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APPLICATION NOTE

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Identification of tiger and lion skulls: An illustrated guide for science and practice

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Abstract
Tiger and lion bones are valuable subjects of trafficking. They can be easily interchanged. The aim of this study was to develop a methodology to quickly distinguish between tiger and lion skulls. From a number of features reported in the literature, a set of seven significant characters was identified that can be recognized even in photographs. A methodology for their assessment was developed. Using a combination of them allows for a very reliable morphological distinction between the two species. A new character, the greater palatine foramen ratio, of both adult and juvenile skulls, has not been described in the context of identification of tiger and lion skulls before.

KEYWORDS
comparative anatomy, craniometry, identification key, morphology, wildlife trafficking

1 | INTRODUCTION

Tiger bones are one of the most lucrative commodities on the wildlife black market (Jha et al., 2022; May & Clough, 2017; Mills & Jackson, 1994; Nowell, 2000), but can easily be mistaken for lion bones due to their strong similarities (Williams et al., 2015, 2021) and given that they cannot be distinguished by size (Boule, 1906; Haltenorth, 1937; Merriam & Stock, 1932; Pocock, 1939; Tilson & Nyhus, 2010). However, quick identification can be crucial in the detection of and solving wildlife crime cases. Various characters have been proposed but in most cases, these have not been accurately defined or tested as to their ability to discriminate.

A wide range of characters typical of tiger and lion skeletons, especially the skulls of both species, are listed in the literature (Blanford, 1888; Boule, 1906; Christiansen, 2007, 2008; Formanova et al., 2024; Haltenorth, 1936, 1937; Hemmer, 1966; Herrington, 1987; Kabitzsch, 1960; Mazák, 1983; Merriam & Stock, 1932; Pocock, 1929, 1939; Williams et al., 2015). Most of these features are highly intraspecifically variable, and none of them on their own allows unambiguous identification of tiger and lion skulls. Yet, the skulls of tigers and lions are distinguishable from each other, as shown by multivariate analyses of morphometric features (Christiansen, 2008; Christiansen & Harris, 2009; Herrington, 1987; Jha et al., 2022; Roy et al., 2022). However, if a skull needs to be identified in the field, morphometric measurements of different variables may be too complicated. For frontline people identifying characters should be sufficiently reliable, easily distinguished and recognizable by an untrained person without any special techniques or training (Williams et al., 2015). This study aimed to review all known morphological characters that can potentially differentiate tiger and lion skulls and to identify a set of morphological characters that will help to improve the identification of tiger and lion skulls in practical situations. This set should include characters suitable for use in juvenile skulls or partially damaged skulls. For example, skulls without teeth or the entire lower jaw may appear on the black market. The second aim of this study was to develop a clear and simple methodology for evaluating the selected characters so that they are always evaluated in the same way and the results of the evaluation can be reproduced. It should be possible for people on the frontline to consult a zoological expert remotely on suspicious specimens. This can be very helpful in detecting illegal trade. Therefore, the selected characters should be easy to capture and distinctive in standardized photographs.

2 | MATERIALS AND METHODS

2.1 | Analysed material

Individuals of all ages were included in the study. A total of 119 dried skulls were evaluated (Table 1). Skulls with fully developed permanent dentition were classified as adults, and skulls with fully or partially deciduous dentition as juveniles.

Owing to a lack of data, the subspecies and geographic origin were not included in the analyses.

Seven tiger and seven lion skulls originated from animals born in the wild, 43 tiger and 38 lion skulls were from animals bred in captivity, and the origin of seven tiger and 17 lion skulls was unknown.

The skulls came from the collections of the Czech National Museum, archives of the zoological gardens of the Czech Republic and Slovakia, University of Veterinary Sciences Brno, Faculty of Science of Charles University in Prague, Czech Environmental Inspectorate, Customs Administration of the Czech Republic, Grammar school Havlickuv Brod, and the private collection of the taxidermist Benjamin Hlivka.

As there is no guarantee that all skulls in osteological collections are correctly identified, no doubtful skulls with no information attached were accepted for the study. The skulls included in the study were divided into two groups in terms of the possibility of misidentification: (a) skulls for which the possibility of misidentification or misdescription was minimal (marked as '0' in the dataset), and (b) all other skulls ('1' in the dataset). The first group included skulls for which species was confirmed by genetic analysis (six skulls seized in illegal activities by law enforcement) and skulls that were unmistakably described or had a well-documented origin (known history of the animal, documented state of the individual prior the taxidermy preparation etc.).

All available data on the origin and identification of the skulls are included in Data S1.

TABLE 1 Species, sex, and age of the tested individuals.

	Tiger ($n=57$)		Lion ($n=62$)	
Sex	Adult ($n=51$)	Juvenile ($n=6$)	Adult ($n=50$)	Juvenile ($n=12$)
Male	15	2	16	4
Female	25	0	20	6
Unknown	11	4	14	2

Using a NIKON D5500 digital camera, a series of five high-resolution (6000 × 4000 pixels) digital photographs of each skull was taken: lateral view from both sides, ventral view, dorsal view, frontal view, and if the mandible was not fixed to the skull, a lateral view of the mandible.

2.2 | Studied characters

All literature sources known to the authors on the morphological differences between tiger and lion skulls were studied (Blanford, 1888; Boule, 1906; Christiansen, 2007, 2008; Christiansen & Harris, 2009; Formanova et al., 2022, 2024; Haltenorth, 1936, 1937; Hemmer, 1966; Herrington, 1987; Jha et al., 2022; Kabitzsch, 1960; Mazák, 1983; Merriam & Stock, 1932; Pocock, 1929, 1939; Tilson & Nyhus, 2010; Williams et al., 2015). All characters were then evaluated on photographs of all skulls in the study. According to how each of these distinct characteristics appeared to be useful for the morphological identification of tiger and lion skulls, they were divided into two categories. The first category included characters that were identified as suitable for determination in practical situations including wildlife crime investigation. The methodology for their assessment was outlined in the preliminary results presented by Formanova et al. (2022). The second category represented characters assessed as unsuitable. The complete summary of characters with reasons for their assignment to one of the categories is provided in Table 2.

Only the following characters included in the first category were used for further analyses:

Greater palatine foramen (GPF) ratio (Figure 1a,b). The GPF is located in the posterolateral region of each of the palatine bones. It is visible from a ventral view of the skull, and it is located closer to the medial palatal suture in tigers (Figure 1a) than in lions (Figure 1b). To easily classify the position of the GPF, a ratio of two distances can be measured in a photograph. The first measurement (indicated by arrow X in Figure 1a,b) is the shortest distance (perpendicular) between the outer edge of the GPF and the medial palatal suture, and the second measurement (indicated by arrow Y in Figure 1a,b) is the total length of the medial

TABLE 2 List of studied characters and their applicability in the morphological determination of tiger and lion skulls.

	Morphological characters	Literature sources	Species-specific description according to literature sources		Result of preliminary evaluation	Reasons for not including the characters into further analyses
			Tiger	Lion		
1	Skull shape	Blanford, 1888; Boule, 1906; Haltenorth, 1936, 1937; Hemmer, 1966; Jha et al., 2022; Mazák, 1983; Merriam & Stock, 1932; Pocock, 1929, 1939; Tilson & Nyhus, 2010	Elevated above the orbits so that its upper profile is much more arched	Flat	Suitable. The exact methodology for assessing these characters has been established.	Included in further analyses.
2	The nasal-frontal suture in relation to the maxillary-frontal suture (N-f/M-f)	Boule, 1906; Haltenorth, 1936, 1937; Hemmer, 1966; Jha et al., 2022; Mazák, 1983; Merriam & Stock, 1932; Pocock, 1929, 1939; Tilson & Nyhus, 2010; Williams et al., 2015	The nasal-frontal suture situated well posterior to the level of the maxillary-frontal suture	The nasal-frontal suture does not reach the maxillary-frontal suture or is aligned		
3	Greater palatine foramen (GPF)	Formanova et al., 2022; Jha et al., 2022	The ratio of the distance of the foramen from the midline to the length of the medial palatal suture is lower	The ratio of the distance of the foramen from the midline to the length of the medial palatal suture is higher		
4	Caudal margin of the palatine bones	Formanova et al., 2022; Haltenorth, 1936, 1937; Hemmer, 1966	Central notch is narrower than the marginal notch	Central notch is as wide or wider than the marginal notch		
5	Foramen ovale orientation (FOO)	Formanova et al., 2024	The opening heading forward	The opening heading to the side		
6	Foramen ovale outlet boundary (FOB)	Formanova et al., 2024	Outlet visible, bounded by semi-circular edge of the os basisphenoidale	Outlet hidden behind the straight edge of the os basisphenoidale		
7	Ventral margin of the mandible	Blanford, 1888; Boule, 1906; Haltenorth, 1936, 1937; Hemmer, 1966; Christiansen, 2008; Mazák, 1983; Merriam & Stock, 1932; Pocock, 1929, 1939; Tilson & Nyhus, 2010; Williams et al., 2015	Straight or even concave, the mandible placed on a flat surface rests on the symphysis and on the angular process and lies steadily	Convex, the mandible placed on a flat surface rests on the central part of the horizontal ramus below the carnassial and is able to 'rock'		

(Continues)

TABLE 2 (Continued)

Morphological characters		Literature sources	Species-specific description according to literature sources: Tiger	Lion	Result of preliminary evaluation	Reasons for not including the characters into further analyses
8	The summit of the frontal process of the maxilla	Boule, 1906; Mazák, 1983; Merriam & Stock, 1932	Truncated	Acute	Unsuitable. The precise methodology for assessing these characters has not been reported in the literature	It was not possible to clearly define the difference between tigers and lions
9	The nasal bones	Boule, 1906; Merriam & Stock, 1932; Pocock, 1929, 1939	Convex throughout their length	Flat or slightly convex, especially toward their frontal extremities		
10	The external opening of the nasal fossae	Boule, 1906; Hemmer, 1966; Christiansen & Harris, 2009; Merriam & Stock, 1932	Narrower, heart-shaped, narrow ventrally and abruptly wider around the middle part	Wider, oval, or almost round in shape, and the lateral edges form a smooth curve		
11	The forehead and interorbital space	Boule, 1906; Hemmer, 1966; Merriam & Stock, 1932; Pocock, 1939	Narrower and more arched (convex)	Wider, flatter, and even commonly excavated (concave)		
12	Presphenoid	Hemmer, 1966	Larger	Smaller		
13	Occipital condyles	Herrington, 1987	Shape of anterior border of occipital condyles—no details described			
14	Mandibular symphysis	Christiansen, 2008	Clearly offset from horizontal ramus	Smoothly rounded		
15	The angular process of the mandible	Christiansen, 2008; Christiansen & Harris, 2009	Larger and more ventrally directed	Short, stumpy and postero-dorsally directed		
16	Foramina incisiva—visibility from the top view	Christiansen & Harris, 2009	At least partially invisible, nasal bones are relatively long from dorsal view so that they completely or partially cover foramina incisiva	Completely visible, nasal bones are relatively short from dorsal view so that foramina incisiva stay visible	Unsuitable	This feature is very sensitive to the exact location and angle from which the skull is observed. Assessment of this feature can be misleading, especially when evaluating photographs
17	The coronoid process of the mandible	Boule, 1906; Merriam & Stock, 1932	Projects behind the condyle	Does not project behind the condyle		
18	The temporal part of the frontals and fronto-parietal sutures	Boule, 1906; Merriam & Stock, 1932	More developed, the fronto-parietal sutures are placed further back	Less developed, the fronto-parietal sutures are placed further forward	Unsuitable	In the vast majority of adult skulls in our dataset, the fronto-parietal sutures could not be distinguished at all

TABLE 2 (Continued)

Morphological characters		Literature sources	Species-specific description according to literature sources: Tiger	Lion	Result of preliminary evaluation	Reasons for not including the characters into further analyses
19	The lambdoidal (parietal-occipital) and squamous (parietal–temporal) sutures	Herrington, 1987	Converge posteriorly	Separate	Unsuitable	In almost half of the skulls evaluated, the sutures were indistinct, and their course could not be determined. In a few cases, the course of these sutures differed on the left and right sides of the skull
20	The nasal length and the snout height	Christiansen, 2008; Pocock, 1939	Nasal bones longer (nasal length/condylobasal skull length ratio higher), snout height smaller (snout height/condylobasal skull length ratio lower)	Nasal bones shorter (nasal length/condylobasal skull length ratio lower), snout height greater (snout height/condylobasal skull length ratio higher)	Unsuitable	These characters are impossible to distinguish from a standard photograph
21	Posterior palatine foramen	Boule, 1906; Hemmer, 1966; Merriam & Stock, 1932	Further removed from the orbital border	Closer to the orbital border	Unsuitable	This character is impossible to distinguish from a standard photograph. In addition, it is a delicate structure that is fractured on many skulls
22	Foramen spheno-palatinum	Hemmer, 1966	Larger	Smaller		
23	Canines	Boule, 1906; Christiansen, 2007, 2008; Merriam & Stock, 1932	Longer (C^1/condylobasal skull length ratio and C_1/lower jaw horizontal ramus length ratio higher); more flattened section, their internal face is less convex, the edges are more separated and sharper	Shorter (C^1/condylobasal skull length ratio and C_1/lower jaw horizontal ramus length ratio lower); more rounded section, their internal face is more convex, the edges are less sharp	Unsuitable	Teeth had often broken crowns and were abraded or completely missing from the skulls. Tooth details are poorly resolved in photographs
24	Molars and premolars	Boule, 1906; Hemmer, 1966; Herrington, 1987; Kabitzsch, 1960; Merriam & Stock, 1932; Pocock, 1929, 1939	Molars are less elevated, have their cusps more compressed transversely, with sharper edges; the premolars are less thick in the posterior part	Molars are slightly more elevated, their cusps less compressed transversely, edges blunter; the premolars are thicker in their posterior part		

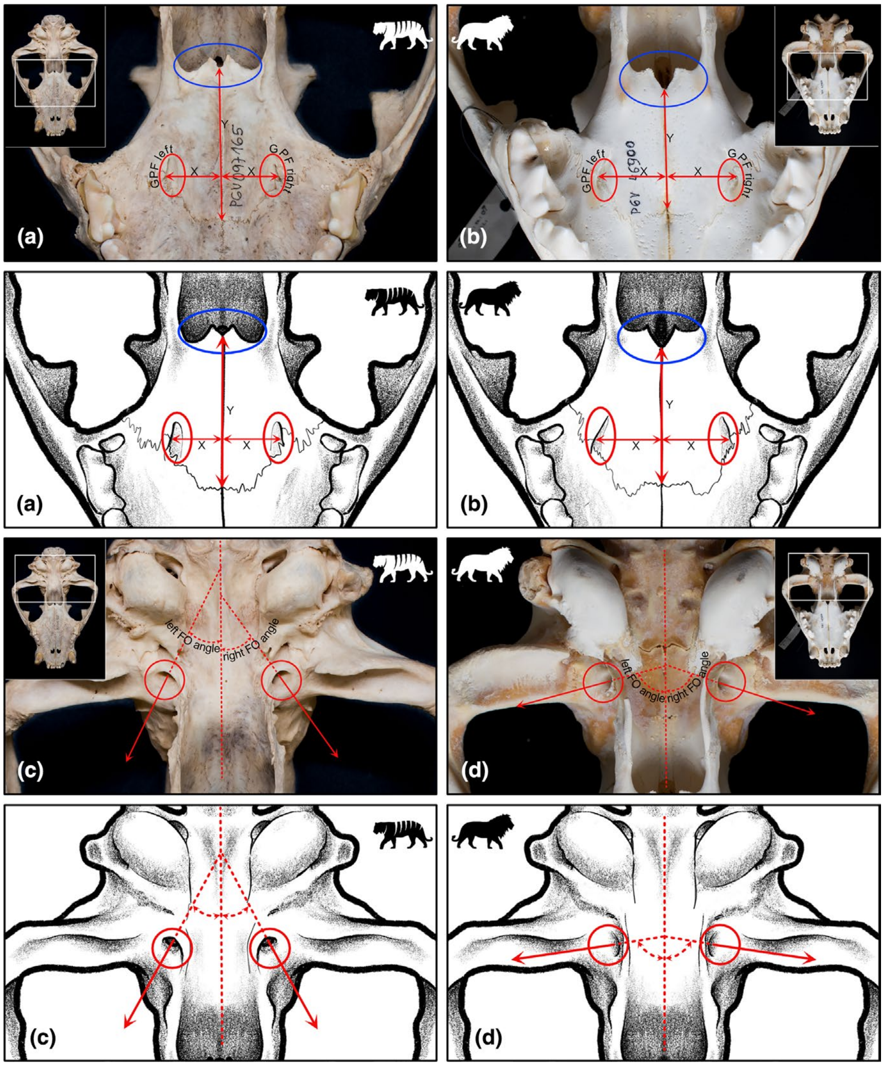

FIGURE 1 a–d. Identifying characters, tiger on the left (a, c photo: *Panthera tigris*, adult female, collection of the Czech National Museum, ID 097165); lion on the right (b, d photo: *Panthera leo leo*, adult male, collection of the Czech National Museum, ID 046900)—a, b. Greater palatine foramen (GPF, highlighted with red circles) ratio and caudal margin of the palatine bones (highlighted with blue circles), detailed view of skulls from the ventral side, the shortest distance between the outer edge of the GPF and the medial palatal suture indicated by arrow X, the total length of the medial palatal suture indicated by arrow Y. (a) tiger skull, photo (GPF ratio X/Y = 0.372, the central (middle) notch of the caudal margin of the palatine bones narrow), and schematic drawing. (b) lion skull, photo (GPF ratio X/Y = 0.550, the central notch wide), and schematic drawing—c, d. Foramen ovale (FO, highlighted with red circles) orientation (FOO) and outlet boundary (FOB), detailed view of skulls from the ventral side (angles of FOO indicated). (c) tiger skull, photo (FOO forward, left angle 32°, right angle 37°, FOB circular and visible), and schematic drawing. (d) lion skull, photo (FOO laterally, left angle 77°, right angle 72°, FOB straight and hidden), and schematic drawing.

palatal suture. The ratio A/B, marked as the 'GPF ratio' in the dataset, is then calculated and evaluated.

Foramen ovale (FO) orientation (FOO) and outlet boundary (FOB) (Figure 1c,d). There are two aspects of this character: 1. orientation of foramina ovalia (FOO) and 2. outlet boundary of foramina ovalia (FOB). Both features can be evaluated from the ventral view of the skull. To evaluate FOO, the angle between two auxiliary lines projected onto the skull was measured. One followed the midline (the exact boundary between the left and right half of the skull) and the other passed in the direction of the outlet of FO. Because the left and right FO can differ significantly in their orientation, it is better to measure the angle on both sides. A FO with an angle of less than 50° is considered as directed rostrally, which was found to be typical of tiger skulls (Figure 1c, marked as 'Forward' in the dataset), and a FO with an angle of more than 70° is considered as directed laterally, which is the character state that is typical of the lion skull (Figure 1d, marked as 'Laterally' in the dataset). Skulls with an angle of between

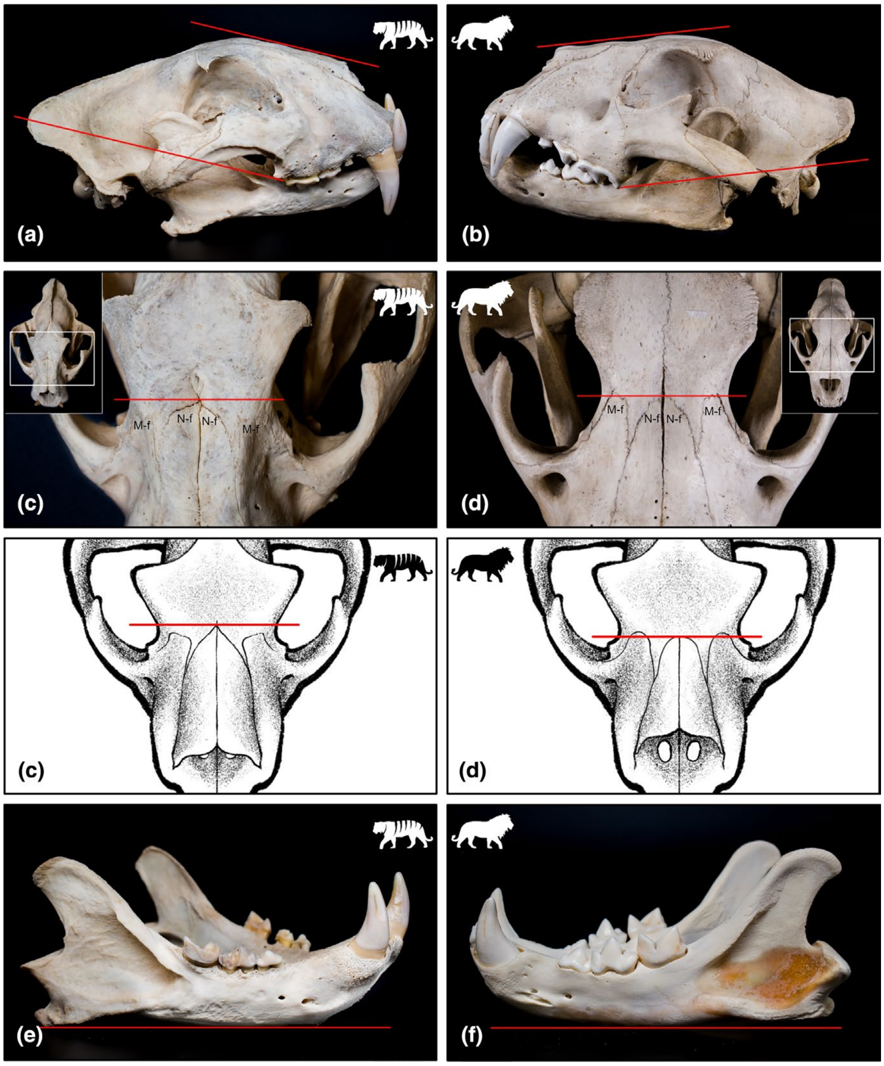

FIGURE 2 a–f. Identifying characters, tiger on the left (a, c, and e photo: *Panthera tigris*, adult female, collection of the Czech National Museum, ID 097165); lion on the right (b and d photo: *Panthera leo*, adult, sex unknown, collection of the Safari Park Dvur Kralove, ID Cakl; f photo: *Panthera leo leo*, adult male, collection of the Czech National Museum, ID 046900)—a, b. Skull shape, auxiliary parallels indicated. (a) tiger skull arched (parallel line crosses the sagittal crest). (b) lion skull flat (parallel line crosses bellow the sagittal crest)—c, d. Nasal-frontal suture in relation to the maxillary-frontal suture (N-f/M-f), detailed view of skulls from above, the outermost point reached by the nasal-frontal and maxillary-frontal sutures indicated. (c) tiger skull, photo (N-f > M-f, N-f sutures exceed the posterior projections of M-f sutures), and schematic drawing. (d) lion skull, photo (N-f < M-f, N-f sutures do not extend beyond M-f sutures), and schematic drawing.—e, f. Ventral margin of the mandible, the line of contact of the mandible with the flat surface indicated. (e) Tiger mandible stable (rests on the symphysis and the angular process). (f) lion mandible rocking (rests on the central part of the horizontal ramus).

50° and 70° and skulls in which the left FOO differs from the right FOO cannot be identified by this character (marked 'Unclear' in the dataset). FOB was also evaluated on both sides of the skulls. From the ventral view of the skull, the outlets of FO may be visible, bounded by a semicircular edge of the os basisphenoidale, which is typical of the tiger skull (Figure 1c, marked as 'Circular' in the dataset). Otherwise, the outlets of FO may be at least partially hidden behind the straight edge of the os basisphenoidale, which is typical of the lion skull (Figure 1d, marked as 'Straight' in the dataset). Intermediate ('Unclear' in the dataset) character states occur.

Caudal margin of the palatine bones (Figure 1a,b). This character can be assessed from the ventral view of the skull. The caudal margin of the fused palatine bones has a typical shape in tigers and lions, which can be described as three notches. The central notch is formed by both the left and right palatine bones, and the medial palatine suture emerges from its centre. If the central (middle) notch is narrower than the outermost notches, it is a tiger character (Figure 1a, marked as 'Narrow' in the dataset). Exceptionally, the middle notch may be completely missing in tigers (marked as 'None' in the dataset). If the middle notch is at least as wide as the outermost notches, but often wider, it is a lion character (Figure 1b, marked as 'Wide' in the dataset).

Skull shape (Figure 2a,b). The skull shape was evaluated from the lateral view. Two auxiliary lines were used for the assessment. The first line runs along the dorsal border of the nasal bone. A parallel line is then drawn so that it passes through the posterior edge of the maxilla. The point at which this parallel line crosses the upper line of the skull is assessed. If the parallel line crosses the skull at the sagittal crest (or at the occipital bone in juveniles), the skull is assessed as 'Arched' as typical for tigers (Figure 2a). If the parallel line projects from the skull below the sagittal crest on the posterior side of the temporal bone, the skull is assessed as 'Flat' as typical for lions (Figure 2b).

The nasal-frontal suture in relation to the maxillary-frontal suture (N-f/M-f) (Figure 2c,d). This character can be assessed from the dorsal view of the skull. If the posterior projections of N-f exceed the posterior projections of M-f, it is a tiger character (Figure 2c, marked as 'N-f > M-f' in the dataset). On the contrary, if the posterior projections of the N-f are in line with, or slightly anterior to the posterior projections of the M-f, it is a lion character (Figure 2d, marked as 'N-f = M-f' or 'N-f < M-f' in the dataset).

Ventral margin of the mandible (Figure 2e,f). The ventral profile of the mandible may be straight or even concave. If a mandible is placed on a flat surface and rests stably on the symphysis and the angular processes of the mandible, this is considered a tiger character. Otherwise, if the ventral margin of the mandible has a rounded convex profile, the mandible rests usually on only one point, and therefore 'rocks' when placed on a flat surface. This is considered a lion character. The stability of the skull on a flat surface can be readily estimated from a lateral view photograph by drawing a straight line under the ventral edge of the mandible (Figure 2e, marked as 'Stable' and Figure 2f, marked as 'Rocking' in the dataset).

2.3 | Measured dimensions

To complement the morphological characteristics and for further analyses, the basic dimensions of all the skulls were measured using a vernier caliper:

Greatest skull length (GSL): the length from the most anterior part of the rostrum (excluding teeth) to the most posterior point of the skull.

Zygomatic breadth (ZB): the greatest distance between the outer margins of the zygomatic arches.

Length of the mandible (LMd): the length between the angular process and the infradentale (for simplicity, the prominent angular process was used rather than the cylinder condylar process).

Height of the mandible (HMd): the greatest distance between the highest point of the coronoid process and the lowest point of the angular process of the mandible.

2.4 | Data analysis

Data from all 119 skulls were included in the analyses unless stated otherwise. A complete set of data is available in Data S1. Some skulls show empty boxes in connection to selected characters in the dataset, owing to their absence in incomplete skulls. All morphological differences between sexes were assessed solely on adult individuals. Decision trees with the CHAID growing method and one ($x=1$) as a minimal number of cases in both parent and child nodes were created in SPSS 28.0.1.0 (IBM SPSS Inc., 2021). All other analyses were processed in TIBCO Statistica 14.0.0.15 (TIBCO Software Inc., 2021). Nonparametric measures and tests were used besides Chi-squared tests. Specifically, Spearman correlation coefficients (ϱ), Kendall rank correlation coefficients (τ), Mann–Whitney U-tests, and Kruskal–Wallis tests were calculated. Fisher's exact tests (two-tailed) were applied when the data did not meet the assumptions for the Chi-squared tests. A significance level $\alpha=.05$ was established for all calculations. All results were rounded to three decimal places except percentage values, which were rounded to one decimal place.

3 | RESULTS

The text refers to individual detailed results (RT1-22, RL1-22, R1-R75) of all analyses that are available in Data S2. Primarily, the study examined possible differences between the skulls with minimal possibility of misidentification or misdescription and all the other skulls included in the study. In lions, no significant difference was found for any of the tested characters (RL1-11). The same results were found in tigers (RT1-9, RT11) except for a significant difference in N-f/M-f (RT10). Both tested groups were dominated by skulls showing N-f > M-f. The difference was caused by the different ratios of N-f = M-f and N-f < M-f. However, the frequency of both variants of this character was low in both groups. Owing to the difference in only one character in one of the tested species and the fact that the detected trend of the dominating type of skull shape was the same in both types of skulls, we did not exclude any skulls from further analyses.

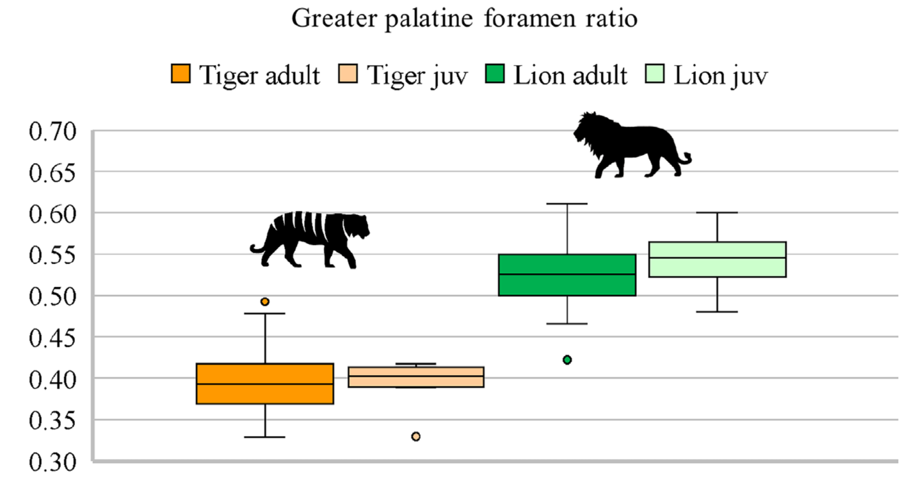

FIGURE 3 Differences between tiger and lion skulls in GPF ratio.

As a next step, possible differences between the skulls originating from wild and captive individuals and skulls with unknown origin were tested. No significant difference was found in tigers (RT12-22). The same results were found in lions (RL12-19, RL21-22) except for a significant difference in the skull shape (RL20), although the flat profile prevailed in all tested groups no matter the origin. Owing to the difference in only one character in one of the tested species and the fact that the detected trend of skull shape was the same in all origins, we decided to analyse all skulls together no matter the origin of the individual.

The skulls of tigers and lions did not significantly differ in their size, no matter which measure (GSL, ZB, LMd, HMd) was considered (R1-4, respectively). Adult skulls reached significantly higher values of GSL, ZB, LMd, and HMd than skulls of juveniles in both tigers (R5-8) and lions (R9-12). In all measured dimensions, male skulls were shown to be bigger than female skulls in both species (tigers R13-16 and lions R17-20, respectively).

3.1 | Studied characters

3.1.1 | Greater palatine foramen ratio

The Greater palatine foramen (GPF) ratio as the only quantitative variable between the studied characters was tested for any relationship with relevant skull dimensions (i.e. GSL and ZB). As tigers and lions did not significantly differ in skull size, according to the obtained results, the correlation was tested for all skulls together. A relationship was found neither between the GPF ratio and GSL (R21-22) nor between the GPF ratio and ZB (R23-24) (Figure 3).

The GPF ratio was significantly lower for tigers than for lions (R25) and the results were the same even when adults (R26) and juveniles (R27) were tested separately. Neither age (tigers R28, lions R29) nor sex (tigers R30, lions R31) significantly influenced the GPF ratio in both species. For 80.7% of all tigers ($n = 46$) GPF ratio was less than 0.42 and there were no lions with similar ratios in the dataset. For 82.3% of all lions ($n = 51$) GPF ratio was higher than 0.50 and there were no tigers with similar ratios in the dataset. About 11 tigers (19.3%) and 11 lions (17.7%) overlapped with a GPF ratios between 0.42 and 0.5.

3.1.2 | Foramen ovale (FO)—orientation (FOO) and outlet boundary (FOB)

Significant differences were found between tigers and lions in FOO (R32) and FOB (R33). The same results were obtained when adult (FOO R34, FOB R35) and juvenile (FOO R36, FOB R37) skulls were tested separately. While FOO and FOB were found to be independent in lions (R38), a relationship between these two was not excluded in tigers (R39).

In tigers, FOO significantly differed between adult and juvenile skulls (R40). In adult tiger skulls, 49.0% ($n = 25$) had both FO oriented forward and the rest of the individuals showed FO of unclear orientation. No tiger had both FO oriented laterally. Juvenile tigers showed almost exclusively unclear orientation of FO. There was only one case of both FOs oriented laterally. In contrast, no difference in FOO was observed between adult and juvenile lions (R41). The majority of adult (52.0%, $n = 26$) and juvenile (75.0%, $n = 9$) lions had both FOs oriented laterally. All the rest showed unclear orientation. No lion had both FOs oriented forward. In adult tigers (R42) and lions (R43), no significant differences in FOO were detected between the sexes.

Adult and juvenile tigers did not significantly differ in FOB (R44). The majority of adult (64.7%, $n = 33$) and juvenile (66.7%, $n = 4$) tigers had both boundaries of FO circular. The rest of the individuals showed unclear FOB, except for one adult tiger with both FOBs assessed as straight. As in tigers, no significant difference was found between the FOB of adult and juvenile lions (R45). Adult lions showed both FOBs as straight in 44.0% ($n=22$) of skulls, unclear in 42.0% ($n=21$) of skulls, and only in 14.0% ($n=7$) of skulls was there circular FOB typical for tigers. Most juvenile lions (83.3%, $n=10$) had straight FOB and only two individuals had FOB evaluated as unclear. In both species, no significant differences in FOB were detected between sexes (tigers R46, lions R47).

3.1.3 | Caudal margin of the palatine bones

Tigers and lions significantly differed in the matter of the caudal margin of the palatine bone assessment (R48). The same result was obtained when only adult individuals were tested (R49). However, it was not confirmed in juveniles (R50). In both species, a relationship between age and this character was found (tigers R51, lions R52). In adult tigers, the central notch was mostly narrow (78.4%, $n=40$), but there were also cases with wide (9.8%, $n=5$) or completely missing (11.8%, $n=6$) central notches. In contrast, almost all skulls of adult lions showed wide central notches (98.0%, $n=49$) except one case with a narrow one (2.0%). No lion (adult or juvenile) was missing a central notch. No significant difference was found between the sexes in both species (tigers R53, lions R54). Central notches of juvenile tigers were the following: three wide, two narrow, and one missing. In lions, eight juveniles showed wide and four showed narrow central notches.

3.1.4 | Skull shape

One adult tiger skull and one adult lion skull were not evaluated because the nasal bones and the back of the skull, respectively, were damaged. The shapes of tiger and lion skulls significantly differed (R55). However, when different age groups were tested separately, only adults showed significant differences (R56) and juveniles did not (R57). The age does not seem to have an impact on the tiger skull shape (R58). The prevailing shape of the tiger skull was arched which occurred in 88.0% of adult ($n=44$) and 100.0% of juvenile ($n=6$) individuals. Only 12.0% of adult tigers ($n=12$) showed flat skulls. In contrast, age influenced the shape of the skull in lions (R59). Although the majority of adult lions had flat skulls (91.8%, $n=45$) and only 8.2% showed arched skulls ($n=4$), juvenile individuals had arched and flat skulls in 58.3% ($n=7$) and 41.7% ($n=5$) of cases, respectively. No significant difference was found between the sexes in both species (tigers R60, lions R61).

3.1.5 | Relation of the nasal-frontal suture to the maxillary-frontal suture (N-f/M-f)

The results of the assessment of this character significantly differed between tested species (R62). Nevertheless, the situation was the same as in skull shape evaluation. While the adults showed significant differences between the species (R63), juveniles did not (R64). Age influenced this character in tigers (R65), but not in lions (R66). Adult tigers showed N-f extended beyond M-f (N-f > M-f) in 76.5% ($n=39$) of cases, N-f in line with M-f (N-f = M-f) in 15.7% ($n=8$) of cases, and N-f slightly anterior to M-f (N-f < M-f) in 7.8% ($n=4$) of cases. In juvenile tigers, N-f slightly anterior to M-f was observed in five skulls and only one skull showed N-f extended beyond M-f. Lion skulls showed the following results: 44.0% ($n=22$) of adults and 33.3% ($n=4$) of juveniles showed N-f anterior to M-f (N-f < M-f), 46.0% ($n=23$) of adults and 50% ($n=6$) of juveniles had N-f and M-f in line (N-f = M-f), and in 10.0% ($n=5$) of adults and 16.7% ($n=2$) of juveniles N-f extended beyond M-f (N-f > M-f). No significant difference was found between the sexes in any of the tested species (tigers R67, lions R68).

3.1.6 | Ventral margin of the mandible

Owing to the lack of a mandible, this character could not be assessed in two skulls of adult tigers and four skulls of adult lions. The shape of the ventral margin of the mandible significantly differed between tigers and lions (R69). When adults and juveniles were tested separately, adults showed significant differences between species (R70), but juveniles did not (R71). As in the case of N-f/M-f relation, age influenced this character in tigers (R72), but not in lions (R73). Most adult tigers (87.8%, $n=43$) had stable mandibles and 12.2% ($n=6$) of adult tiger mandibles rocked. In contrast, only one juvenile tiger skull was stable, and the rest ($n=5$) rocked. Stable mandibles occurred in 23.9% ($n=11$) of adult lions and only one juvenile lion (8.3%). The rocking mandible was most prevalent in lions. It was found in 76.1% ($n=35$) of adults and 91.7% ($n=11$) of juveniles. No significant difference was found between the sexes in both species (tigers R74, lions R75).

3.2 | Combination of all studied characters

All seven studied characters were input as independent variables in the decision trees. Two decision trees were created (Data S3). The first one was based on data from all skulls with valid values in all tested characters ($n=111$). The second one was based only on the data from the skulls of adults with valid values in all tested characters ($n=93$). The overall predictability of correct species identification reached 100% in both cases. The characters common to both decision trees are the GPF ratio and FO. Nevertheless, different characteristics of FO were important for individual trees. GPF ratio and FOB were accompanied by N-f/M-f when all skulls were analysed together. In adult skulls only, the GPF ratio and FOO were accompanied by the shapes of the caudal margin of the palatine bone and the ventral margin of the mandible. Skull shape did not appear in the decision tree of any dataset.

Regarding the total number of identifying characters found on individual skulls, 87.7% of tiger skulls ($n=50$) and 83.9% of lion skulls ($n=52$) in the overall dataset, had at least four species-specific characters. If only adult skulls were considered, this was 90.2% of tiger ($n=46$) and 86.0% of lion skulls ($n=43$). At the same time, 82.5% of tiger ($n=47$) and 90.3% of lion skulls ($n=56$) had none or only one character typical of the second species. If only adult skulls were considered, this was 92.2% of tiger ($n=47$) and 94.0% of lion skulls (n=47). In the total tiger skull collection, there were seven skulls, including four juvenile skulls, that had two lion characters (12.3% of all tigers) and three skulls, including two juvenile skulls, that had three lion characters (5.3% of all tigers). In the total lion skull collection, there were four skulls, including two juvenile skulls, that had two tiger characters (6.5% of all lions) and two skulls, including one juvenile skull, that had three lion characters (3.2% of all lions). No skull was found to have more than three characters of the other species.

4 | DISCUSSION

Until now the methodology recommended for customs officers inspecting large felid skeletal shipments has been based on the examination of two morphological features: N-f/M-f and ventral margin of the mandible, which were applicable only to adult skulls (Williams et al., 2015), or the method of Jha et al. (2022), which relies on the measurement of only 16 tiger skulls and one lion skull. Our results have revealed that the two features used by Williams et al. (2015) fail for 14.7% of adult skulls if used on their own (for more detailed results see Data S4). Further, Herrington (1987) stated that the distinction between tiger and lion skulls should be possible by the relative positions of the lambdoidal and squamosal sutures. However, in light of our research and in agreement with Christiansen (2008), this feature alone cannot be used to make a confident determination (see Table 2). Reliable identification of tiger and lion skulls, using morphological characters was described by Christiansen (2008), but this method is closer to a complex morphometric measurement and its application in the field can be problematic. Besides, it was developed only for adult skulls.

This study describes individual morphological characters that can be used in the identification of adult and juvenile skulls. Only skulls for which the original species determination should have been correct were included in the research, but it cannot be excluded that there are skulls in the set that were misidentified. However, a comparison of the group of skulls for which misdetermination was most likely ruled out with the group of all other skulls showed no difference in any of the morphological characters observed that could affect the use of these characters in the species identification described in this study.

The characters recommended have great potential to distinguish between tiger and lion skulls in a very reliable yet simple manner. The advantage for frontline people is that the proposed characters need not be correlated to the overall size of the skulls and that they are identifiable in photographs. The disadvantage is that they were only tested on a small sample of skulls from juvenile animals (six juvenile tigers, four of which belonged to the subspecies *Panthera tigris altaica*, and 12 juvenile lions, seven of which were from the Indian population) and a small sample of skulls from wild animals (seven tigers and seven lions, with the lions being only individuals from the African region). Cooper et al. (2022, 2023) found that the skull morphology of both tigers and lions is influenced by the type of diet, and the skull phenotype of captive animals differs from that of wild animals. In our study, there was no difference in the observed characters between the skulls of wild and captive animals, but only 14 skulls originating from wild animals were included, six of which were subsequently kept in zoos. It would therefore be worthwhile to test and validate the described characters on a larger sample of wild animal skulls and also on a larger sample of juvenile skulls. The differences between subspecies and between individuals from separate geographical areas should also be tested.

The GPF ratio alone distinguished the vast majority of lion and tiger skulls with high accuracy. This character can be tentatively evaluated by a cursory glance, but precise measurement is preferred. This character is not affected by skull size or sex and is usually preserved even in cases of extensive skull damage. Our results for this character are consistent with the findings of Jha et al. (2022). In addition, the GPF ratio appears to be able to discriminate species also in the skulls of juveniles, although the sample size of juveniles was very limited. Though skulls of adults are crucial for legal as well as illegal trade, even sporadic data referring to juveniles can be very useful for wildlife crime detection. This is especially so as most morphological studies completely ignore juvenile skulls. Of the characters analysed in detail in this study, only the GPF ratio and foramina ovalia (FOO and FOB) have the potential to correctly identify the species of juvenile skulls.

The foramen ovale (FO) was discussed by Formanova et al. (2024).

The caudal margin of the palatine bone was mentioned in Haltenorth (1936, 1937); Hemmer (1966), but has not been used in other studies dealing with the identification of big cats' skulls since. This character can be used for preliminary differentiation of all larger felid species (Formanova, unpublished data, see also Haltenorth, 1936, 1937; Hemmer, 1966).

The N-f/M-f ratio and the ventral margin of the mandible are two characters that are widely referenced in original studies distinguishing tiger and lion skulls (Blanford, 1888; Boule, 1906; Christiansen, 2008; Haltenorth, 1936, 1937; Hemmer, 1966; Jha et al., 2022; Merriam & Stock, 1932; Pocock, 1929, 1939; Williams et al., 2015). Both of these characters were indeed found to be very easy to use, in line with Williams et al. (2015), but did not prove to be very reliable in our dataset when used separately from other characters, even if we only include adult skulls in the analysis (see Data S4).

The shape of the skull also appears extensively in studies of tiger and lion anatomy (Blanford, 1888; Boule, 1906; Haltenorth, 1936, 1937; Hemmer, 1966; Jha et al., 2022; Mazák, 1983; Merriam & Stock, 1932; Pocock, 1929, 1939; Tilson & Nyhus, 2010), but it has not been specified how to decide whether the skull is more arched or flat.

The differences in skull shape and the ventral margin of the mandible in juveniles and adults of both species found in our study can be explained by ontogenetic development. The cubs of both species are born with an arched cranial and shortened facial part of the skull, and only gradually approach the species-typical skull shape. In adult tigers, it consists of an arched skull and straight ventral margin of the mandible, while in adult lions develops a flat skull and convex ventral margin of the mandible. Therefore, in tigers, adults differ from juveniles in the ventral margin of the mandible but not in the skull shape, and in lions, adults differ from juveniles in the skull shape but not in the ventral margin of the mandible.

All morphological characters presented in this study can be freely combined in the determination of tiger or lion skulls. The identification key based on the decision trees calculated from all of the above determination characters was surprisingly simple when applied universally to all skulls, including juvenile ones than when only adult skulls were included. The universal key works in two steps, differentiating 100% of the skulls and eliminating characters that were problematic in terms of data availability – the mandible, which was missing from some skulls, and skull shape, which cannot be examined if the upper part of the skull is damaged. However, it is always good to have multiple options to distinguish skulls precisely because not all features are always available. The identification of a skull should always use as many morphological features as possible. Genetic analysis can always be used as a back-up for species identification in case of doubt (Williams et al., 2015).

5 | CONCLUSIONS

Seven morphological characters of the skulls of tigers and lions were determined as being able to distinguish between the two species. These can be used to quickly identify skulls in practical situations. Typically, tiger characters are as follows: GPF ratio<0.42; FOO forward, with angle <50° in both FO; FOB circular and visible in both foramina; central notch in the caudal margin of the palatine bones narrow; skull shape arched; N-f > M-f; mandible stable. In contrast, the following characteristics are typical of lions: GPF ratio>0.50; FOO lateral, with angle >70° in both FO; FOB straight and invisible in both foramina; central notch in the caudal margin of the palatine bones wide; skull shape flat; N-f < M-f; mandible rocks.

AUTHOR CONTRIBUTIONS

Data collection and study on skulls was carried out by Dominika Formanova. Statistical analysis was performed by Anna Kubatova. Both authors drafted and finalized the manuscript and approved the final version.

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CONFLICT OF INTEREST STATEMENT

None known for all authors.

DATA AVAILABILITY STATEMENT

A complete dataset is available in the supplementary material of this article (Supplementary file 1). The original photographs are archived at the Environmental Forensic Sciences Centre, Institute for Environmental Studies, Faculty of Science, Charles University, Prague, Czech Republic, and can be provided on request from the corresponding author.

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SUPPORTING INFORMATION

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

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