



## OPEN Skull morphology analysis suggests the extinct Cape lion, *Panthera leo melanochaita* (Smith, 1842), is not distinctive

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The lion (*Panthera leo*) was extirpated from the Cape region of South Africa during the mid-nineteenth century. Whilst historically classified as a distinct subspecies known as the Cape lion (*P. l. melanochaita*), recent molecular studies challenge the distinctiveness of this population, suggesting that it represents the southernmost population of the species' Southern Clade. The Cape lion is often cited as having a distinctive skull morphology, which has justified its subspecific classification, but only a limited number of specimens have been available for examination, so that the Cape lion's skull morphology has not been satisfactorily understood. In this study we collected morphometric data from a greatly enlarged sample of 22 Cape lion skulls, including 12 adults, constituting the largest sample size analysed for this possible subspecies. The results suggest that (1) morphological characteristics of the skull previously thought to distinguish the Cape lion are not diagnostic, and (2) nor is the skull morphology of male and female Cape lions distinct from that of males and females of other southern African lions. Our results independently support those based on molecular investigations, which suggest that the Cape lion was not distinct from other lions within the Southern Clade.

**Keywords** Conservation, Cranial, Lion, Restoration, Subspecies, South Africa

The lion, as a phylogenetically coherent species complex, is one of the most charismatic mammals<sup>1</sup> Molecular and fossil evidence suggest that the lion diverged from its closest living relative, the leopard, approximately 1.9 (0.9–4.1) million years ago, with the earliest lion appearing in East Africa during the early Pleistocene shortly after 2 million years ago<sup>2–4</sup>. During the Late Pleistocene it likely held the largest geographical range amongst terrestrial apex predators<sup>2,5–7</sup>. However, a sudden, massive range contraction occurred at the end of the Pleistocene, leading to the extinction of all lion populations except the "modern" lion (*Panthera leo*, hereafter referred to simply as the lion) in Africa and southwest Eurasia<sup>2,5–8</sup>. Subsequently, over the past few thousand years, human persecution has further decimated lion populations, leaving them surviving only in fragmented populations in Sub-Saharan Africa and India<sup>8</sup>. In the last c. 150 years, this human-induced decline has resulted in the extinction of several lion populations, including that of the Cape provinces, South Africa, which held prominence during the early days of modern natural history in the eighteenth and nineteenth centuries, and was designated as a distinct subspecies known as the Cape lion *P. l. melanochaita* (Smith, 1842)<sup>10–17</sup>.

The Cape lion inhabited the southern regions of the former Cape Province (currently Eastern Cape, Western Cape, and the southern part of the Northern Cape Provinces) and the former Orange Free State Province (current Free State) in the Republic of South Africa<sup>14,18–21</sup>. Until relatively recently, the classification of lions

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was based upon their geographical provenance and very small sample sizes biased towards males. The Cape lion was described as a distinct lion subspecies based upon morphological characteristics including large size, the presence of a huge black/dark mane extending behind the shoulders and covering the belly, and a facial appearance said to resemble that of a bulldog<sup>13,20</sup>. However, observations and anecdotes of other contemporaries, including locals and travellers, are not consistent with such distinctions, including the colour of the mane<sup>11,12,22</sup>. Additionally, the size and colour of a lion's mane is strongly influenced by environmental factors and the animal's condition, including ambient temperature and age<sup>23–26</sup>. Therefore, identification of lions to subspecies based on mane morphology is fraught with problems. For example, a male lion that died in 1809 in the menagerie of King Louis Napoleon Bonaparte of the Netherlands was initially identified as a Cape lion based on its large mane<sup>27</sup>, only to be later reclassified as an Asian lion (*P. l. persica*) based on skull morphology and genetic analysis<sup>28</sup>. From an ecological perspective the existence of a distinct (largely isolated) lion population in the Cape region appears improbable given the lion's historical ubiquity throughout the Cape region and into north-eastern South Africa, and the suitability of favourable habitat between the ranges of the Cape lion and other populations<sup>29,30</sup>. Recent genetic research indicates that the Cape lion is likely not as phylogenetically distinct as previously thought, based on analyses of mitochondrial DNA<sup>16,22,31</sup> and whole genome analyses<sup>22,32</sup>, and that lions from the Cape region form part of the Southern Clade of the species<sup>16,33–35</sup>. It is worth noting that *P. l. melanochaita*, the name originally given to the Cape lion by Smith in 1842, represents the oldest trinomial available for any member of the Southern Clade<sup>36</sup>. Consequently, according to the rule of priority in zoological nomenclature, *P. l. melanochaita* is now validated and used for the Southern Clade<sup>9,35</sup>.

Although the Cape lion does not seem to constitute a phylogenetically distinct lion population, it has been suggested that the Cape lion is distinguishable from other lion populations based on skull morphological traits, such as the presence of the lower second premolar ( $pm_2$ ), a greater ratio of condylobasal length to greatest length of the skull, and a greater ratio of rostral depth to greatest length of the nasal bone, as well as multivariate analyses based on skull morphometrics<sup>20,38,39</sup>. In the tiger (*Panthera tigris*), an intriguing disparity between molecular-based phylogeny and skull morphology has been observed amongst Southeast Asian populations, particularly those in the Sunda Islands, where closely-related island populations, Sumatran tiger (*P. t. sumatrae*), Javan tiger (*P. t. sondaica*), and Bali tiger (*P. t. balica*), exhibit marked differences in skull morphologies<sup>39–43</sup>. It is important to adopt an integrative approach to such interpretations by bringing together independent lines of evidence, in order to understand phenotypic (e.g. morphological) differences among closely-related species/populations and to contribute to our understanding of evolutionary processes, as well as aiding conservation decision-making<sup>44,45</sup>. Therefore, investigating the morphological characteristics of the Cape lion and its distinctiveness compared to other southern African lion populations will greatly contribute to our understanding of lion evolution and lion conservation in the region. However, the very limited sample size of Cape lion specimens examined in previous research<sup>19,20,38,46</sup> is problematic for investigating morphological differences at the population level, and hence, an investigation based on a larger sample size is needed.

To fill this knowledge gap, we located and physically examined Cape lion skulls in natural history collections in Europe and southern Africa, and conducted an analysis of Cape lion skull morphology based on the largest-ever sample set of Cape lion skulls. We aim to determine 1) whether previous assertions regarding Cape lion skull characteristics are valid, 2) if the Cape lion is distinguishable from other lion populations in southern Africa based on skull morphology, and 3) to consider potential implications for the restoration of the Cape lion.

## Results

### Putative Cape lion characteristics

Of the 22 Cape lion skulls, 12 were classified as adult (Table 1), six of which exhibited major damage and/or missing parts, preventing us from taking every measurement. Although the original description by Lundholm (1952)<sup>47</sup> identified the Port Elizabeth specimen of the Cape lion (1468/62; see Table 1) as female, the sexual dimorphism of upper canine size in lions (Fig. S1) suggests that it is a male. We examined the mandibles of 16 Cape lions, and only two specimens possessed  $pm_2$  (Table 1). However, it is important to note that out of the other (i.e. non-Cape lion) 732 lion mandibles we examined, only one specimen had  $pm_2$  alveolus (on the left mandible). The ratio of condylobasal length to greatest length of the skull of wild adult Cape lions was not statistically significantly greater than those of other wild adult southern African lions (ANOVA, males:  $df=3$ ,  $F=1.76$ ,  $p=0.16$ , females:  $df=3$ ,  $F=1.33$ ,  $p=0.27$ , Fig. S2a). Similarly, the ratio of rostral depth to nasal length of adult Cape lions was not statistically significantly greater than those of other wild adult southern African lions, although a statistically significant difference was detected amongst wild adult male southern African lions (ANOVA, males:  $df=3$ ,  $F=5.39$ ,  $p=0.002$ , females:  $df=3$ ,  $F=0.70$ ,  $p=0.56$ , Fig. S2b).

### Can Cape lion skulls be distinguished from those of other southern African lions?

Principal Component Analysis on all five nominal subspecies extracted five PCs whose eigenvalues were greater than one for males, explaining 51.9%, 11.7%, 5.3%, 4.4%, and 3.7% of the variance, and six such PCs for females (explaining 47.0%, 12.0%, 6.0%, 5.1%, 4.3%, and 3.5%) (Fig. 1). The scatter plots based on the first two PCs suggest that the skulls of Cape lions cannot be differentiated from those of males and females of other southern African lions, whilst Asian lion skulls are clearly distinguishable from those of southern African lions (Fig. 1). However, because the foregoing PCA may have been heavily influenced by the differences between Asian lions and southern African lions, we also conducted PCA only on the four nominal subspecies from southern Africa. Principal Component Analysis on those four nominal subspecies extracted six PCs, whose eigenvalues were greater than one for males (explaining 53.9%, 6.8%, 5.1%, 4.0%, 3.8%, and 3.4% of the variance), and seven such PCs for females (explaining 50.3%, 7.4%, 6.0%, 4.3%, 4.0%, 3.6%, and 3.2%) (Fig. S3). Discriminant Function Analysis on all five nominal subspecies extracted four Canonical Discriminant Functions (CDFs) for males, each of which explained 91.4%, 5.8%, 1.8%, and 1.0% of the variance, and four CDFs for females (explaining 88.9%,

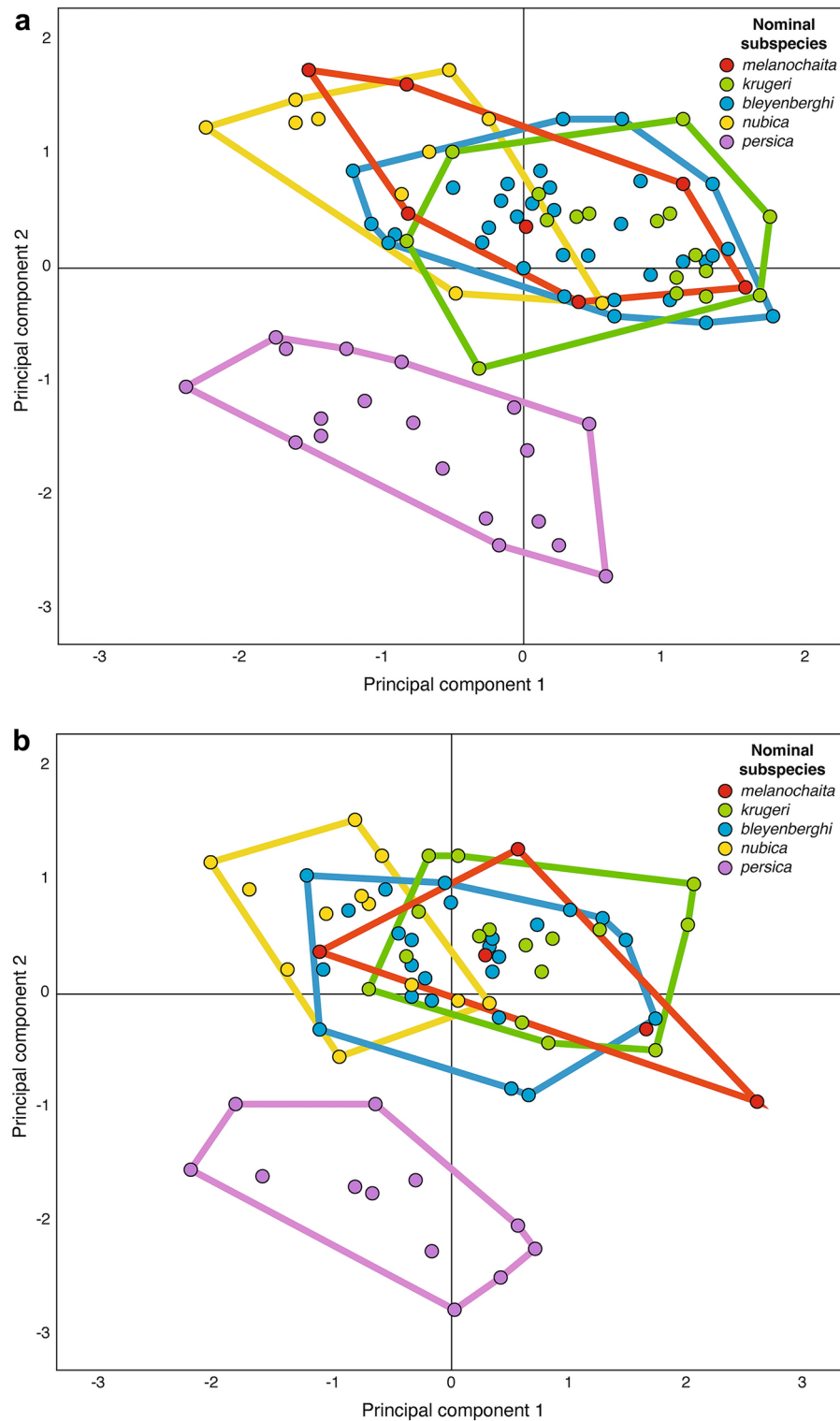
Museum	Museum ID #	Year	Captive?	Locality	Sex	Age	Comments
Bulawayo	61,463	1966	no	Port Elizabeth, Cape, South Africa	m	a	No mandible, picked up in a sand dune near the sea
Cape Town	7529		no	Beaufort West, Cape, South Africa	f	a	No mandible
Cape Town	33,425	1938	no	Studtis, Willowmore, Cape, South Africa	m	a	Found in a limestone cave
Cape Town	35,121		yes?	Unknown	f	a	Labelled "Cape lion", donated by the South African Institute
Copenhagen	1570		no?	Unknown	m	a	No mandible, labelled " <i>Felis leo</i> Kap", acquired from the South African Museum, described in Christiansen (2008) <sup>38</sup>
King Williams Town	15,904	1940	no	King Williams Town, Cape, South Africa	m	sa	No mandible, found in Welcome Wood, near King William's Town
King Williams Town	19,150	1962	no	Betty's Bay, Western Cape, South Africa	m	sa	No mandible, dug up from sand hills near the beach, labelled "female", but likely male
Leiden	Cat i (663 Br)	< 1860	no	Kaap	m	sa	Anatom. Cab. 1860, referred to in Hemmer (1966) <sup>19</sup>
Leiden	Cat k (664 Br)	< 1860	no	Kaap	f	a	Anatom. Cab. 1860, referred to in Hemmer (1966) <sup>19</sup>
Leiden		< 1887	no	<i>Panthera leo melanochaita</i>	m	sa	
Leiden		1871	yes	<i>Panthera leo melanochaita</i> 1871, born at the Amsterdam Zoo?	m	j	No mandible, donated by Amsterdam Zoo, labelled female juvenile but likely a male juvenile
London	1846.7.2.6		no	Cape?	f	a	Turner Collection
London	1918.5.23.2	c. 1873	no	Cape	m	a	Lower pm <sub>3</sub> is present on the left mandible, shot in c. 1873 (Murray), referred to by Mazak (1975) <sup>20</sup>
London	1936.5.26.6	1848	no	Cape Town	m	a	Lower pm <sub>s</sub> are present on both mandibles, shot in 1848 (Murray), referred to by Mazak (1975) <sup>20</sup>
Oxford	14,181		no	The Cape	m	sa	
Oxford	14,178	1800s	no	South Africa?	f	a	Burchell collection
Oxford	14,179	1800s	no	South Africa?	m	sa	Burchell collection
Paris	A1838		yes	Cap	m	sa	Brought back from the Cape by a merchant
Port Elizabeth	1468/62 (I-1453?)	1952	no	Tonderskraal, Murraysburg, Cape, South Africa	m	sa	Picked up at Tonderskraal, labelled "female", but likely male, described in Lundholm (1952) <sup>47</sup> , referred to in Mazak (1964, 1975) <sup>57,20</sup> , Hemmer (1966) <sup>19</sup>
Stockholm	A58 (3712)	1874	no	South Africa	m	a	Labelled <i>melanochaitus</i>
Stockholm	A59 (1310)	1845	no	Caffraria interior	m	a	Skin length (c. 285 cm), mane very well developed, tail tuft big, no belly mane except a small patch of dark hairs at the groin (Yamaguchi unpublished), referred to in Mazak (1975) <sup>20</sup>
Vienna	1422	1892	yes	Unknown (zoo?)	m	a	A deformed skull, described in Hemmer (1966) <sup>19</sup>

**Table 1.** Specimens of the Cape lion that we physically examined. Museums: Natural History Museums of Zimbabwe (Bulawayo), Natural History Museum of Denmark (Copenhagen), Amathole Museum (King William's Town), Naturalis Biodiversity Centre (Leiden), Natural History Museum (London), Oxford Museum of Natural History (Oxford), National Museum of Natural History (Paris), Port Elizabeth Museum (Port Elizabeth), Swedish Museum of Natural History (Stockholm), Museum of Natural History Vienna (Vienna). Sex: male (m), female (f). Age: adult (a), subadult (sa), juvenile (j). Vienna1422, highlighted in yellow, was not included in the analysis as it is a deformed skull.

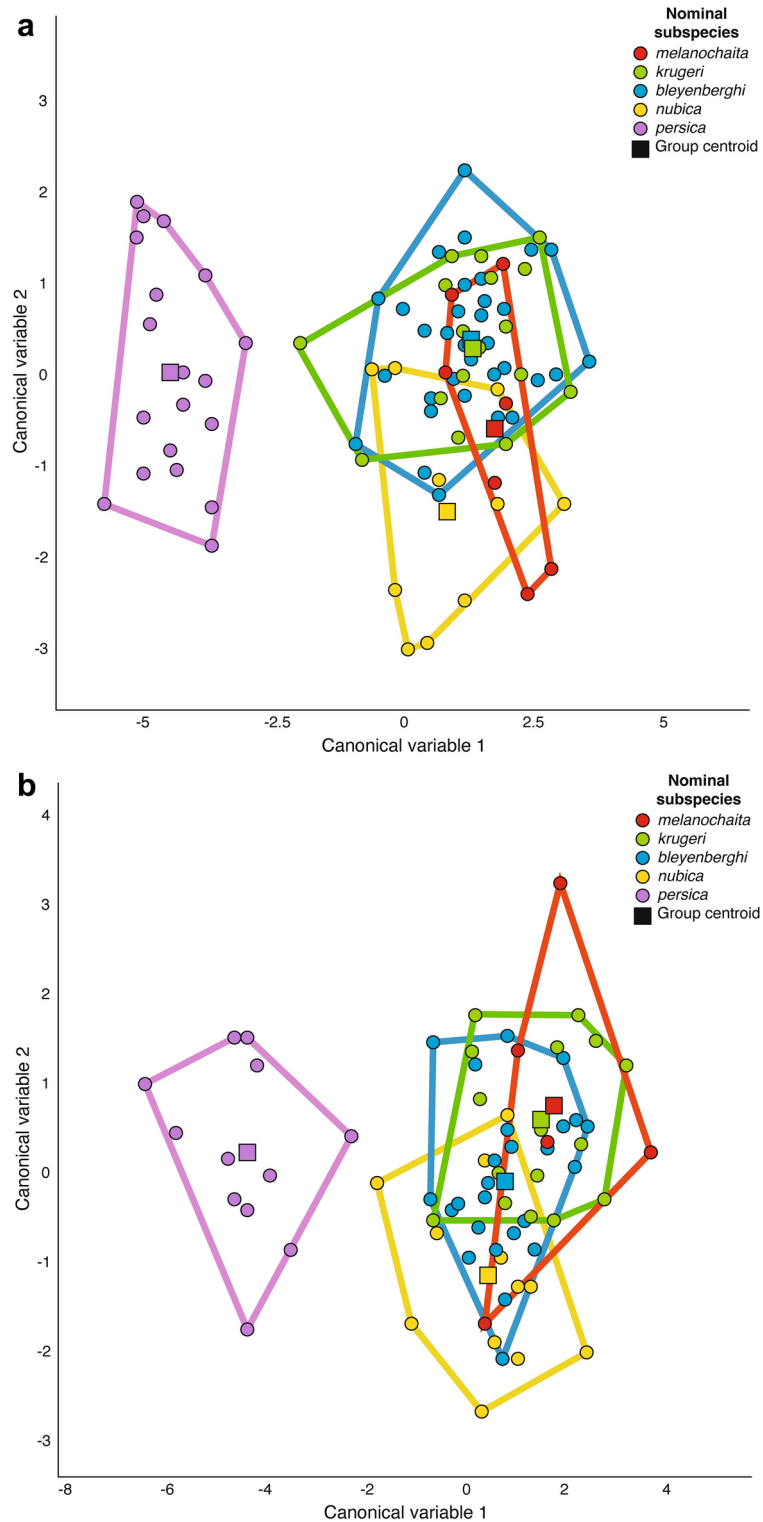
7.2%, 3.8%, and 0.1%). The scatter plots based on the first two CDFs (Fig. 2) and the classification table (Table 2) suggest that Cape lion skulls are not distinguishable from those of other male and female southern African lions, whilst Asian lion skulls were clearly distinguishable from those of southern African lions, whilst the skulls of the nominal subspecies *P. l. nubica* may be tentatively distinguishable from those of other southern African lions. Discriminant Function Analysis on the four nominal subspecies from southern Africa extracted three CDFs for males, each of which explained 63.3%, 22.1%, and 14.6% of the variance, and three CDFs for females (explaining 78.1%, 20.7%, and 1.2%). The scatter plots based on the first two DFAs (Figs. S14, S18) and the classification table (Table 3) suggest that Cape lion skulls are not distinguishable from those of other male and female southern African lions, whilst skulls of the nominal subspecies *P. l. nubica* may be tentatively distinguished from those of other southern African lions.

## Discussion Analysis

One of the most critical challenges in interpreting Cape lion skull morphology has been the small number of surviving specimens, particularly when conducting separate analyses for sex and age classes<sup>19,20,38,46,47</sup> (Table 4). Here, we examined the largest known number of Cape lion skulls (Table 1), and yet, with such a small sample



**Fig. 1.** (a) Scatter plots of male *Panthera leo melanochaita*, *P.l. krugeri*, *P.l. bleyenberghi*, and *P.l. nubica*, and *P. l. persica* based on the first two Principal Components (PCs) extracted from 30 raw variables (see Table S4). The PCs-1 and -2 explain 51.9% and 11.7% of the variance, respectively. (b) Scatter plots for females based on the first two PCs extracted from 31 raw variables (see Table S5). The PCs-1 and -2 explain 47.0% and 12.0% of the variance, respectively.



**Fig. 2.** (a) Scatter plots of male *Panthera leo melanochaita*, *P.l. krugeri*, *P.l. bleyenberghi*, and *P.l. nubica*, and *P.l. persica* based on the first two Canonical Discriminant Functions (CDFs) extracted from the five Principal Components (PCs) (Table S1 Model-3c). (b) Scatter plots for females based on the first two CDFs extracted from 4 PCs (Table S1 Model-4c).

size, we faced difficulties in identifying appropriate DFA models (Table S1, Figs. S4–S18). Discriminant Function Analysis is sensitive to the ratio of sample size to the number of predictor variables, and, at a minimum, the smallest group size should exceed the number of predictor variables<sup>48,49</sup>. A statistically significant *p*-value for Box's *M* test indicates the violation of an assumption for DFA, which is equal variance/covariance matrices

Sex	Subspecies	Predicted group membership					Total
		mel	kru	ble	nub	per	
Male	mel	2 (28.6)	1 (14.3)	3 (42.9)	1 (14.3)	0 (0)	7 (100)
	kru	0 (0)	7 (41.2)	8 (47.1)	1 (5.9)	1 (5.9)	17 (100)
	ble	0 (0)	4 (12.1)	29 (87.9)	0 (0)	0 (0)	33 (100)
	nub	0 (0)	1 (10.0)	4 (40.0)	5 (50.0)	0 (0)	10 (100)
	per	0 (0)	0 (0)	0 (0)	0 (0)	19 (100)	19 (100)
Female	mel	2 (40.0)	1 (20.0)	1 (20.0)	1 (20.0)	0 (0)	5 (100)
	kru	1 (6.3)	7 (43.8)	8 (50.0)	0 (0)	0 (0)	16 (100)
	ble	2 (8.0)	4 (16.0)	17 (68.0)	2 (8.0)	0 (0)	25 (100)
	nub	0 (0)	0 (0)	7 (58.3)	5 (41.7)	0 (0)	12 (100)
	per	0 (0)	0 (0)	0 (0)	0 (0)	12 (100)	12 (100)

**Table 2.** The classification results, number and (%), of Discriminant Function Analyses for the nominal “subspecies”: *Panthera leo melanochaita* (mel), *Pl. krugeri* (kru), *Pl. bleyenberghi* (ble), *Pl. nubica* (nub), and *Pl. persica* (per). For males, the DFA was based on 5 Principal Components (PCs) extracted by a Principal Component Analysis (PCA) whose eigenvalues were greater than 1. The PCA was performed on the 30 raw variables (Table S4). For females, the DFA was based on the 4 Principal Components (PCs) extracted by a Principal Component Analysis (PCA) whose eigenvalues were greatest. The PCA was performed on the 31 raw variables (Table S5).

Sex	Subspecies	Predicted group membership					Total
		mel	kru	ble	nub	Total	
Male	mel	3 (42.9)	1 (14.3)	3 (42.9)	0 (0)	7 (100)	
	kru	0 (0)	7 (41.2)	9 (52.9)	1 (5.9)	17 (100)	
	ble	0 (0)	2 (6.1)	30 (90.9)	1 (3.0)	33 (100)	
	nub	0 (0)	1 (10.0)	4 (40.0)	5 (50.0)	10 (100)	
Female	mel	2 (40.0)	1 (20.0)	2 (40.0)	0 (0)	5 (100)	
	kru	1 (6.3)	6 (37.5)	8 (50.0)	1 (6.3)	16 (100)	
	ble	0 (0)	5 (20.0)	18 (72.0)	2 (8.0)	25 (100)	
	nub	0 (0)	0 (0)	3 (25.0)	9 (75.0)	12 (100)	

**Table 3.** The classification results, number and (%), of Discriminant Function Analyses for the four southern African “subspecies”: *Panthera leo melanochaita* (mel), *Pl. krugeri* (kru), *Pl. bleyenberghi* (ble), and *Pl. nubica* (nub). For males, the DFA was based on the 5 Principal Components (PCs) extracted by a Principal Component Analysis (PCA) whose eigenvalues were greater than 1. The PCA was performed on the 30 raw variables (Table S4) and only on the 4 nominal “subspecies” of the Southern Africa. For females, the DFA was based on the 4 Principal Components (PCs) extracted by a Principal Component Analysis (PCA) whose eigenvalues were the largest. The PCA was performed on the 31 raw variables (Table S5) and only on the 4 nominal “subspecies” of the Southern Africa.

Publication	Male	Female	Total
Lundholm (1952) <sup>47</sup>	1 (initially classified as female)	0	1
Hemmer (1966) <sup>19</sup>	4(+1)*	1(+1)*	7*
Mazák (1975) <sup>20</sup>	3(+2)**	0	5**
Christiansen (2008) <sup>38</sup>	3	0	3
J. Mazák (2010) <sup>46</sup>	2	0	2
This study	17 (8 adults)	5 (5 adults)	22

**Table 4.** Sample sizes of the Cape lion specimens examined in the previous studies. \*Limited tooth measurements are available for two mounted specimens in Stuttgart. \*\*Two lions from the geographical area of the Cape lion were not categorised as such, because they did not exhibit morphological characteristics that were expected.

across groups<sup>48,49</sup>. Also, the greater the difference in the classification results between the original data set and cross-validation, the lower the reliability of the model (e.g. overfit to the original set of variables)<sup>48,49</sup>. In this context, we employed PCA to reduce the number of predictor variables before conducting a DFA, which improved the DFA (Table S1). When the PCA extracted a larger number of PCs with eigenvalues greater than one compared to the smallest group size (female Cape lions,  $n = 5$ ), we suggest including only  $(n - 1)$  or fewer PCs with the greatest eigenvalues (compare Models 4b and 4c in the Supplementary Information). We highlight the ability of models created using high dimension, low sample size data to categorise individuals into distinct groupings using DFA (Models 3a and 4a in Table S1). However, cross validation indicates overfitting of the data (Table S1, Figs S12, S15), Box's  $M$  test shows that the variance/covariance matrices are not equal across groupings, and therefore such analyses are not statistically appropriate<sup>48,49</sup>. The results presented in Christiansen (2008)<sup>38</sup>, where Cape lion skulls were clearly and accurately distinguished from those of other lion subspecies by DFA, may be partially attributed to the use of 27 predictor variables whilst even the largest comparative group consisting of only 24 specimens.

### Are Cape lion skulls distinctive?

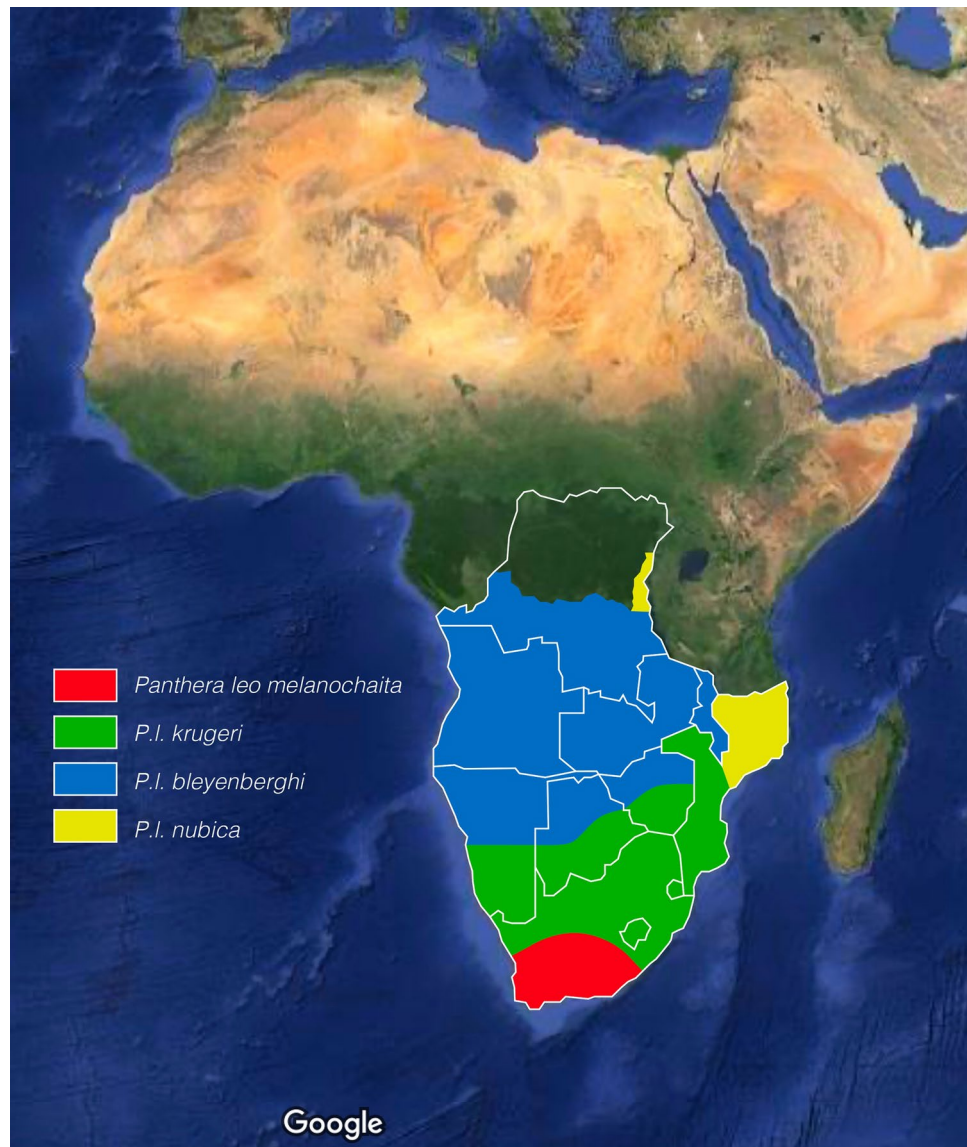
We tested skull characteristics suggested by previous authors to distinguish Cape lions from those of other lion subspecies (presence of the lower  $pm_2$ , greater ratio of condylobasal length to greatest length of skull, and greater ratio of rostral depth to greatest length of nasals<sup>20</sup>), as well as conducting multivariate statistics to determine if Cape lion skulls could be distinctive. The results indicate that these skull characteristics are not useful for distinguishing Cape lion skulls, and DFA does not differentiate Cape lion skulls from those of other lion subspecies in southern Africa, at least based on the variables included in the analysis. This lack of distinction is especially evident among the three subspecies distributed in southern Africa (*P. l. melanochaita*, *P. l. krugeri*, and *P. l. bleyenberghi*), while *P. l. nubica*, whose range extends widely to East Africa, appears to be somewhat distinguishable (Tables 2, 3, Figs. 2, S2b). Phenotypic plasticity has been suggested to explain differences in the morphology of both the lion and tiger, including areas around the rostrum, mandible, and sagittal crest, by comparing wild and captive animals<sup>50–52</sup>. This may suggest that variables such as the ratios of rostral depth to nasal length or condylobasal length to greatest length may not be very useful for demonstrating the distinctiveness of the Cape lion, because they are likely influenced by an animal's behaviour and ecology, although we do not reject the influence of phylogeny. We also obtained, for the first time, a sample size of female Cape lions ( $n = 5$ ) large enough for more rigorous statistical analyses than previous investigations. There is no evidence from either sex to consider the Cape lion a distinct lion population among southern African lions based on skull morphology. Our results independently support the results obtained by molecular analyses based on mitochondrial DNA<sup>16,22,31</sup> and whole genomes<sup>22,32</sup>.

### Avoiding circular arguments

Until the nineteenth century, lions were often classified by geographical provenance, such as Asian, Barbary, Senegal, or Cape lions<sup>10,12,53,54</sup>. Early authors classified lions into subspecies based on their provenances a priori rather than testing a posteriori whether geographical varieties/populations were diagnosably distinct. The distinctiveness of the Cape lion was based upon descriptions of some individuals, typically males, including arguably the most famous mounted skin kept in the Natural History Museum, London (museum accession number: NHMUK ZD.1968.268) that was first described by Pocock (1931)<sup>55</sup>. Probably, one of the most ambitious attempts to “define” the Cape lion was made by Mazák (1975)<sup>20</sup>. However, the procedure taken by Mazák (1975)<sup>20</sup> is circular because 1) the author started his arguments by assuming that the Cape lion was special, 2) identified characteristics which distinguished some (i.e. NOT all) skulls from the Cape region from other lion skulls from southern Africa, and used these characteristics to define the Cape lion, and then 3) this arbitrary definition was used for claiming that the Cape lion was distinctive. For example, Mazák (1975)<sup>20</sup> assigned some specimens (London NHMUK ZD.1918.5.23.2, NHMUK ZD.1936.5.26.6, Port Elizabeth 1468/62, in Table 1) from the southern parts of South Africa to the Cape lion based on his definition, whilst others (Stockholm A59 (1310), Leiden Cat i (663 Br), in Table 1) from the same region were not. It appears that Mazák (1975)<sup>20</sup> did so by (1) assuming the Cape lion was a distinct subspecies, (2) identifying morphological characteristics for separating the former three specimens from other lions from southern Africa, and (3) classifying the latter two as non-Cape lions based on these characteristics—in other words, the author probably assigned the latter two lion specimens to non-Cape lion to provide a basis for claiming the distinction of the Cape lion, which was the author's conclusion a priori. Here we did not a priori assume the distinctiveness of the Cape lion in the way Mazák (1975)<sup>20</sup> did, and included all the specimens in the analysis as Cape lions on the condition that they originated from the range ascribed to this putative form<sup>14,18–21</sup>, see Fig. 3). The results suggest that all three main skull characteristics suggested by Mazák (1975)<sup>20</sup> to distinguish the Cape lion fail to do so, and the DFA fails to separate skulls of lions from the apparent former range of the Cape lion from those of other southern African lions.

### Paradigm shift from nostalgic sentiment to restoration

Despite the lack of positive supportive evidence, the Cape lion was customarily considered a distinct lion subspecies, where males were huge and possessed large black manes by some authors<sup>5,13,20,37,55–57</sup>. This sentiment may have been maintained due to societal preference and nostalgia for the charismatic image of the Cape lion<sup>58</sup> along with apparent unfounded emphases on the heritability of males' manes by some early authors, despite mane morphology likely exhibiting variation due to environmental factors<sup>2,23,24,26,28</sup>. However, ecologically and phylogenetically, it is unlikely that a distinct lion population existed in the Cape region<sup>16,22,29–32</sup>. Two “Cape” lions analysed by De Flamingh et al. (2024)<sup>22</sup> are clustered with East African lions based on mitochondrial DNA, and one of them is classified as an East African lion also based on the whole genome, whilst other molecular results,



**Fig. 3.** The distribution ranges of the nominal "subspecies" from the southern Africa, *Panthera leo melanochaita*, *P.l. krugeri*, *P.l. bleyenberghi*, and *P.l. nubica*, that we defined and analysed in this study based on Roberts (1951)<sup>18</sup>, Mazák (1968)<sup>65</sup>, and Hemmer (1974)<sup>5</sup>. White lines delineate the national borders of the countries considered part of the southern Africa in this study.

using Cape lions whose localities were known, put them very close to the other southern African lions<sup>16,31,32</sup>. Assuming those "Cape" lions analysed by De Flamingh et al. (2024)<sup>22</sup> are genuine, the Cape region might have been home to a genetically variable lion population, as suggested for eastern-southern Africa<sup>59,60</sup>, and not one supporting an isolated distinct lion subspecies. This notion may apply to other regional lion populations too as the current regional differentiations in the lion based on nuclear DNA may have been largely the results of anthropogenic fragmentation<sup>61</sup>.

To judge by the large number of hits on the internet ("Cape lion" resulted in c. 66,300,000 hits on Google in April 2024), the Cape lion myth remains prominent in public sentiment. Our results do not support the distinctiveness of the Cape lion. However, its membership as part of the southern African lion clade may make the restoration of the (Cape) lion in the southernmost part of Africa more acceptable both scientifically and politically, through lion translocation into areas of its former range from the adjacent regions without concern about population distinctions<sup>62</sup>. In this context, we consider retrospectively that it is appropriate that many lion populations have been established in the Western, Eastern and Northern Cape Provinces and also the Free State Province through such translocations<sup>63</sup>. Nonetheless, some might argue that we should never overlook the possibility that the Cape lion was subject to region-specific selection, about which we currently have little information. For example, since 2000, 13 populations of lions have been established in the Eastern Cape Province of South Africa<sup>63</sup> (also, Kerley, unpublished), mostly sourced from the Kgalagadi Transfrontier Park in the Northern Cape and Botswana. Despite these populations now being within the former range of the Cape

lion, by 2024 so far only one of the males and their male offspring had developed a large mane extending behind the shoulders and along the belly as is the traditional description of the male Cape lion (Kerley, unpublished, Simeonovski, unpublished, Yamaguchi, unpublished). These findings may suggest that the famous external morphology of the male Cape lion, which was manifest in all six of the male Cape lion specimens examined by Mazák (1975)<sup>20</sup> (but, see above concerning circular argument), may be the result of some Cape-specific selection, rather than being characteristic of the routine development of male southern African lions in the environment where the Cape lion used to occur. However, it is also possible that the (male-biased) morphological descriptions of the Cape lion were always exaggerated, and never truly reflected the population-level pattern; furthermore, previous researchers may not have assigned a male specimen to the Cape lion if its mane was not luxuriantly developed. Also, one might argue that changes in the region's climate during the last c. 200 years may have influenced the lion's mane. Despite the lack of information on these various possibilities, the question arises as to whether conservationists should argue for selective breeding of the imagined stereotype of the Cape lion, such as male's huge black mane? This is more a question of consumer choice than of any fundamental precept of biodiversity conservation, but it prompts us to point out that artificial selection for a specific phenotype often leads to a loss of genetic diversity in a population insofar as it is typically achieved through inbreeding. In the absence of genetic and morphometric evidence that the Cape lion ever existed as a separate distinct subspecies, a more appropriate conservation goal would seem to be the carefully-planned management of Southern African lions as a functioning metapopulation.

## Material and methods

### Data collection

Lion skulls were examined, and up to 75 morphometric measurements per skull were collected, at natural history collections in Europe and Africa, following methodologies of Barnett et al. (2008)<sup>64</sup> (Table S2, Fig. S19). We examined 242 lion skulls originated from southern Africa, which we defined as currently comprising Angola, Botswana, the southern half of the Democratic Republic of the Congo (DRC), Eswatini, Lesotho, Malawi, Mozambique, Namibia, the Republic of South Africa, Zambia, and Zimbabwe (Fig. 3), and 37 from Asia for comparison (Table 4). Concerning the presence of the mandibular pm<sub>2</sub> we examined 748 lion mandibles from all over its geographical range. The provenance of a specimen was determined based on labels and records kept in the collections. To facilitate groupings other than national borders and increase the sample size of each group, we classified specimens into nominal "subspecies", or local populations, based on Roberts (1951)<sup>18</sup>, Mazák (1968)<sup>65</sup>, and Hemmer (1974)<sup>5</sup>, as summarised in Table 5 and Fig. 3. Animals were assigned to age categories (adult, sub-adult, or juvenile) according to the criteria outlined by Yamaguchi et al. (2013)<sup>41</sup>. Sex was identified from museum labels and records when available, and sexual dimorphism in canine size was utilised for verification and to determine the sex of unsexed specimens (Yamaguchi et al., 2013)<sup>41</sup>. We also assessed whether an animal was wild or captive based on museum label, record, and skull morphology characteristics known to be associated with captivity, such as a thicker zygomatic arch<sup>50,66,67</sup>.

### Data analysis

Only skulls of wild adult animals were included in the analysis, except for Asian lions, for which the insufficient sample size of wild specimens compelled us to include also the skulls of captive individuals. Regarding adult Cape lions, we excluded a male specimen (Vienna 1422: see Table 1) due to skull deformity, but included a male (Copenhagen 1570: see Table 1) and a female (Cape Town 35,121: see Table 1) specimen, despite possible captive origins, to increase sample size. Morphological characteristics previously suggested to distinguish Cape lion skulls from those of other lions (presence of lower pm<sub>2</sub>, ratio of condylobasal length to greatest length, and ratio of rostral depth to nasal length) were compared amongst southern African lion subspecies using ANOVA. For this analysis, due to differences in measurement orientation, nasal length as defined by Mazák (1975)<sup>20</sup> was calculated as follows based on our measurements of nasal length-I and nasal breadth.

$$\text{Nasal length (Mazak 1975)} = \sqrt{((\text{nasal length} - I)^2 - ((\text{nasal breadth})/2)^2)}$$

Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) were conducted to assess whether Cape lion skulls could be differentiated from those of other southern African lions and Asian lions (see the Supplementary Information for the details). Unfortunately, 13 Cape lion skulls were at least partially damaged (including one of the most famous Cape lion skulls kept in the Natural History Museum London

Nominal "subspecies"	Geographic area	#
<i>Panthera leo melanochaita</i>	Southern Cape, southern Natal, and southern Free State, of South Africa	22
<i>P. l. krugeri</i>	South Africa (except southern Cape, southern Natal, and southern Free State), southern half of Mozambique, southern half of Namibia, southern half of Botswana, and southern half of Zimbabwe	83
<i>P. l. bleyenberghi</i>	Southern DRC, Angola, Zambia, northern half of Botswana, northern half of Zimbabwe, and northern half of Namibia	109
<i>P. l. nubica</i>	East Africa between Sudan and the northern half of Mozambique, including Somalia and central eastern part of the Democratic Republic of the Congo (DRC)	28
<i>P. l. persica</i>	Asia	38

**Table 5.** Definition of the nominal "subspecies" (or local populations) that we used in this study based on Roberts (1951)<sup>18</sup>, Mazák (1968)<sup>65</sup>, and Hemmer (1974)<sup>5</sup>. Number of specimens examined: (#).

(1918.5.23.2: see Table 1) whose occiput area has been cut off) and the mandible was missing in six specimens, so that we were unable to measure every variable on those skulls. Consequently, we performed analyses on sample sets that either maximised the number of Cape lions by including only variables measured in all Cape lion specimens, resulting in 19 variables for both sexes combined, 30 for males, and 31 for females (Tables S3–S5), or maximised the number of variables, while minimising the exclusion of Cape lions from the analysis, resulting in 48 variables for both sexes combined (Table S6). Preliminary analyses indicated that DFAs, using smaller sets of variables (principal components: PCs) extracted by PCA based on correlations, were statistically more appropriate than using larger sets of original variables, with higher proportions of cases correctly classified when males and females were analysed separately (for details, see Table S1 and the Supplementary Information). Therefore, separate analyses were conducted for each sex, and DFA was based on PCs. Given the limited number of adult female Cape lion specimens ( $n = 5$ ), only the first four PCs with the highest eigenvalues were used, whilst six PCs with eigenvalues greater than one were extracted (see the Supplementary Information for the details including Table S1). All statistical analyses were performed using SPSS 29 (IBM, Armonk).

## Data availability

Data is provided on request. Contact Nobuyuki Yamaguchi (nobuyuki.yamaguchi@umt.edu.my) for more information.

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## References

- Macdonald, E. A. *et al.* Conservation inequality and the charismatic cat: *Felis felis*. *Glob. Ecol. Conserv.* **3**, 851–866 (2015).
- Yamaguchi, N., Cooper, A., Werdelin, L. & Macdonald, D. W. Evolution of the mane and group-living in the lion (*Panthera leo*): a review. *J. Zool. Lond.* **263**, 329–342 (2004).
- Werdelin, L. & Peigné, S. *Carnivora in Cenozoic Mammals of Africa* (University of California Press, 2010).
- Li, G., Davis, B. W., Eizirik, E. & Murphy, W. J. Phylogenomic evidence for ancient hybridization in the genomes of living cats (*Felidae*). *Genome Res.* **26**, 1–11 (2016).
- Hemmer, H. Untersuchungen zur Stammesgeschichte der Pantherkatzen (Pantherinae). Teil III. Zur Artgeschichte des Löwen *Panthera (Panthera) leo* (Linnaeus 1758). *Veröffentlichungen der Zoologischen Staatssammlung München* **17**, 167–280 (1974).
- Barnett, R. *et al.* Phylogeography of lions (*Panthera leo*) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity. *Mol. Ecol.* **18**, 1668–1677 (2009).
- Salis, A. T. *et al.* Lions and brown bears colonized North America in multiple synchronous waves of dispersal across the Bering Land Bridge. *Mol. Ecol.* **31**, 6407–6421 (2022).
- Cooper, D. M. *et al.* A kingdom in decline: Holocene range contraction of the lion (*Panthera leo*) modelled with global environmental stratification. *PeerJ*. <https://doi.org/10.7717/peerj.10504> (2021).
- Nicholson, S. *et al.* *Panthera leo*. *The IUCN Red List of Threatened Species* <https://doi.org/10.2305/IUCN.UK.2023-1.RLTS.T15951A231696234.en> (2023).
- Newbery, J. *A Historical Account of the Curiosities of London and Westminster*. (Bible and Sun, 1753).
- Burchell, W. J. *Travels in the Interior of Southern Africa* Vol. 2. (Longman, Hurst, Rees, Orme, and Brown, 1824).
- Bennett, E. T. *The Tower Menagerie: Comprising the natural history of the animals contained in that establishment, with anecdotes of their characters and history* (R. Jennings, 1892).
- Smith, C. H. *The Naturalist's Library* Vol. XIII *Introduction to Mammalia*. (W. H. Lizars, and Stirling and Kenny, 1842).
- Harper, F. *Extinct and Vanishing Mammals of the Old World* (American Committee for International Wild Life Protection New York Zoological Park, 1945).
- Yamaguchi, N. & Haddane, B. The North African Barbary lion and the Atlas Lion Project. *Int. Zoo News* **49**, 465–481 (2002).
- Barnett, R. *et al.* Lost population and preserving genetic diversity in the lion *Panthera leo*: implications for its ex situ conservation. *Conserv. Genet.* **7**, 507–514 (2006).
- Patterson, B. D. On the nature and significance of variability in lions (*Panthera leo*). *Evol. Biol.* **34**, 55–60 (2007).
- Roberts, A. *The Mammals of South Africa* (The Trustee of the Mammals of South Africa, 1951).
- Hemmer, H. Mitteilung über ein weiteres Exemplar des Kaplöwen – *Panthera leo melanochaita* (Smith, 1842). *Zeitschr. Säugetierkunde* **31**, 57–61 (1966).
- Mazák, V. Notes on the black-maned lion of the Cape, *Panthera leo melanochaita* (Ch. H. Smith, 1842) and a revisited list of the preserved specimens. *Verh. Kon. Ned. Akad. Wet.* **64**, 1–44 (1975).
- Skead, C. J. *Historical Incidence of the Larger Land Mammals in the Broader Eastern Cape* 2nd edn. (Nelson Mandela Metropolitan University, 2007).
- De Flamingh, A. *et al.* Genomic analysis supports Cape Lion population connectivity prior to colonial eradication and extinction. *J. Hered.* <https://doi.org/10.1093/jhered/esad081> (2024).
- Kays, R. W. & Patterson, B. D. Mane variation in African lions and its social correlates. *Can. J. Zool.* **80**, 471–478 (2002).
- West, P. M. & Packer, C. Sexual selection, temperature, and the lion's mane. *Science* **297**, 1339–1343 (2002).
- Patterson, B. D. *The Lions of Tsavo: Exploring the Legacy of Africa's Notorious Man-Eaters* (McGraw-Hill, 2004).
- Patterson, B. D. *et al.* Developmental effects of climate on the lion's mane (*Panthera leo*). *J. Mammal.* **87**, 193–200 (2006).
- Van Bree, P. J. H. On a mounted skeleton of apparently the extinct Cape lion, *Panthera leo melanochaita* (Ch. H. Smith, 1842). *Contrib. Zool.* **68**, 67–71 (1998).
- Barnett, R. *et al.* Using ancient DNA techniques to identify the origin of unprovenanced museum specimens, as illustrated by the identification of a 19th century lion from Amsterdam. *Contrib. Zool.* **76**, 87–94 (2007).
- Boshoff, A. F. & Kerley, G. I. H. Potential distributions of medium- to large-sized mammals in the Cape Floristic Region, based on historical accounts and habitat requirements. *Afr. Zool.* **36**, 245–273 (2001).
- Boshoff, A., Landman, M. & Kerley, G. Filling the gaps on the maps: historical distribution patterns of some larger mammals in part of southern Africa. *Trans. R. Soc. South Africa* **71**, 23–87 (2016).
- Barnett, R. *et al.* The origin, current diversity, and future conservation of the modern lion (*Panthera leo*). *Proc. R. Soc. B* **273**, 2119–2125 (2006).
- De Manuel, M. *et al.* The evolutionary history of extinct and living lions. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 10927–10934 (2020).
- Barnett, R. *et al.* Revealing the maternal demographic history of *Panthera leo* using ancient DNA and a spatially explicit genealogical analysis. *BMC Evol. Biol.* **14**, 70 (2014).
- Bertola, L. D. *et al.* Phylogeographic patterns in Africa and high resolution delineation of genetic clades in the lion (*Panthera leo*). *Sci. Rep.* <https://doi.org/10.1038/srep30807> (2016).

35. Kitchener, A. C. et al. A revised taxonomy of the Felidae. *Cat News Special Issue* **11**, 71–73 (2017).
36. Dubach, J. M. et al. Genetic perspectives on “Lion Conservation Units” in Eastern and Southern Africa. *Conserv. Genet.* **14**, 741–755 (2013).
37. Mazák, V. & Husson, A. M. Einige bemerkungen über den Kaplöwen, *Panthera leo melanochaitus* (Ch. H. Smith, 1842). *Zool. Meded. uitgeg. door het Rijksmuseum van Natuurlijke Historie te Leiden* **37**, 102–111 (1960).
38. Christiansen, P. On the distinctiveness of the Cape lion (*Panthera leo melanochaita* Smith, 1842) and a possible new specimen from the Zoological Museum. *Copenhagen. Mammal Biol.* **73**, 58–65 (2008).
39. Hemmer, H. Zur stellung des Tigers (*Panthera tigris*) der Insel Bali. *Z. Säugetierkd.* **34**, 216–223 (1969).
40. Mazák, J. H. & Groves, C. P. A taxonomic revision of the tiger (*Panthera tigris*) of Southeast Asia. *Mamm. Biol.* **71**, 268–287 (2006).
41. Yamaguchi, N. et al. Locating specimens of extinct tiger (*Panthera tigris*) subspecies: Javan tiger (*P. t. sondaica*), Balinese tiger (*P. t. balica*), and Caspian tiger (*P. t. virgata*), including previously unpublished specimens. *Mammal Study* **38**, 187–198 (2013).
42. Xue, H.-R. et al. Genetic Ancestry of the Extinct Javan and Bali Tigers. *J. Hered.* **106**, 247–257 (2015).
43. Sun, X. et al. Ancient DNA reveals genetic admixture in China during tiger evolution. *Nat. Ecol. Evol.* <https://doi.org/10.1038/s41559-023-02185-8> (2023).
44. Crandall, K. A., Bininda-Emonds, O. R. P., Mace, G. M. & Wayne, R. K. Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**, 290–295 (2000).
45. Padiál, J. M. et al. The integrative future of taxonomy. *Front. Zool.* **7**, 16 (2010).
46. Mazák, J. H. Geographical variation and phylogenetics of modern lions based on craniometric data. *J. Zool. Lond.* **281**, 194–209 (2010).
47. Lundholm, B. A skull of a Cape lioness (*Felis leo melanochaitus* H. Smith). *Ann. Transv. Mus.* **22**, 21–24 (1952).
48. Tabachnick, B. G. & Fidell, L. S. *Using Multivariate Statistics* 7th edn. (Pearson, 2019).
49. Lavine, B. K. & Rayens, W. S. Statistical Discriminant Analysis. In *Comprehensive Chemometrics: Chemical and Biochemical Data Analysis* (ed. Lavine, B. K.) (Elsevier, 2020).
50. Saragusty, J. et al. Comparative skull analysis suggests species-specific captivity-related malformation in lions (*Panthera leo*). *PLoS One* **9**, e94527 (2014).
51. Cooper, D. M. et al. Phenotypic plasticity determines differences between the skulls of tigers from mainland Asia. *R. Soc. Open Sci.* <https://doi.org/10.1098/rsos.220697> (2022).
52. Cooper, D. M. et al. Getting to the meat of it: The effects of a captive diet upon the skull morphology of the lion and tiger. *Animals* **13**, 3616. <https://doi.org/10.3390/ani13233616> (2023).
53. La Cépède, M., Cuvier, G. L. C. F. D., Maréchal, N. & Miger, S. C. *La Ménagerie de Muséum National d'Histoire Naturelle*. (Miger, Patris, Grandcher and Dentu, 1801).
54. Jardine, W. *The Naturalist's Library Vol. II the Natural History of Felinae*. (W. H. Lizars, and Stirling and Kenny, 1834).
55. Pocock, R. I. The lion's mane. *The Field* **158**, 208 (1931).
56. Pocock, R. I. *Mammalia in Natural History*, (Ward (Lock and Co., 1936).
57. Mazák, V. Preliminary list of the specimens of *Panthera leo melanochaitus* Ch. H. Smith, 1842, preserved in the museums of the whole world in 1963. *Z. Säugetierkunde* **29**, 52–58 (1964).
58. Monsarrat, S. & Kerley, G. I. H. Charismatic species of the past: Biases in reporting of large mammals in historical written sources. *Biol. Conserv.* **223**, 68–75 (2018).
59. Dubach, J. et al. Molecular genetic variation across the southern and eastern geographic ranges of the African lion. *Panthera leo. Conserv. Genet.* **6**, 15–24 (2005).
60. Antunes, A. et al. The evolutionary dynamics of the lion *Panthera leo* revealed by host and viral population genomics. *PLoS Genet.* **4**(11), e1000251. <https://doi.org/10.1371/journal.pgen.1000251> (2008).
61. Curry, C. J. et al. Spatiotemporal genetic diversity of lions reveals the influence of habitat fragmentation across Africa. *Mol. Biol. Evol.* <https://doi.org/10.1093/molbev/msaa174> (2021).
62. Bertola, L. D. et al. Genetic guidelines for translocations: Maintaining intraspecific diversity in the lion (*Panthera leo*). *J. Biogeogr.* <https://doi.org/10.1111/eva.13318> (2021).
63. Banasiak, N. M., Hayward, M. W. & Ten Kerley, G. I. H. years on: have large carnivore reintroductions to the Eastern Cape Province, South Africa worked?. *Afr. J. Wildl. Res.* **51**, 111–126 (2021).
64. Barnett, R., Yamaguchi, N., Shapiro, B. & Sabin, R. Ancient DNA analysis indicates the first English lions originated from North Africa. *Contrib. Zool.* **77**, 7–16 (2008).
65. Mazák, V. Der Löwe. *Das Pelzgewerbe* **19**, 3–27 (1968).
66. Hollister, N. Some effects of environment and habit on captive lions. *Proc. U. S. Natl. Museum* **53**, 177–193 (1917).
67. Yamaguchi, N., Kitchener, A. C., Gilissen, E. & Macdonald, D. W. Brain size of the lion (*Panthera leo*) and the tiger (*P. tigris*): Implications for intrageneric phylogeny, intraspecific differences, and the effects of captivity. *Biol. J. Linn. Soc.* **98**, 85–93 (2009).

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## Author contributions

Conceived and general planning: NY, ACK, BDP, and DWM, Data collection: NY, Data analysis: NY, ON, DMC, Writing of the manuscript: NY, ON, GIHK, Unpublished information provided: NY, GIHK, TPG, JCKP, and VS, Creation of figures: NY, and ON, Discussion and review of the paper: NY, DMC, ACK, GIHK, TPG, JCKP, VS, BDP, DWM, All authors reviewed the manuscript submitted.

## Declarations

### Competing interests

The authors declare no competing interests.

## Additional information

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