Shape changes related to increasing size can be subdivided into two, probably related, groups: increased muscle force and growth. The first group somewhat parallels the differences between both mink species, while the second group of traits includes an anterodorsal expansion of the face, and the neurocranium shifting from a globous shape in small individuals to a dorsoventrally flattened ellipse in the largest ones. Finally, the sexual dimorphism trend, while also accounting for differences in muscle force, seems to be related to the observed dietary differences between males and females. Overall, differences between species and sexes, and shape changes with increasing size, seem to mainly relate to differences in masticatory-muscle volume and therefore muscle force and bite force, which, in turn, relate to a wider range of potential prey (bigger prey, tougher shells). Thus, muscle force (and dietary range) would be

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Journal of Anatomy* published by John Wiley & Sons Ltd on behalf of Anatomical Society

larger in American mink than in European mink, in males than in females, and in larger individuals than in smaller ones.

KEYWORDS

American mink, cranium, European mink, geometric morphometrics, sexual dimorphism, shape variation

1 | INTRODUCTION

The European mink (*Mustela lutreola* Linnaeus, 1761) is a small mustelid (weasels, badgers, martens, and otters) that originally populated almost all of Europe. Its range has severely declined by over 90% since the mid-19th century and nowadays it can only be found in northern Spain and southern France, the Danube Delta, and in some areas of Ukraine and Russia (Amstislavsky et al., 2008; Maran et al., 2016). It has been classified as Critically Endangered on the IUCN Red List since 2011, and it is currently considered the second most threatened mammal in Europe (Palazón & Melero, 2014). The main cause of this decline is thought to be the invasive American mink (*Neovison vison* Schreber, 1777; Maran et al., 2016; Palazón & Melero, 2014), a closely related mustelid species native to North America but now widespread in Europe (Bonesi & Palazón, 2007). This alien pest species is in direct competition with the European mink for habitat and food resources, and their interaction usually ends in the displacement of the native species. Furthermore, the American mink is a transmitter of the Aleutian mink disease parvovirus (AMDV; fatal for the European mink) and it has been reported to prey on European mink (Maran, MacDonald, et al., 1998; Pödra et al., 2013; Sidorovich et al., 1999).

Several traits of the American mink might give it the advantage over the European mink in any competition scenario, causing the decline of the latter. For instance, American mink are larger, both in length and weight (Sidorovich et al., 1999; Wilson & Mittermeier, 2009), and thus can overcome the native species in physical confrontation. Furthermore, although the American mink has been described as a strictly carnivorous, generalist, and opportunistic predator, there is potential dietary overlap or prey competition between the two species (García Bustos, 2018; Maran, Kruuk, et al., 1998; Sidorovich et al., 2010).

So far, conservation plans focus on enhancing the survivability of European mink populations and managing American mink populations (Amstislavsky et al., 2008; Bonesi & Palazón, 2007; Maran et al., 2016; Martin & Lea, 2020; Palazón & Melero, 2014). Feeding ecology provides a control checkpoint for both strategies. However, although dietary differences have been studied, knowledge of the biomechanics of feeding in both species is lacking. The present study represents a first step in that direction, providing a thorough

analysis of cranial shape variation between both species, which will serve as a basis for future studies on feeding biomechanics in minks.

The form of a structure is closely related to its function, and many studies have demonstrated how bone size and shape variations can impact functional capabilities and behavior (Dickson & Pierce, 2019; Hautier et al., 2012; Pigot et al., 2020). This is particularly true of the skull in relation to feeding performance, especially so in predators that use their jaws to kill prey, like most mustelids (Ewer, 1973; Wilson & Mittermeier, 2009). Any change to the morphology of the skull will alter the lever mechanics of the masticatory system (Cox et al., 2012; McIntosh & Cox, 2016; Radinsky, 1981; Van Valkenburgh, 2007), thus affecting a number of important biomechanical parameters, namely: bite force, and hence the ability to deliver the kill bite and/or crush food items; maximum gape, which can alter the range of accessible prey; and stresses (i.e., internal distributions of applied forces) and strains (i.e., deformations) across the skull generated by biting or by prey struggling to escape. Skull form, therefore, has a major influence on the range of prey that can be exploited by a species, and therefore the ability of a species to thrive in a particular habitat, adopt new niches, and withstand environmental change. Several studies have been carried out in Carnivora to describe morphological adaptations to different diets in this order (e.g., Dumont et al., 2016; Gittleman & Van Valkenburgh, 1997; Goswami et al., 2011; Holliday & Steppan, 2004; Michaud et al., 2018; Radinsky, 1981; Sicuro & Oliveira, 2010; Van Valkenburgh, 2007). However, most of these studies focus on medium to large carnivorans, so it remains unclear whether the adaptations they describe also apply to small carnivorans, such as minks.

Nevertheless, feeding biomechanics is only one of the many factors related to skull shape. For instance, size and phylogenetic history tend to be the main factors influencing the shape of a structure (Gálvez-López, 2021; Goswami, 2006; Machado et al., 2018). Of those, size is particularly important in small mustelids, since these species usually present a high degree of sexual dimorphism, not only in size (e.g., Moors, 1980; but specifically for minks see Abramov & Tumanov, 2003; Palazón et al., 2006; Tamlin et al., 2009; Wiig, 1982a) but also in body proportions (Lawes & Andrews, 1987; Loy et al., 2004; Wiig, 1986). Additionally, other functions of the skull, such as protecting the brain and sensory organs, are also related to its shape (e.g., Dumont et al., 2016). Thus, in order to understand how skull shape differences between minks could be related to differences in feeding biomechanics, we also need to understand whether those differences are also related to other factors.

Unfortunately, although plenty of studies have analyzed intraspecific variation in skull size and proportions in minks (e.g., Abramov & Tumanov, 2003; Lynch & Hayden, 1995; Palazón et al., 2006; Tamlin et al., 2009; Wiig, 1982a, 1985, 1986; Wiig & Lie, 1979), the only comparison between both species is a qualitative description in Heptner et al. (2002). In that translation of an earlier work, originally from 1967, the authors described the European mink as carrying “infantile features” compared to the American mink. Additionally, all quantitative studies have been carried out with traditional morphometrics (i.e., comparing direct measurements and ratios using univariate or multivariate approaches), so no analysis of overall shape or form has been carried out. Geometric morphometric methods (GMM) allow the separation of form into size and shape (i.e., all the geometric features except for its size, position, and orientation), which, in turn, enables a deeper study on the relationship between shape and a multitude of factors. Recently, there has been an increase in interspecific studies on mustelids (Dumont et al., 2016; Law, Duran, et al., 2018; Law & Mehta, 2018), but we still have much to learn about shape variation within mustelid species. As with traditional morphometrics studies, GMM studies on intraspecific variation in cranial shape in mustelids found significant differences in cranial size both between sexes and among populations. However, results for cranial shape are varied with significant differences in shape between sexes being reported in fishers (*Pekania pennanti*; Law, 2020) and two marten species (*Martes martes*, *M. foina*; Loy et al., 2004), but no such differences being found in Neotropical otters (*Lontra longicaudis*; Hernández-Romero et al., 2015), lesser grisons (*Galictis*

cuja; Migliorini et al., 2020) or American martens (*Martes americana*; Law, 2020). Finally, some of these intraspecific studies also found significant differences in cranial shape among populations (Hernández-Romero et al., 2015; Loy et al., 2004), and between age classes (Law, 2020; Law et al., 2017).

The aim of this study is to analyze and compare cranial shape variation in both mink species in order to understand how factors such as size, sexual dimorphism, and function (particularly feeding) can be related to shape differences between European and American minks. This broad objective can be subdivided into the following research questions (RQ):

RQ1 *Are European mink more similar to American mink or European polecat?* Since both mink species have a very similar phenotype and ecology, we expect to observe similar functional adaptations in cranial shape (i.e., morphological convergence). However, since both species last shared a common ancestor about 8.6 Mya, and the European mink split from their sister group just 1.24 Mya (Law, Slater, et al., 2018), such similar cranial morphology could be a common feature to musteline species more generally. Previous studies indicate a wide diversity in cranial shape in mustelids (Dumont et al., 2016; Michaud et al., 2018), so ecomorphological convergence would be the expected cause for these similarities.

RQ2 *Can differences in cranial shape be identified between European and American mink?* The crania of both species are so similar visually, that one must rely on anatomical traits such as the appearance of the tympanic bullae (S. Palazón, pers. comm.) or the

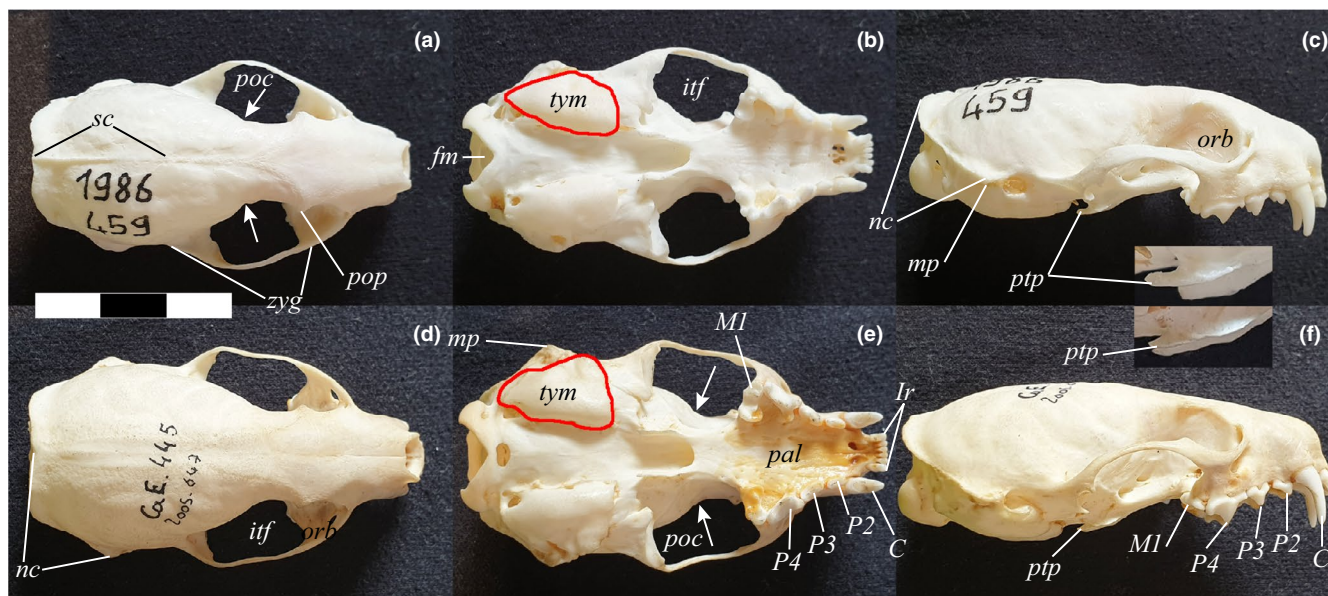


FIGURE 1 Cranial anatomy in minks. European mink MNHN 1986-459 in dorsal (a), ventral (b), and lateral views (c). American mink MNHN 2005-647 in dorsal (d), ventral (e), and lateral views (f). See Table S2 for additional information on both specimens. Scale bar is 3 cm long. Red lines represent the outline of the auditory bulla, while the small boxes provide a closer look at the pterygoid processes, both traits can be used to identify each species. Some of the anatomical features mentioned throughout the text are illustrated here using the following abbreviations: C, canine; *fm*, foramen magnum; *Ir*, incisor row; *itf*, infratemporal fossa; *M1*, molar 1; *mp*, mastoid process; *nc*, nuchal crest; *orb*, orbit; *P2–P4*, premolars 2–4; *pal*, palate; *poc*, postorbital constriction (marked by arrows); *pop*, postorbital process; *ptp*, pterygoid process; *sc*, sagittal crest; *tym*, tympanic bulla; *zyg*, zygomatic arch

pterygoid processes (E. Gálvez-López, pers. obs.) to differentiate them (Figure 1). Thus, we would expect a high overlap in shape variation between both species, which, according to Heptner et al. (2002), would occur between American mink juveniles and European mink adults.

RQ3 *Are shape differences between both mink species caused by differences in size?* Given the size differences between both species and between sexes within each species, any shape differences found between the species could be the result of common allometric shape changes.

RQ4 *Is there significant sexual dimorphism in cranial shape and size in either mink species?* While sexual dimorphism in size is expected in both mink species as it is common in mustelids, previous studies have reported different findings regarding sexual dimorphism in cranial shape, with no clear pattern arising from them. It is hoped that the results from this study will shed light on the underlying reasons.

We also wondered whether any observed shape differences between both mink species would translate into potential differences in feeding biomechanics, and, if they did, whether these differences would allow the American mink to outcompete the European mink. We expect to find some morphofunctional differences in cranial shape between both species that can be attributed to the reported differences in diet between the two species. Particularly, American mink should present larger gapes and produce higher bite forces, which would allow them to exploit a wider range of prey than European mink. We believe that this, coupled with the aggressive behavior of American mink towards the native species, could result in European mink being displaced towards food sources that they cannot process optimally.

Finally, we hope that the results of the present study would provide a basis of comparison for future intraspecific studies on cranial shape variation in minks, allowing the identification of shape features common to the mink ecotype and others exclusive to each species.

2 | MATERIAL & METHODS

2.1 | Sample

The sample consisted of 144 adult mink crania, of which 62 belonged to European minks (Mlu) and 82 to American minks (Nvi). The number of specimens of each sex and species were similar except for female European minks (Table 1), which are uncommon in museum collections. In order to capture the widest possible range of shape variation in each species, we sampled both male and female individuals from the three extant populations of European mink, and from wild and feral American mink populations (North America and Europe, respectively). Additionally, the American mink sample included individuals from different regions of their native distribution, and from different European countries (since feral populations deriving from

individuals escaped from different farms might present slightly different morphologies; Lynch & Hayden, 1995). Finally, the sample was completed with the crania of three juvenile American minks: if Heptner et al. (2002) description was accurate, we would expect those juveniles to cluster with European mink adults. Unfortunately, we were unable to find any juvenile European mink specimens.

Additionally, in order to test whether cranial shape in the European mink was more similar to the American mink or to its sister lineage, we sampled 11 specimens of European polecat (*Mustela putorius*; Mpu). Since we are not interested in the shape variation of this species, we only sampled enough individuals so that statistical comparisons could be performed.

The specimens studied were housed in the collections of the Naturhistorisches Museum Basel (Switzerland), the Museum für Naturkunde (Berlin, Germany), the Bristol City Museum and Art Gallery (Bristol, United Kingdom), the Hungarian Natural History Museum (Budapest, Hungary), the National Museum of Scotland (Edinburgh, United Kingdom), the Muséum d'Histoire Naturelle de Genève (Switzerland), the Natural History Museum at the University of Oslo (Norway), the Muséum National d'Histoire Naturelle (Paris, France), the Estonian Museum of Natural History (Tallinn, Estonia), the Department of Archaeology at the University of York (United Kingdom), and the private collection of Dr Santiago Palazón (Flora and Fauna Service, Generalitat de Catalunya, Barcelona, Spain). Catalogue numbers and additional information (sex, locality, etc.) for each specimen are available in Table S1. While browsing those collections, visual inspection revealed an unusually high number of specimens that had probably been assigned to the wrong species. We sampled 44 of those to test whether cranial shape variation could be used to classify specimens of unknown species. The methods and results for that test can be found as supplementary information (Appendix S1). Finally, we also found two juvenile polecat × European mink hybrids (one male, one female). Since European minks and polecats hybridize in the wild, we decided to scan and plot those rare specimens in the shape morphospace as a curiosity, but they were not included in the analyses.

All specimens were imaged using micro-computed tomography. Imaging was carried out at the Museum für Naturkunde (Berlin, Germany), the Natural History Museum at the University of Oslo (Norway), the Biomaterials Science Center at the University of Basel (Switzerland), the X-ray tomography facilities at the University of Bristol (United Kingdom), and ScanoMed Debrecen (Hungary). Voxels were isometric and the voxel resolution usually ranged from 0.039 to 0.050 mm, with the total number of slices per scan ranging between 1270 and 1762 (Table S2). The scans were segmented using the Dragonfly software (Version 3.7.3 for Windows; Object Research Systems Inc). The automated "split at Otsu" function was used to isolate bone material, then the selection was manually revised to verify that no scanning artefacts were included, nor bone excluded. The resulting meshes were decimated to 2 million faces in Avizo Standard Edition (Version 7.1.0 for Windows; Visualization Sciences Group). Finally, to avoid issues with semilandmark placement (see below), the decimated meshes were imported to Geomagic

Studio (Version 2013.0.1 for Windows; Geomagic Inc) to remove small spikes, holes, and tunnels using the “Mesh Doctor” function.

2.2 | Landmark configuration

In order to quantify the morphology of the skull, the 3D coordinates of 38 homologous landmarks and 107 semilandmarks were digitized on the left side of each specimen (Figure 2). The repeatability of homologous landmarks was analyzed using the intraclass correlation coefficient (Fisher, 1958; Fruciano, 2016), with detailed information on methods and results available as supplementary information (Appendix S1). Avizo was used to capture the homologous landmarks (Table 2) and five surface paths along curves. Equidistant semilandmarks were placed along each surface path (five on the orbit, 10 on the dorsal midline, eight on the nuchal crest, six on the dorsal border of the zygomatic arch, eight on its ventral border), and to create a patch of semilandmarks on the surface of the face (20 semilandmarks) and vault (50) regions of each specimen (Figure 2). Finally, based on the homologous landmarks, the semilandmarks were slid on the surfaces in order to minimize the bending energy of the thin-plate spline (Gunz et al., 2005). In other words, this process minimizes the shape variation due only to the arbitrary spacing

of semilandmarks. After the sliding, the landmark configurations of all specimens can be compared with standard GMM. All subsequent analyses were carried out using both R (Version 4.0.3 for Windows; R Core Team, 2020) and the MorphoJ software (Klingenberg, 2011). All calculations in R were carried out within the RStudio environment (Version 1.3.1093 for Windows; RStudio Team, 2020), and the following R packages were used: Arothron (Profico et al., 2021), geomorph (Adams et al., 2020), Morpho (Schlager, 2017), magick (Ooms, 2020), rgl (Adler et al., 2020), RRPP (Collyer & Adams, 2018, 2020), and stringr (Wickham, 2019). R code for all the analyses is available at <https://git.io/JCq5M>.

2.3 | Shape variation

The different landmark configurations were standardized in size, position, and rotation by performing a Generalized Procrustes Analysis (GPA; Rohlf & Slice, 1990). Before standardization, the centroid size of each specimen was calculated (i.e., the square root of the sum of the squared distances of all points of the configuration to their centroid), and this was used to represent size in all size comparisons. Then, a principal component analysis (PCA) was carried out on the Procrustes coordinates to summarize cranial shape variation in the main axes of the morphospace. This set of analyses was carried out separately for the whole sample, an adult subsample (no juveniles), and a mink subsample (adult European and American minks), with each subsample addressing different RQ.

The *whole sample* provided an exploratory framework of interspecific and ontogenetic shape variation. If interspecific differences were substantially larger than intraspecific differences (RQ1), all three species would occupy different regions of the morphospace.

TABLE 1 Sampled specimens

Species	Females	Males	Unsexed	Total
European mink (Mlu)	16	24	22	62
American mink (Nvi)	23	24	38 ^a	85 ^a
European polecat (Mpu)	6	5	—	11

^aIncludes three juvenile specimens of unknown sex.

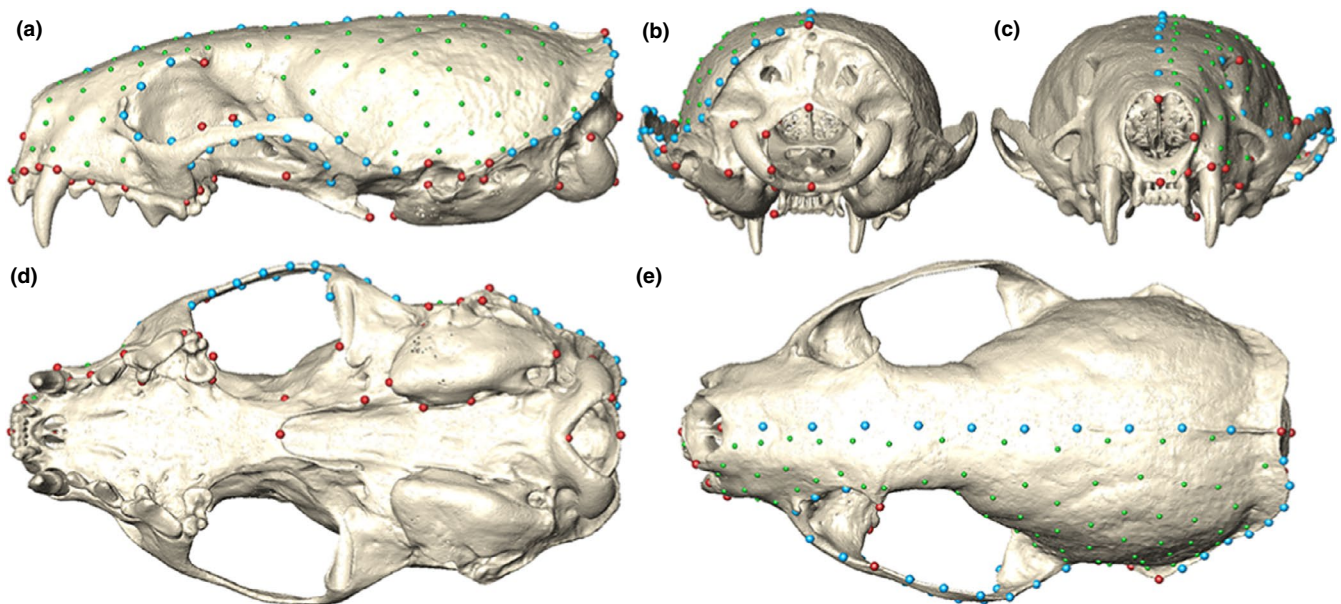


FIGURE 2 Landmark configuration. (a) lateral view; (b) caudal view; (c) cranial view; (d) ventral view; (e) dorsal view. Red dots represent landmarks (as defined in Table 2), blue dots represent semilandmarks along curves, and green dots illustrate semilandmark patches on surfaces

TABLE 2 Landmark definitions

Landmark	Definition
1	Left I1-right I1 cranial contact point
2	Cranialmost point between nasal bones
3	Mediodorsal tip of nuchal crest
4	Midpoint of dorsal margin of foramen magnum
5	Midpoint of ventral margin of foramen magnum
6	Midpoint of posterior edge of palatine torus
7	Lateralmost point of nasal opening on premaxilla
8	Most laterodorsal point of I3 alveolus
9	Cranialmost point of C alveolus
10	Lateralmost point of C alveolus
11	C-P2 lateral contact point
12	P2-P3 lateral contact point
13	P3-P4 lateral contact point
14	P4-M1 lateral contact point
15	Most laterocaudal point of M1
16	Most mediocaudal point of M1
17	Most mediocranial point of M1
18	Point of flexus between M1 paracone and protocone
19	P4-M1 medial contact point
20	Most mediocranial point of P4 protocone
21	Point of maximum curvature at palatine-pterygoid suture
22	Tip of pterygoid process
23	Cranialmost point of optical foramen
24	Tip of postorbital process on frontal bone
25	Tip of postorbital process on jugal bone
26	Point of insertion of zygomatic arch on braincase
27	Cranialmost point of external auditory meatus
28	Caudalmost point of external auditory meatus
29	Lateralmost point of mastoid process
30	Ventralmost point of mastoid process
31	Ventralmost point of jugular process
32	Most laterodorsal point of occipital condyle
33	Intersection between occipital condyle and cranial border of foramen magnum
34	Most caudoventral point of occipital condyle

(Continues)

TABLE 2 (Continued)

Landmark	Definition
35	Most mediocaudal point of anterior carotid foramen
36	Most mediocranial point of posterior carotid foramen
37	Cranialmost point of auditory bulla
38	Intersection between dorsal lip of glenoid fossa and braincase

Additionally, if American mink juveniles and adult European mink shared placement in the morphospace (RQ2), the description of paedomorphic features in European mink by Heptner et al. (2002) could be considered accurate.

The *adult subsample* aimed to address whether cranial shape in European mink was more similar to that of American mink (shared phenotype) or European polecat (closest relative) (RQ1). To distinguish between these potential scenarios, a Procrustes ANOVA by species was performed on the Procrustes coordinates of the adult subsample, and pairwise differences in mean Procrustes distances between species were calculated (Goodall, 1991). The significance was determined through randomization of residuals using permutation procedures (RRPP; Anderson, 2001; Collyer et al., 2015). The number of permutations was set to 10,000 for all analyses.

Finally, the *mink subsample* was the main focus of this manuscript. It was used to provide a detailed description of cranial shape variation in European and American mink (RQ2), and to relate potential shape differences to factors, such as size and sex (RQ3-4). Procrustes ANOVA was used to investigate the relationship between shape and size and sex, while non-parametric ANOVAs were used to analyze the effect of those factors on individual principal components (PCs) and to test for size differences between species and sexes. Non-parametric ANOVA is more robust than traditional ANOVA in unbalanced, heteroscedastic designs such as the present study (Cohen, 2001; Gálvez-López, 2021). Post hoc tests were carried out to assess pairwise differences between subgroups, and the Holm-Bonferroni method was used to adjust *p*-values to account for multiple comparisons (Holm, 1979). Again, significance in all tests was determined with RRPP (10,000 permutations). Once the effect of the different factors (species, sex, size) was analyzed individually, whenever more than one factor played a significant role in the variation of a particular variable (shape, centroid size, PCs) their interaction was further explored using Procrustes or non-parametric ANOVAs, as required. Finally, the effect of any significant interaction was quantified using phenotypic trajectory analysis (PTA), a technique that allows us to identify differences in patterns of shape change between groups in multivariate data (Collyer & Adams, 2013). When studying shape changes along two-level factors (such as sex), each pattern (or trajectory) can be defined by three parameters: location, magnitude, and orientation. Comparing the first parameter is equivalent to testing for shape differences between groups, as we just did with Procrustes ANOVA

and pairwise comparisons. The magnitude of the trajectory indicates how far apart in the morphospace are the means of the two groups, while its orientation informs us of whether both groups follow a similar (parallel) pattern of shape change, or whether they diverge, by comparing the angle between both trajectories. When one of the factors is continuous (such as size), trajectory analysis is equivalent to comparing regression slopes (magnitude) and the angle between them (orientation).

3 | RESULTS

3.1 | Morphological convergence or family-wide morphotype? (RQ1)

The first three PCs for the whole sample (adults and juveniles of all three species) explained 51.45% of total shape variation, with each species clustering in a different region of the morphospace defined by these three axes and the European polecat cluster clearly separated from the minks (Figure 3A,B and Figure S1), suggesting that cranial shape in European mink, American mink, and European polecat, is indeed different and that cranial shape is more similar between mink species than between the European mink and the European polecat (RQ1). All juveniles (hybrid or otherwise) clustered together in the same region of the morphospace, away from the adults (RQ2).

The morphospace for the adult-only subsample further confirmed these findings. The first three PCs still explained about 50% of total shape variation, and the three species were clearly separated (Figure 3C,D). Additionally, males of each species clustered at the top of each cloud and females at the bottom. The Procrustes ANOVA by species was highly significant ($F = 31.854$; $p < 0.001$), and pairwise distance comparisons between species revealed that both minks were closer together in the morphospace (i.e., more similar) than either of them was to the European polecat (Mlu–Nvi distance: 0.034; Mlu–Mpu distance: 0.061; Nvi–Mpu distance: 0.056; $p < 0.001$ for all comparisons) (RQ1).

3.2 | Cranial shape differences in adult minks (RQ2)

When analyzing the subsample containing only adult minks (i.e., excluding polecats), the first three PCs explained about 50% of total shape variation (48.02%; Figure 4, Figures S2–S4), as in previous analyses. PC1 clearly separated both species, with positive values representing American mink and negative values for European mink (Figure 4A). Accordingly, PC1 scores were significantly different between both species ($F = 1113.749$; $p < 0.001$). The shape changes along this axis indicated that, relative to American mink, European mink presented the following morphology (PC1 in Figure 4, also Figure S2): a smaller face with more rounded forehead; a shorter and wider palate; smaller P2 and M1; a longer neurocranium; less developed sagittal and nuchal crests, particularly at the mastoid processes; deeper and wider orbits; broader, shorter, and less

convex zygomatic arches; and antero-medially placed tympanic bullae, with a anteriorly expanded cranial border. On the other hand, both PC2 and PC3 represented trends in shape variation common to both species (Figure 4A,B), as no significant differences between species were found in either axis (PC2: $F = 0.117$, $p = 0.728$; PC3: $F = 0.758$, $p = 0.385$).

3.3 | Cranial size variation and its effect on cranial shape (RQ3)

The Procrustes ANOVA of size on shape was significant ($F = 12.568$; $p < 0.001$), but indicated that only 8.13% of shape variation was related to allometric changes. As for the individual PCs, PC1 was not significantly related to size ($F = 0.409$; $p = 0.526$), suggesting that the shape differences between both species are not a product of their different sizes. Conversely, both PC2 and PC3 were significantly related to size ($p < 0.001$ in both cases; $F = 54.918$ and 60.309 , respectively), with 27.89% and 29.81% of the shape variation along those axes, respectively, being associated with size variation between specimens. Finally, mean centroid size was not significantly different between both species ($F = 1.401$; $p = 0.240$).

3.4 | Sexual dimorphism (RQ4)

The Procrustes ANOVA by sex was significant ($F = 3.634$; $p < 0.001$), denoting that there were shape differences between sexes when pooling individuals from both species. As for the individual PCs, again PC1 was not related to sex ($F = 0.766$; $p = 0.385$), and PC2 was only slightly significantly related to sex ($F = 6.463$; $p = 0.013$). On the other hand, the shape changes described by PC3 were highly related to sex ($F = 20.972$; $p < 0.001$). Finally, significant differences in mean centroid size were found between pooled sexes ($F = 134.784$, $p = 0.001$).

As expected for mustelids, sexual dimorphism in size was significant within species (species–sex interaction: $F = 8.696$; $p = 0.003$), and all pairwise comparisons were also significant except between females of both species (Table 3). However, sexual dimorphism in shape was only supported for the American mink. A two-way Procrustes ANOVA by species and sex failed to recover a significant interaction between sex and species ($F = 1.607$; $p = 0.060$), but pairwise comparisons were significant between all groups except male and female European minks (Table 3). Since the interaction's p -value was almost significant and most species–sex categories were significantly different from each other, we decided to analyze it further using trajectory analysis. PTA revealed that shape differences between males and females in American mink were larger than in European mink (i.e., larger trajectory magnitude in the former: Mlu = 0.013, Nvi = 0.020; $Z = 2.486$, $p = 0.020$) (Figure 5A). Additionally, cranial shape differences between sexes were different in both species, as their trajectories had different orientations (angle = 59.61° ; $Z = 6.021$, $p < 0.001$).

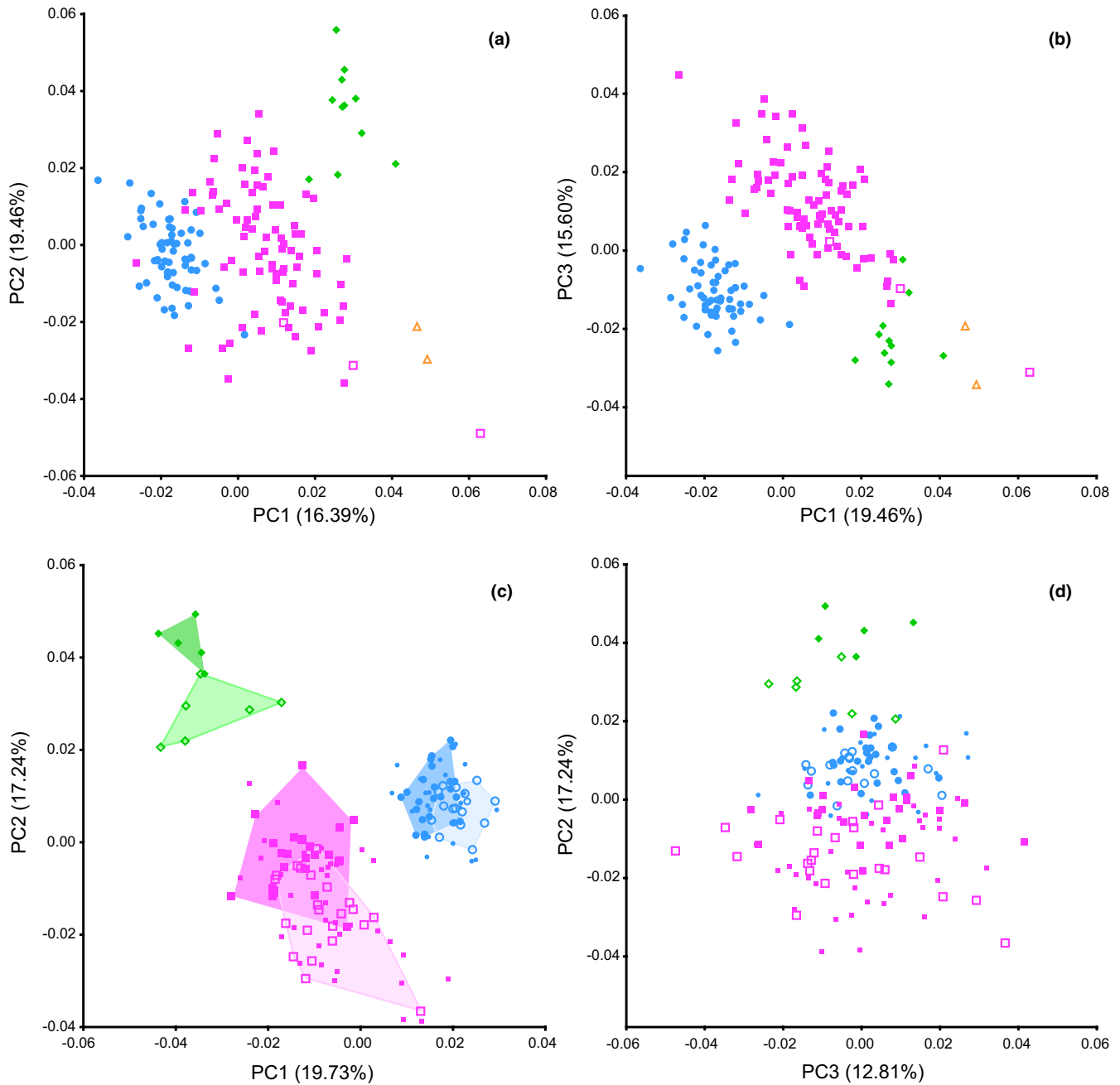


FIGURE 3 Principal component (PC) analyses of the whole sample (a, b) and the adult subsample (c, d). The percentage of total variance explained by each PC is given in parentheses. European mink are represented in blue circles, American mink in pink squares, European polecats in green diamonds, and hybrids in orange triangles. In panels (a, b) adults are represented by full symbols, and juveniles with open symbols. In panels (c, d) adult specimens of known sex are represented by large symbols (females: open; male: full), while small symbols are used for those with unknown sex. Minimum convex polygons for males (darker shades) and females (lighter shades) of each species are drawn on panel (c)

3.5 | Common trends in cranial shape (RQ2-4)

Since all studied factors (species, sex, and size) had a significant effect on cranial shape, a more thorough exploration of the potential interactions between these factors and cranial shape was carried out. A Procrustes ANOVA revealed a significant interaction between species and centroid size, indicating that shape allometry is different between mink species in both magnitude and orientation (Table 4). On the other

hand, no significant interaction was recovered between centroid size and sex (Table 4), which can be interpreted as pooled sexes of both species following parallel allometric trajectories (i.e., no differences in magnitude or orientation between the shape allometries of each sex; Table 4). Finally, the triple interaction among species, sex, and centroid size was also significant ($F = 1.682$; $p = 0.048$), but PTA did not reveal significant differences between trajectories in either magnitude ($Z = 0.097$, $p = 0.427$) or direction ($Z = -1.012$, $p = 0.837$).

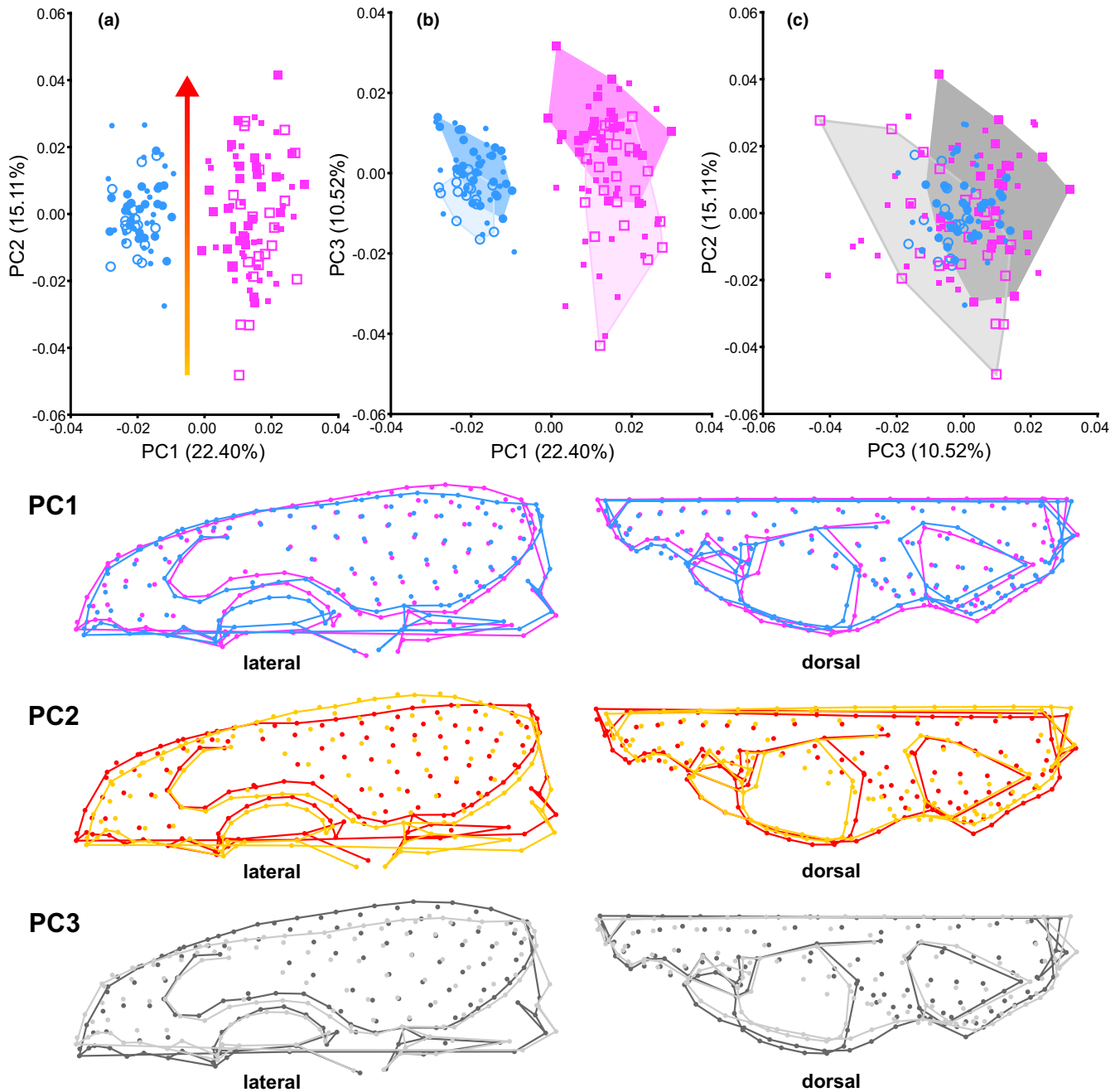


FIGURE 4 Principal component (PC) analysis of the adult mink subsample. The percentage of total variance explained by each PC is given in parentheses. European mink are represented in blue circles, American mink in pink squares, while large open symbols represent females, large full symbols males, and small full symbols unsexed specimens. The wireframes illustrate the shape of the specimen with lowest and highest score for each PC in lateral and dorsal view. The arrow in panel (a) signals that specimen size increases along PC2 (orange for small, red for large). Minimum convex polygons for males (darker shades) and females (lighter shades) are drawn for each species (b), and pooling both species (c)

Similarly, both PC2 and PC3 were significantly related to sex and centroid size. However, non-parametric ANOVAs did not reveal a significant interaction between species and sex for either PC ($F = 0.691$, $p = 0.404$, and $F = 0.947$, $p = 0.337$, for PC2 and PC3, respectively). Thus, we examined shape variation along those axes based on centroid size and sex alone. The significant relationship between PC2 and size, together with the wide overlap of male and female specimens along PC2 (Figure 4A), suggests that the shape

changes along that axis describe an allometric pattern common to both species. That would also explain the slightly significant relationship with sex, as males had significantly higher centroid size values than females in each species. With increasing values of PC2 (i.e., as size increased), we observed an antero-dorsal expansion of the face (shifting the palate anteriorly relative to the orbit) and a relative contraction of the braincase, which went from globous with a slightly sunken dorsal midline, to a dorsoventrally flattened ellipse

TABLE 3 Sexual dimorphism in size and shape

Factor	Shape	Size
Spp	13.751 (<0.001)	0.089 (0.768)
Sex	1.680 (0.048)	45.789 (<0.001)
Spp × Sex	1.607 (0.060)	8.696 (0.003)
Pairwise		
Mlu.F–Mlu.M	0.210	0.001
Mlu.F–Nvi.F	0.001	0.869
Mlu.F–Nvi.M	0.001	0.001
Mlu.M–Nvi.F	0.001	0.001
Mlu.M–Nvi.M	0.001	0.011
Nvi.F–Nvi.M	0.002	0.001

Note: Results from two-way Procrustes ANOVA (cranial shape) and non-parametric ANOVA (centroid size) with species and sex as categorical variables. For each factor, Goodall's *F* values are provided together with its associated *p*-value (in brackets). Results of post-hoc pairwise tests used to assess differences between each category are also provided, with non-significant *p*-values in grey.

Abbreviations: F, female; M, male; Mlu, European mink; Nvi, American mink; spp, species; Spp × Sex, interaction between species and sex.

with well-developed crests (PC2 in Figure 4, also Figure S3). Along with those changes, larger specimens were characterized by deeper, more forwardly oriented orbits; more laterally projected postorbital processes; wider, broader, longer, and more convex zygomatic arches; slightly larger I1-3, C and P2; a dorsally projected basicranium relative to the palate; and a narrower postorbital constriction.

In contrast to PC2, there is only a slight overlap in PC3 scores between males and females (Figure 4B). This, together with increasing PC3 values with increasing centroid size, suggests that this axis represents shape changes due to sexual size dimorphism (i.e., common sexual allometry). Additionally, since neither species nor the interaction term significantly affected PC3 scores, it is possible that these

changes correspond to a common trend between both species. As low PC3 scores correspond to female shapes, male shapes (i.e., high PC3 scores) are characterized by the following morphology (PC3 in Figure 4, also Figure S4): a well-developed sagittal crest; larger canines; wider, more convex and laterally projected zygomatic arches; narrower, deeper, and more forwardly oriented orbits; more laterally projected postorbital and mastoid processes; laterally displaced tympanic bullae; a narrower postorbital constriction; and a ventrally projected palate relative to the basicranium.

As a final note on these trends in shape variation common to both mink species, since the trends observed along PC2 and PC3 seemed to combine to create a gradient of female–male cranial shape variation while separating individuals by size (i.e., common intraspecific sexual allometry; Figure 4C), we used PTA to further explore these common pattern of shape change along these two PCs simultaneously (Figure 5B). PTA confirmed that both mink species follow a common pattern of shape change between males and females along those axes (i.e., parallel trajectories; $Z = -0.694$, $p = 0.700$), but revealed that the magnitude of this shape change is larger in American mink (Mlu = 0.008, Nvi = 0.017; $Z = 2.380$, $p = 0.025$).

4 | DISCUSSION

This is the first study comparing cranial shape between mink species and, as such, it has clarified some issues regarding the degree of morphological similarity of these two species. While both mink species could be identified based on cranial shape alone, our results clearly suggest ecomorphological convergence between the two species. Furthermore, we were able to identify both divergent patterns of shape variation between both species (sexual shape dimorphism, intraspecific allometry) and common trends in cranial shape (intraspecific sexual allometry) (Figures 4 and 5).

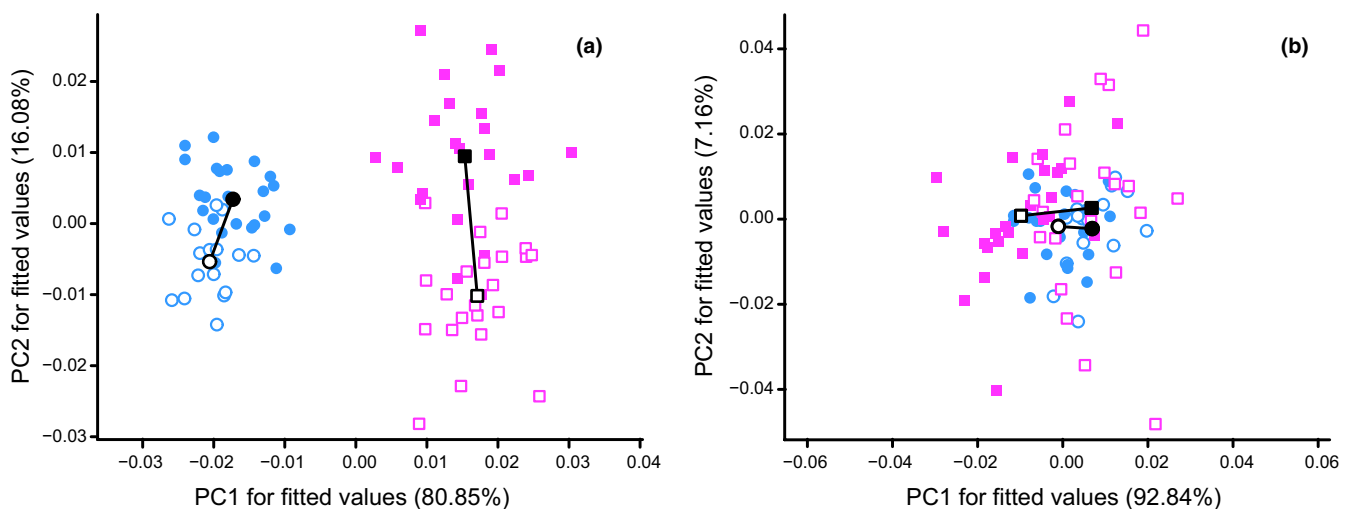


FIGURE 5 Phenotypic trajectory analysis for species and sex in adult mink cranial shape (a) and PC2 and PC3 combined (b). To visualize the trajectories, multivariate data are summarized using a PCA on the fitted values, and the two first PCs are shown for each analysis (with percentage of total variance explained in parentheses). Symbols as in Figure 4, with mean scores for each group in black

TABLE 4 Intraspecific and sexual allometry in cranial shape

Factor	Magnitude		Orientation
Spp	2.634 (0.002)	Mlu	0.0006 Angle: 48.99°
CS	2.824 (0.001)	Nvi	0.0008 3.971 (0.001)
Spp × CS	2.282 (0.006)	2.102 (0.040)	
Sex	1.546 (0.105)	F	0.0007 Angle: 62.19°
CS	0.823 (0.592)	M	0.0012 1.664 (0.068)
Sex × CS	1.387 (0.158)	1.471 (0.092)	

Note: Results from two-way Procrustes ANOVAs to test the potential interactions between size and species, and size and sex. For each factor, Goodall's *F* values are provided together with its associated *p*-value (in brackets). Results of *z*-tests used to assess differences in trajectory magnitude and orientation are also provided, with non-significant *p*-values in grey.

Abbreviations: CS, centroid size; F, female; M, male; Mlu, European mink; Nvi, American mink; spp, species; Sex × CS, interaction between sex and size; spp, species; Spp × CS, interaction between species and size.

4.1 | Juvenile morphology

Adult European mink were widely separated from the juvenile region of the morphospace, refuting the claim that they present pedomorphic features relative to American mink, as proposed by Heptner et al. (2002). Additionally, the fact that all the juveniles clustered on the same region of the morphospace regardless of their species suggests that juvenile cranial shape may be similar across Mustelinae (minks, polecats, and weasels). Shapes in this juvenile region of the morphospace were characterized by a flatter, almost concave forehead; a more ventrally oriented foramen magnum, and a dorsolaterally expanded braincase presenting a convex midline and an anteriorly displaced occipital (i.e., shorter and globous relative to adult shapes). Similar shapes were described for sea otter (*Enhydra lutris*) pups by Law et al. (2017), who also reported that during growth the occipital expanded postero-dorsally and the dorsal midline flattened (i.e., the braincase became longer and less globous). Thus, juvenile cranial shape could potentially be similar, not just across mustelines, but across the whole Mustelidae. Further analyses including juveniles of more species should be carried out to investigate whether this pattern can be generalized.

4.2 | Morphological convergence

European mink are more similar in cranial shape to American mink than to European polecats (their sister lineage). This result could be explained either by morphological convergence between mink species due to similar functional requirements or by the European polecat having a highly derived cranial shape and mink retaining a more basal condition. However, the second hypothesis seems unlikely, since in a previous study the European polecat occupied a

central position in the cluster of musteline and ictonychine mustelids within the musteloid morphospace (figure S2 in Dumont et al., 2016), indicating that its cranial shape is shared among many species in those clades (some of those even more distant phylogenetically than the American mink). Additionally, the more rounded forehead of the European mink is reminiscent to that of its sister species (PC1 in Figure 4).

While this reported morphological convergence between European and American mink could be an adaptation to life in riparian habitats, a gradual shift in diet from small mammals to aquatic prey (fish, amphibians, invertebrates) seems a more likely explanation. Like the European polecat, most musteline and ictonychine mustelids feed on small terrestrial vertebrates (mammals, birds; Wilson & Mittermeier, 2009), which agrees with their similar placement in the morphospace of Dumont et al. (2016). In both mink species, aquatic prey usually represent the largest percentage of their diet. However, this percentage is bigger in the European mink than in the American mink, since the latter tends to rely more on rodents and waterfowl (e.g., Maran, Kruuk, et al., 1998; Sidorovich et al., 2010). The fact that the distance between American mink and European polecat in the morphospace of this study is shorter than the distance between the European mink and the European polecat supports this hypothesis, as it suggests a dietary gradient among the three species (Figure 3A).

4.3 | Interspecific differences

Most of the differences in cranial shape between European and American mink can be related to feeding performance and potential diets. When comparing the cranial shapes characteristic of each species (PC1 in Figure 4), several traits suggest that American mink have relatively larger masticatory and neck muscles (Ewer, 1973; Radinsky, 1981): more developed sagittal and nuchal crests, together with a slightly narrower braincase indicate a larger temporalis; a longer and more curved zygomatic arch provides a larger insertion area for the masseter, while a larger infratemporal fossa suggests a bigger volume for the masseter-ptyergoid complex; and finally, an expanded mastoid region coupled with the more developed nuchal crests relates to stronger neck muscles. Thus, barring major differences in muscular architecture, shape differences suggest that muscle force production is higher in American mink. One exception to these findings would be the relatively broader zygomatic arches of the European mink, which according to Ewer (1973) suggest a stronger masseter in this species even though both its attachment surfaces and volume are relatively smaller. This seems to indicate differences in muscle architecture between both species. Additionally, it is worth remembering that these shape comparisons have been made after scaling to unit centroid size, but when looking at actual cranial size, American mink are significantly larger than European mink (Table 3). Given the positive allometry of muscle forces (e.g., Christiansen & Adolphsen, 2005), size alone would also suggest higher muscle force in American mink (but see Law [2020] on the relationship between size-corrected bite forces and muscle

volume). Thus, combining shape and size differences, muscle force in American mink should be substantially higher than in European mink. As a final note on muscle forces and their relationship with size, the limitations of estimating bite forces from bone morphology should be addressed. Recent studies suggest that muscle volume (and thus potential muscle force) is better predicted by morphological indicators of muscle cross-section (e.g., area of infratemporal fossa, narrower braincase) than of muscle attachment (e.g., crest development, process lengths), as the latter tend to scale with negative allometry relative to body mass (Dickinson et al., 2021 and references therein). Since in this study both types of indicators were considered simultaneously, we believe that our estimations of muscle force should be a realistic approximation.

In order to fully discuss interspecific differences in expected bite force and muscle power, more data on differences in mandible morphology between both species are needed (i.e., in-levers and out-levers). However, the relatively longer and narrower face of American mink indicates that they have larger out-levers at the anterior teeth, which suggests a relative reduction of bite force with those teeth (i.e., killing bite). Preliminary results on mandible shape variation in minks highlight differences in the configuration of masticatory moment arms between both species, which suggest that each species would favor stronger bites at different teeth (Gálvez-López, pers. obs.). In any case, assuming that larger muscle forces translate into stronger bite forces, this would allow the American mink to exploit a wider dietary range: stronger bite forces with the anterior dentition (incisors and canines) would enable the killing of larger prey (with more robust nuchal/cervical regions), while stronger bites at the carnassials would penetrate thicker hides, and at the molars would crush harder materials (i.e., harder arthropod shells, thicker bones). The relatively larger M1 of the American mink would further support a more marked adaptation for crushing in this species (Biknevicius & Van Valkenburgh, 1996).

4.4 | Common allometric trend

Shape changes related to increasing size can be split into two, probably related, groups: increased muscle force and growth. Some of the traits in the first group have been described above for the American mink: more developed sagittal and nuchal crests, longer and more convex zygomatic arches, and a larger infratemporal fossa. Three additional traits further emphasize the relationship between larger size and more powerful masticatory muscles: a narrower postorbital constriction, which has been related to increased volume of the anterior part of the temporalis (Sicuro & Oliveira, 2010; Wiig, 1982b); deeper zygomatic arches, which as discussed above for the European mink relate to stronger masseter muscles (Ewer, 1973); and more laterally projected postorbital processes on the frontal bone. According to Ewer (1973), this last trait is related to both a more powerful temporalis and larger orbits but, since no changes in orbit shape were observed along PC2 (Figure 4), we could assume that in this case it strictly relates to the former. Thus, two size-related shape changes

can be linked to a stronger anterior temporalis, which exerts the main force when the jaws are fully opened (Ewer, 1973; Wiig, 1982b). This means that larger specimens are better equipped to deal with the more robust skulls of larger prey. Additionally, the alveoli for the anterior dentition (incisors, canine, and P2) become relatively larger as size increases, suggesting that the teeth are reinforced in larger specimens, which could be associated with the higher stresses placed on those teeth by bigger prey struggling to get free during the killing bite (Biknevicius & Van Valkenburgh, 1996).

The second group of traits includes an anterodorsal expansion of the face and the neurocranium shifting from a globous shape in small individuals to a dorsoventrally flattened ellipse in the largest ones. Similar shape changes were reported by Law et al. (2017) when studying the postnatal growth of the sea otter. Since no data on age were available for any mink specimen, we identified adult specimens based on all the cranial sutures being closed, which in mink happens around 6 months after birth (Wiig, 1985; Wiig & Lie, 1979). However, the observed shape changes suggest that we have specimens of different ages in our adult sample (i.e., from young adults to more mature specimens), and that adult growth is still an important factor in the development of cranial shape in minks. In agreement with this, a broadening of the zygomatic arches (described in the first group above) was one of the main shape changes observed between subadult and adult fishers (Law, 2020). This finding, together with the similar features of juveniles and young adults (Law et al., 2017; this study, see above), may explain the partial overlap between juveniles and adults in the morphospace for the whole sample (Figure S1).

4.5 | Sexual dimorphism

The fact that a significant triple interaction among species, sex, and size was found in cranial shape, but PTA recovered no differences in magnitude or orientation between trajectories, suggests the existence of a common sexual allometry in cranial shape in both species. This trend seemed to be represented along PC3. As with the common allometric trend discussed above, the larger males presented several traits indicating higher muscle force than the smaller females: more developed sagittal crests and mastoid processes, a narrower postorbital constriction and more acute postorbital processes, wider and more convex zygomatic arches, and larger infratemporal fossae (in this case due to the lateral expansion of the zygomatic arches). As both the purely allometric and the sexual allometry trends are size-related shape changes, it is not surprising that some traits are common to both (Figure 5B). As discussed above, larger muscles can be related to a wider range of potential prey, in this case in males relative to females.

Another trait of the sexual dimorphism common trend was an increase in canine alveolus length and width. Dayan and Simberloff (1994, 1996) undertook extensive studies on the sexual variation of this trait and used it as evidence of character displacement in mustelids (see below). Canine cross-sectional properties (and alveolus length and width as a proxy) are indicative of the magnitude

and direction of the loadings it experiences (Biknevicius & Van Valkenburgh, 1996). Thus, males having larger canine alveoli suggests that their teeth are adapted to withstand larger stresses, which, as stated above, can be related to them catching larger prey.

Even though direct comparisons with previous studies are not possible due to the different methodologies employed, some commonalities can be seen. First, some of the traits related to sexual shape dimorphism in this study were also highlighted in previous traditional morphometric studies, e.g., sagittal crest (Abramov & Tumanov, 2003; He et al., 2002), zygomatic breadth (He et al., 2002; Palazón et al., 2006; Wiig, 1982a, 1986), and mastoid breadth (Palazón et al., 2006; Wiig, 1982a, 1986). Second, some other traits highlighted by those studies were identified as strictly allometric in this study: condylobasal length (Palazón et al., 2006; Wiig, 1982a), palatal length (Abramov & Tumanov, 2003; Wiig, 1982a, 1986), and maxillary tooththrow length (Abramov & Tumanov, 2003; Wiig, 1982a). Thus, if the comparison between sexes had been carried out between the similar-sized smallest males and largest females, no significant differences should have been observed. This evidences the need to use size-corrected values when comparing direct measurements (Gálvez-López, 2021). In this section, the width of the postorbital constriction deserves special attention, as traditional studies found that it was widest in males (Suzuki et al., 2011; Wiig, 1982a), but later “form” studies (PCAs on cranial measurements) showed that, relative to condylobasal length, it was widest in females (Tamlin et al., 2009; Wiig, 1986). In agreement with this, both the present study and Loy et al. (2004) found that female crania had a broader postorbital constriction. Finally, some dental characteristics (P4 length, M1 width) highlighted as sexually dimorphic in traditional morphometrics studies (Abramov & Tumanov, 2003; Palazón et al., 2006; Wiig, 1982a) were not recovered in this study, either in the allometric or sex trends. This might be due to those traits being sexually dimorphic at the intraspecific level instead of following a trend common to both mink species as in the other characters (i.e., part of the divergent trajectories in intraspecific sexual dimorphism in cranial shape; Table 4, Figure 5A). The fact that Loy et al. (2004) found shape differences in the P4 and M1 in their intraspecific comparison of two marten species seems to support this argument.

Given the striking size differences between males and females in most mustelids, sexual dimorphism studies in this group are numerous. Most early studies adhered to one of two hypotheses explaining this phenomenon: resource partitioning (e.g., Moors, 1980) or mating system (e.g., Wiig, 1982a), but see also Wiig (1986). The first hypothesis is based on the concept of character displacement (Brown & Wilson, 1956; Dayan & Simberloff, 1996) applied to sexes as morphospecies, and it states that sexual size dimorphism evolved so that males and females could feed on different-sized prey, thus reducing intraspecific competition (i.e., trophic segregation). The second hypothesis states that, since most mustelids are polygynous, sexual selection would have favored larger males because they would be more successful in fighting other males (Moors, 1980). The fact that the magnitude of sexual dimorphism in size would be higher in species with more aggressive male–male encounters strongly supports this

hypothesis (Johnson et al., 2000; Lynch & Hayden, 1995). However, later studies agreed that resource partitioning appears to explain most of the morphological differences between sexes (e.g., Abramov & Tumanov, 2003; Loy et al., 2004). Additionally, the magnitude of sexual dimorphism in size was linked to diet, with more carnivorous species being the most dimorphic (Gittleman & Van Valkenburgh, 1997), which was attributed to competition for vertebrate prey being probably greater than competition for the relatively more abundant plant material and non-vertebrate prey. Recent studies on the evolution of sexual dimorphism in Musteloidea have confirmed that trophic segregation plays a stronger role than sexual selection (Law & Mehta, 2018; Noonan et al., 2016). The results of the present study agree with those findings, since most of the differences in cranial shape between males and females can be related to feeding biomechanics. On a similar note, the degree of carnivory could explain, to some extent, the variation in sexual dimorphism in cranial shape within Mustelidae. In both the present study and that of Loy et al. (2004), the more carnivorous species had a stronger degree of cranial shape dimorphism (American mink and stone marten, respectively), while it was lower in pine marten (for which plant material, carrion, and non-vertebrate prey play a major part in the diet), and non-existent in European mink (which specializes in aquatic prey). Similarly, no cranial shape dimorphism between sexes was found for the Neotropical otter (Hernández-Romero et al., 2015), which feeds almost strictly on aquatic prey. The exception to this trend would be the American marten, as Law (2020) found significant sexual dimorphism in cranial shape in fishers but not American martens, and both species have a similar diet based mainly on vertebrate prey (Wilson & Mittermeier, 2009; Zielinski & Duncan, 2004).

4.6 | Trophic segregation, displacement, and conservation

Most of the cranial shape differences outlined above suggest that muscle force (and potential dietary range) would be larger in American mink than in European mink, in males than in females, and in larger individuals than in smaller ones. Thus, this would create a gradient from female European mink to male American mink, with male European mink and female American mink together in an intermediate position due to their similar size. Dietary studies on American mink and on European mink seem to support this gradient: while both species target a similar range of prey types overall, European mink are relatively more stenophagous (preying mostly on fish and amphibians) and American mink are generalist with preferred prey varying across localities and seasons (e.g., Abramov & Tumanov, 2003; Arnold & Fritzell, 1987; Bartoszewicz & Zalewski, 2003; Birks & Dunstone, 1985; Palazón et al., 2004). Also in relation to wider prey range, males of both species tend to capture larger prey than females (Birks & Dunstone, 1985; Palazón et al., 2004).

However, when two predators which target a similar range of prey coexist, one of them (usually the smaller and/or less aggressive) is generally displaced by the other towards less energetic or

abundant prey (Dayan & Simberloff, 1996; Ewer, 1973). This has been studied both within and between both mink species (Birks & Dunstone, 1985; Palazón et al., 2004; Sidorovich et al., 2010), and complemented with studies on social interaction also within and between species (Maran, MacDonald, et al., 1998; Sidorovich et al., 1999). As, the smaller sex of the smaller species, female European mink could be being displaced to an increasingly narrower, poorer diet when both species coexist. If this is indeed the case, it would paint a bleak picture for the future of European mink populations as the American mink extends throughout Europe. It leads to a decrease in the survivability of females and even potentially to higher infant deaths due to malnutrition, adding to the compounding list of adverse effects that makes the American mink the highest threat for European mink conservation.

5 | CONCLUSIONS

As the first quantitative comparison of cranial morphology between European and American mink, we provide a thorough description of the differences in cranial shape between both species and discuss how these might affect their feeding biomechanics and dietary range. Our results negate the previous observation that European mink presents paedomorphic features relative to American mink and suggest that a similar cranial shape is shared by all juvenile mustelines (and potentially across the whole Mustelidae). As expected given their similar phenotype and ecology, European mink are more similar in cranial shape to American mink than to European polecats (its sister lineage), with a gradual shift in diet from small mammals to aquatic prey (fish, amphibians, and invertebrates) as the most likely explanation for this evolutionary convergence. Both size and sex are important factors influencing shape variation in minks, and we found two trends common to both species: one describing allometric changes mainly related to individual growth, and the other related to differences in size between the sexes of each species. Most of the observed cranial shape variation can be related to feeding performance and potential diets, as several traits indicate either more robust teeth (i.e., higher loadings) or larger masticatory and neck muscles (and therefore higher bite forces), both leading to wider potential dietary ranges. Thus, potential dietary range is larger in American mink than in European mink, in males than in females, and in larger individuals than in smaller ones. These results support recent findings that trophic segregation plays a stronger role than sexual selection in the evolution of sexual dimorphism in mustelids. Finally, together with previous studies on other species, our results suggest that sexual dimorphism in cranial shape in Mustelidae is related to the degree of carnivory, with species preying on terrestrial vertebrates being highly dimorphic and those with a high percentage of aquatic prey or plant matter in their diet being less so (or not at all).

ACKNOWLEDGEMENTS

This work would not have been possible without access to museum collections, so we would like to thank the following curators

and collection managers: Loïc Costeur (Naturhistorisches Museum Basel), Christiane Funk (Museum für Naturkunde), Tamás Görföl (Hungarian Natural History Museum), Lars Erik Johannessen (Natural History Museum at the University of Oslo), Andrew Kitchener and Jerry Herman (National Museum of Scotland), Lennart Lennuk (Estonian Museum of Natural History), Rhian Rowson (Bristol City Museum and Art Gallery), Manuel Ruedi (Muséum d'Histoire Naturelle de Genève), and Géraldine Veron (Muséum National d'Histoire Naturelle). We also thank the technicians of the following scanning facilities for the astounding quality of their work: Lars Erik Johannessen (Natural History Museum at the University of Oslo), Kristin Mahlow (Museum für Naturkunde), Liz Martin-Silverstone and Tom Davies (X-ray tomography facilities at the University of Bristol), and Georg Schulz (Biomaterials Science Center at the University of Basel). We are also thankful to Santiago Palazón for both giving us access to his personal collection and his insightful comments on European mink ecology and conservation. The comments provided by Chris J. Law and an anonymous reviewer greatly improved the quality of this manuscript. EGL would like to sincerely thank Antonio Profico, Lou Albessard-Ball, and Lisa Genochio for their help with coding and their invaluable support. Finally, this work was completed with the assistance of funds from the Marie Skłodowska-Curie grant agreement No 835736 funded by European Union's Horizon 2020 research and innovation program.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

EGL and PGC designed the study; all authors provided specimens; EGL analyzed data, drafted manuscript, created figures; all authors made revisions to the original draft.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Eloy Gálvez-López  <https://orcid.org/0000-0002-6211-966X>

REFERENCES

- Abramov, A.V. & Tumanov, I.L. (2003) Sexual dimorphism in the skull of the European mink *Mustela lutreola* from NW part of Russia. *Acta Theriologica*, 48, 239–246.
- Adams, D.C., Collyer, M.L. & Kaliantzopoulou, A. (2020) Geomorph: software for geometric morphometric analyses. R package version 3.2.1. Available at: <https://cran.r-project.org/package=geomorph>.
- Adler, A., Murdoch, D., Nenadic, O., Urbaneck, S., Chen, M. & Gebhardt, A. et al. (2020) rgl: 3D Visualization Using OpenGL. R package version 0.103.5. Available at: <https://CRAN.R-project.org/package=rgl>.
- Amstislavsky, S., Lindeberg, H., Aalto, J. & Kennedy, M.W. (2008) Conservation of the European mink (*Mustela lutreola*): focus on reproduction and reproductive technologies. *Reproduction in Domestic Animals*, 43, 502–513.

- Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26, 32–46.
- Arnold, T.W. & Fritzell, E.K. (1987) Food habits of prairie mink during the waterfowl breeding season. *Canadian Journal of Zoology*, 65, 2322–2324.
- Bartoszewicz, M. & Zalewski, A. (2003) American mink, *Mustela vison* diet and predation on waterfowl in the Stońsk Reserve, western Poland. *Folia Zoologica*, 52, 225–238.
- Biknevicius, A.R. & Van Valkenburgh, B. (1996) Design for killing: craniodental adaptations of predators. In: Gittleman, J.L. (Ed.) *Carnivore behavior, ecology and evolution*. 2, Ithaca: Cornell Univ. Press, pp. 393–428.
- Birks, J.D.S. & Dunstone, N. (1985) Sex-related differences in the diet of the mink *Mustela vison*. *Holarctic Ecology*, 8, 245–252.
- Bonesi, L. & Palazón, S. (2007) The American mink in Europe: status, impacts, and control. *Biological Conservation*, 134, 470–483.
- Brown, W.L. & Wilson, E.O. (1956) Character displacement. *Systematic Zoology*, 5, 49–64.
- Christiansen, P. & Adolphsen, J.S. (2005) Bite forces, canine strength and skull allometry in carnivores (Mammalia, Carnivora). *Journal of Zoology*, 266, 133–151.
- Cohen, B.H. (2001) *Explaining psychological statistics*, 2nd edition. New York: John Wiley & Sons Inc.
- Collyer, M.L. & Adams, D.C. (2013) Phenotypic trajectory analysis: comparison of shape change patterns in evolution and ecology. *Hystrix*, 24, 75–83.
- Collyer, M.L. & Adams, D.C. (2018) RRPP: an R package for fitting linear models to high-dimensional data using residual randomization. *Methods in Ecology and Evolution*, 9, 1772–1779.
- Collyer, M.L. & Adams, D.C. (2020) RRPP: linear model evaluation with randomized residuals in a permutation procedure. Available at: <https://cran.r-project.org/web/packages/RRPP>.
- Collyer, M.L., Sekora, D.J. & Adams, D.C. (2015) A method for analysis of phenotypic change for phenotypes described by high-dimensional data. *Heredity*, 115, 357–365.
- Cox, P.G., Rayfield, E.J., Fagan, M.J., Herrel, A., Pataky, T.C. & Jeffery, N. (2012) Functional evolution of the feeding system in rodents. *PLoS One*, 7, e36299.
- Dayan, T. & Simberloff, D. (1994) Character displacement, sexual dimorphism, and morphological variation among British and Irish mustelids. *Ecology*, 75, 1063–1073.
- Dayan, T. & Simberloff, D. (1996) Patterns of size separation in carnivore communities. In: Gittleman, J.L. (Ed.) *Carnivore behavior, ecology and evolution*. 2, Ithaca: Cornell University Press, pp. 243–266.
- Dickinson, E., Davis, J.S., Deutsch, A.R., Patel, D., Nijhawan, A., Patel, M. et al. (2021) Evaluating bony predictors of bite force across the order Carnivora. *Journal of Morphology*, 282, 1499–1513.
- Dickson, B.V. & Pierce, S.E. (2019) Functional performance of turtle humerus shape across an ecological adaptive landscape. *Evolution*, 73, 1265–1277.
- Dumont, M., Wall, C.E., Botton-Divet, L., Goswami, A., Peigné, S. & Fabre, A.-C. (2016) Do functional demands associated with locomotor habitat, diet, and activity pattern drive skull shape evolution in musteloid carnivorans? *Biological Journal of the Linnean Society*, 117, 858–878.
- Ewer, R.F. (1973) *The carnivores*. London: Weidenfeld and Nicolson.
- Fisher, R.A. (1958) *Statistical methods for research workers*. Edinburgh: Oliver and Boyd.
- Fruciano, C. (2016) Measurement error in geometric morphometrics. *Development Genes and Evolution*, 226, 139–158.
- Gálvez-López, E. (2021) Quantifying morphological adaptations using direct measurements: the carnivoran appendicular skeleton as a case study. *Anatomical Record*, 304, 480–506.
- García Bustos, K. (2018) *Ecología poblacional e interacciones entre tres especies de mustélidos semiacuáticos en España* (PhD Thesis). Barcelona: Universitat de Barcelona.
- Gittleman, J.L. & Van Valkenburgh, B. (1997) Sexual dimorphism in the canines and skulls of carnivores: effects of size, phylogeny, and behavioural ecology. *Journal of Zoology*, 242, 97–117.
- Goodall, C.R. (1991) Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society: Series B (Methodological)*, 53, 285–339.
- Goswami, A. (2006) Morphological integration in the carnivoran skull. *Evolution*, 60, 169–183.
- Goswami, A., Milne, N. & Wroe, S. (2011) Biting through constraints: cranial morphology, disparity and convergence across living and fossil carnivorous mammals. *Proceedings of the Royal Society B: Biological Sciences*, 278, 1831–1839.
- Gunz, P., Mitteroecker, P. & Bookstein, F.L. (2005) Semilandmarks in three dimensions. In: Slice, D.E. (Ed.) *Modern morphometrics in physical anthropology*. New York: Kluwer Academic/Plenum Publishers, pp. 73–98.
- Hautier, L., Lebrun, R. & Cox, P.G. (2012) Patterns of covariation in the masticatory apparatus of hystricognathous rodents: implications for evolution and diversification. *Journal of Morphology*, 273, 1319–1337.
- He, T., Friede, H. & Kiliardis, S. (2002) Macroscopic and roentgenographic anatomy of the skull of the ferret (*Mustela putorius furo*). *Laboratory Animals*, 36, 86–96.
- Heptner, V.G., Naumov, N.P., Yurgenson, P.B., Sludskii, A.A., Chirkova, A.F., Bannikov, A.G. et al. (2002) *Mammals of the Soviet Union, Vol. II, Part 1b – Carnivora (Weasels; Additional Species)*. (BAM Botros et al., trans.) Washington, DC: Smithsonian Institution Libraries and The National Science Foundation (Original work published 1967).
- Hernández-Romero, P.C., Guerrero, J.A. & Valdespino, C. (2015) Morphological variability of the cranium of *Lontra longicaudis* (Carnivora: Mustelidae): a morphometric and geographic analysis. *Zoological Studies*, 54, e50.
- Holliday, J.A. & Steppan, S.J. (2004) Evolution of hypercarnivory: the effect of specialization on morphological and taxonomic diversity. *Paleobiology*, 30, 108–128.
- Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 6, 67–70.
- Johnson, D.D.P., Macdonald, D.W. & Dickman, A.J. (2000) An analysis and review of models of the sociobiology of the Mustelidae. *Mammal Review*, 30, 171–196.
- Klingenberg, C.P. (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 11, 353–357.
- Law, C.J. (2020) Sex-specific ontogenetic patterns of cranial morphology, theoretical bite force, and underlying jaw musculature in fishers and American martens. *Journal of Anatomy*, 237, 727–740.
- Law, C.J., Baliga, V.B., Tinker, M.T. & Mehta, R.S. (2017) Asynchrony in craniomandibular development and growth in *Enhydra lutris neireis* (Carnivora: Mustelidae): are southern sea otters born to bite? *Biological Journal of the Linnean Society*, 121, 420–438.
- Law, C.J., Duran, E., Hung, N., Richards, E., Santillan, I. & Mehta, R.S. (2018) Effects of diet on cranial morphology and biting ability in musteloid mammals. *Journal of Evolutionary Biology*, 31, 1918–1931.
- Law, C.J. & Mehta, R.S. (2018) Carnivory maintains dimorphism between males and females: evidence for niche divergence in extant Musteloidea. *Evolution*, 72, 1950–1961.
- Law, C.J., Slater, G.J. & Mehta, R.S. (2018) Lineage diversity and size disparity in Musteloidea: testing patterns of adaptive radiation using molecular and fossil-based methods. *Systematic Biology*, 67, 127–144.
- Lawes, I.N.C. & Andrews, P.L.R. (1987) Variation of the ferret skull (*Mustela putorius furo* L.) in relation to stereotaxic landmarks. *Journal of Anatomy*, 154, 157–171.
- Loy, A., Spinosi, O. & Carlini, R. (2004) Cranial morphology of *Martes foina* and *M. martes* (Mammalia, Carnivora, Mustelidae): the role of size and shape in sexual dimorphism and interspecific differentiation. *The Italian Journal of Zoology*, 71, 27–35.

- Lynch, J.M. & Hayden, T.J. (1995) Genetic influences on cranial form: variation among ranch and feral American mink *Mustela vison* (Mammalia: Mustelidae). *Biological Journal of the Linnean Society*, 55, 293–307.
- Machado, F.A., Zahn, T.M.G. & Marroig, G. (2018) Evolution of morphological integration in the skull of Carnivora (Mammalia): changes in Canidae lead to increased evolutionary potential of facial traits. *Evolution*, 72, 1399–1419.
- Maran, T., Kruuk, H., MacDonald, D.W. & Polma, M. (1998) Diet of two species of mink in Estonia: displacement of *Mustela lutreola* by *M. vison*. *Journal of Zoology*, 245, 218–222.
- Maran, T., MacDonald, D.W., Kruuk, H., Sidorovich, V. & Rozhnov, V.V. (1998) The continuing decline of the European mink, *Mustela lutreola*: evidence for the intra-guild aggression hypothesis. *Symposia of the Zoological Society of London*, 71, 297–324.
- Maran, T., Skumatov, D., Gómez, A., Pödra, M., Abramov, A.V. & Dinets, V. (2016) *Mustela lutreola*. *The IUCN Red List of Threatened Species*. 2016: e.T14018A45199861.
- Martin, A.R. & Lea, V.J. (2020) A mink-free GB: perspectives on eradicating American mink *Neovison vison* from Great Britain and its islands. *Mammal Review*, 50, 170–179.
- McIntosh, A.F. & Cox, P.G. (2016) The impact of gape on the performance of the skull in chisel-tooth digging and scratch digging mole-rats (Rodentia: Bathyergidae). *Royal Society Open Science*, 3, 160568.
- Michaud, M., Veron, G., Peigné, S., Blin, A. & Fabre, A.-C. (2018) Are phenotypic disparity and rate of morphological evolution correlated with ecological diversity in Carnivora? *Biological Journal of the Linnean Society*, 124, 294–307.
- Migliorini, R.P., Fornel, R. & Kasper, C.B. (2020) Geographic variation in the skull morphology of the lesser grison (*Galictis cuja*: Carnivora, Mustelidae) from two Brazilian ecoregions. *PeerJ*, 8, e9388.
- Moors, P.J. (1980) Sexual dimorphism in the body size of mustelids (Carnivora): the roles of food habits and breeding systems. *Oikos*, 34, 147–158.
- Noonan, M.J., Johnson, P.J., Kitchener, A.C., Harrington, L.A., Newman, C. & Macdonald, D.W. (2016) Sexual size dimorphism in musteloids: an anomalous allometric pattern is explained by feeding ecology. *Ecology and Evolution*, 6, 8495–8501.
- Ooms, J. (2020) Magick: advanced graphics and image-processing in R. R package version 2.5.2. Available at: <https://CRAN.R-project.org/package=magick>.
- Palazón, S. & Melero, Y. (2014) Status, threats and management actions on the European mink *Mustela lutreola* (Linnaeus, 1761) in Spain: a review of the studies performed since 1992. *Munibe Monographs. Nature Series*, 3, 109–118.
- Palazón, S., Ruiz-Olmo, J. & Gosálbez, J. (2004) Diet of European mink (*Mustela lutreola*) in Northern Spain. *Mammalia*, 68, 159–165.
- Palazón, S., Ruiz-Olmo, J. & Gosálbez, J. (2006) Body and skull morphology of European mink (*Mustela lutreola*) in northern Spain. *Proceedings of the International Congress on the Conservation of European mink (Mustela lutreola)*. Logroño: Gobierno de La Rioja, pp. 167–178.
- Pigot, A.L., Sheard, C., Miller, E.T., Bregman, T.P., Freeman, B.G., Roll, U. et al. (2020) Macroevolutionary convergence connects morphological form to ecological function in birds. *Nature Ecology & Evolution*, 4, 230–239.
- Pödra, M., Gómez, A. & Palazón, S. (2013) Do American mink kill European mink? Cautionary message for future recovery efforts. *European Journal of Wildlife Research*, 59, 431–440.
- Profico, A., Buzi, C., Castiglione, S., Melchionna, M., Piras, P., Veneziano, A. et al. (2021) Arothron: an R package for geometric morphometric methods and virtual anthropology applications. *American Journal of Physical Anthropology*, 176, 144–151.
- R Core Team. (2020) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: <https://www.R-project.org/>.
- Radinsky, L.B. (1981) Evolution of skull shape in carnivores. 1. Representative modern carnivores. *Biological Journal of the Linnean Society*, 15, 369–388.
- Rohlf, F.J. & Slice, D.E. (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, 39, 40–59.
- RStudio Team. (2020) *RStudio: integrated development for R*. Boston, MA: RStudio, Inc. <http://www.rstudio.com/>.
- Schlager, S. (2017) Morpho and Rvcg - shape analysis in R. In: Zheng, G., Li, S. & Szekely, G. (Eds.) *Statistical shape and deformation analysis*. Orlando: Academic Press, pp. 217–256.
- Sicuro, F.L. & Oliveira, L.F.B. (2010) Skull morphology and functionality of extant Felidae (Mammalia: Carnivora): a phylogenetic and evolutionary perspective. *Zoological Journal of the Linnean Society*, 161, 414–462.
- Sidorovich, V.E., Kruuk, H. & MacDonald, D.W. (1999) Body size, and interactions between European and American mink (*Mustela lutreola* and *M. vison*) in Eastern Europe. *Journal of Zoology*, 248, 521–527.
- Sidorovich, V.E., Polozov, A.G. & Zalewski, A. (2010) Food niche variation of European and American mink during the American mink invasion in north-eastern Belarus. *Biological Invasions*, 12, 2207–2217.
- Suzuki, S., Abe, M. & Motokawa, M. (2011) Allometric comparison of skulls from two closely related weasels, *Mustela itatsi* and *M. sibirica*. *Zoological Science*, 28, 676–688.
- Tamlin, A.L., Bowman, J. & Hackett, D.F. (2009) Separating wild from domestic American mink *Neovison vison* based on skull morphometrics. *Wildlife Biology*, 15, 266–277.
- Van Valkenburgh, B. (2007) Déjà vu: the evolution of feeding morphologies in the Carnivora. *Integrative and Comparative Biology*, 47, 147–163.
- Wickham, H. (2019) Stringr: simple, consistent wrappers for common string operations. R package version 1.4.0. Available at: <https://CRAN.R-project.org/package=stringr>.
- Wiig, Ø. (1982a) Sexual dimorphism in the skull of the feral American mink (*Mustela vison* Schreber). *Zoologica Scripta*, 11, 315–316.
- Wiig, Ø. (1982b) Bone resorption in the skull of *Mustela vison*. *Acta Theriologica*, 27, 358–360.
- Wiig, Ø. (1985) Multivariate variation in feral American mink (*Mustela vison*) from Southern Norway. *Journal of Zoology*, 206, 441–452.
- Wiig, Ø. (1986) Sexual dimorphism in the skull of minks *Mustela vison*, badgers *Meles meles* and otters *Lutra lutra*. *Zoological Journal of the Linnean Society*, 87, 163–179.
- Wiig, Ø. & Lie, R.W. (1979) Metrical and non-metrical skull variations in Norwegian wild mink (*Mustela vison* Schreber). *Zoologica Scripta*, 8, 297–300.
- Wilson, D.E. & Mittermeier, R.A. (Eds.) (2009) *Handbook of the mammals of the world. Carnivores*. Vol. 1, Barcelona: Lynx Edicions.
- Zielinski, W.J. & Duncan, N.P. (2004) Diets of sympatric populations of American martens (*Martes americana*) and fishers (*Martes pennanti*) in California. *Journal of Mammalogy*, 85, 470–477.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Gálvez-López, E., Kilbourne, B. & Cox, P.G. (2022) Cranial shape variation in mink: Separating two highly similar species. *Journal of Anatomy*, 240, 210–225. <https://doi.org/10.1111/joa.13554>