



ORIGINAL INVESTIGATION

**Craniometric variation in the tiger (*Panthera tigris*):
Implications for patterns of diversity, taxonomy and conservation**

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Abstract

Patterns of geographical variation in tigers are reviewed extensively by a morphometric analysis based on 273 skulls of certain wild origin. The following principal observations emerging from this investigation are found:

1. Modern tigers contain two basic forms: the mainland Asia tiger and the Sunda Island tiger. They are differentiated markedly in skull morphology as well as other morphological characters, the characteristic skull shape and small body size in Java/Bali tigers can be interpreted as adaptational responses to a particular island landscape type and prey species fauna, an evolutionary process known as insular dwarfism.
2. The Sumatran tiger (*P.t. sumatrae*) probably represents a hybrid of mainland and Island tigers, which originated from mainland Southeast Asia, colonized Indonesia and hybridized with the Sunda island tigers (Java tiger) during the late Pleistocene, and was subsequently completely isolated from both the mainland Southeast Asia and Java/Bali populations.
3. Among the mainland Asia tigers, the Amur or Siberian tiger is the most distinct; India, Indochinese and South China tigers are craniometrically distinguishable on average, but with clear overlaps; the Caspian tiger, on the other hand, is indistinguishable from other mainland forms and extensively overlaps with both the Northern and Southern Asia subspecies.
4. Most proportional craniometric differences among tigers observed from this study are mainly allometric; the pattern of craniometric variation in mainland tigers is clearly clinal; craniometric variation and sexual dimorphism are closely related.
5. Skull morphometric characters are quite effective for discriminating major tiger geographical populations, but further analysis using other phenetic craniodental characters (shape of sagittal crest, degree of convexity of the frontal, endocranial volume, detailed carnassial morphology) as well as molecular genetic sources, rather than this purely metric study, would certainly be of considerable value in understanding the evolutionary relationships among mainland Asia tigers and their appropriate taxonomic designations.

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Introduction

The tiger (*Panthera tigris*) is the largest and one of the most critically endangered felids in the world (Nowell and Jackson 1996; Seidensticker et al. 1999; Sunquist and Sunquist 2002). As a widely recognized symbol of wildlife conservation (Luo et al. 2004), every aspect of its biology (evolutionary history, systematics, ecology, behavior, molecular biology and biogeography) are of great significance in conservation and management projects. Of the eight traditionally accepted putative tiger subspecies, hunting, habitat loss and fragmentation caused three or probably four to go extinct, while the remaining four are close to extinction (Nowell and Jackson 1996; Seidensticker et al. 1999). The determination of subspecies and patterns of geographical variation are of critical importance for global tiger conservation and management (Cracraft et al. 1998; Kitchener 1999; Luo et al. 2004; Mazák and Groves 2006). Traditionally putative tiger subspecies are defined largely on body size, pelage coloration and striping patterns, skull dimensions and craniological details (Hemmer 1978; Kitchener 1999; Mazák 1979, 1981; Nowell and Jackson 1996; Pocock 1929). The recent widely used biochemical (Goebel and Whitmore 1987; Newman et al. 1985) and molecular genetic analysis (Cracraft et al. 1998; O'Brien et al. 1987; Wayne et al. 1989; Wentzel et al. 1999), however, yield a different image in intraspecific recognition, although the latest genetic investigation using 134 “voucher specimens” suggests that recognition of the six taxonomic units or subspecies of tigers can be confirmed, with one further subspecies new to science (Luo et al. 2004, 2006). There are, however, as Luo et al. (2006) suggested, some aspects of tiger phylogeny and subspecific recognition which still remain uncertain. For instance, whether the significant subdivision in mtDNA among the putative subspecies could be supported from the evidence of morphological analysis? Whether the degree of differentiation of the three extinct subspecies (Caspian tiger *P.t. virgata*, Java tiger *P.t. sondaica*, Bali tiger *P.t. balica*) are comparable to those revealed from the living subspecies?

Understanding the nature of geographic variation morphologically in tigers is thus of major importance in their conservation and management (Kitchener 1999; Mazák and Groves 2006). Kitchener (1999), in his comprehensive review largely based on analysis on variations of pelage marking and body size and biogeographic analysis, strongly maintained that the pattern of geographical variation in tigers is basically clinal, while J. Mazák (2004) and J. Mazák and Groves (2006) found that there are considerable craniodental distinctions among some of the putative subspecies, and separated the modern tigers into two major species-level groups (mainland group and Island group) by applying the Phylogenetic Species Concept (PSC). In short, two

points we still need to know: Are the patterns of geographic variation in tigers really clinal? Can the cranial distinctions among the putative subspecies be confirmed when a comprehensive morphometric review over the entire geographic range, with more specimens, is undertaken?

A brief review of the geographic variation and taxonomy of tigers

Our understanding of geographic variation and intraspecific taxonomy in tigers ranged from Linnaeus's type description (but not supported by specimens) of this species, through Pocock's fundamental review (Pocock 1929) and V. Mazák's series of voluminous works (V. Mazák 1967, 1968, 1976, 1978, 1979, 1981), to the current both morphological and molecular genetic analyses (Phenotypic variation: Herrington 1987; Kitchener 1999; J. Mazák and Groves 2006; molecular genetics: O'Brien et al. 1987, Luo et al. 2004, 2006; Wentzel et al. 1999).

The first scientific description of the tiger, with its scientific name *Felis tigris*, came from Linnaeus's type description in 1758, which is neither supported by specimens nor by mention of any exact locality (the type locality was fixed as Bengal by Thomas 1911). The second accepted tiger subspecies was the Caspian tiger, which was described by Illiger in 1815 based on fur characters and an unknown number of specimens (Illiger 1815); then in 1844 the Amur and the Javan tigers were made known to science by Temminck on the basis of two mounted skins (Temminck 1844). In the early twentieth century, the South China tiger, Bali tiger and Sumatran tiger were named, each based on a limited number of specimens (Hilzheimer 1905; Schwarz 1912; Pocock 1929). Pocock (1929), who reviewed the subspecies of tigers extensively, based on a number of skins and skulls preserved in the British Museum (Natural History), provided the basic features for distinguishing subspecies (body size, stripe pattern and ground coloration, skull characters and dimensions). The later named Indochinese tiger was based on an examination of a relatively large sample size (19 skulls and 13 skins) compared to the other subspecies, with comparisons with the previously described nominate subspecies (V. Mazák 1968). More subspecies are still being proposed today which clearly have no chance to be valid (Kirk 1994; see Kock 1995). Lately, the Malay tiger has been regarded as distinct, and proposed as new subspecies based on molecular genetic analysis (Luo et al. 2004). It should also be noted that the characters, which have been used to distinguish between subspecies by early authors are various, but mainly concern striping patterns, ground coloration of the pelage, and fur length (Illiger 1815; Temminck 1844). We now

know, certainly, that pelage coloration and markings are highly polymorphic in tigers, and variations within putative subspecies are greater than variation between them (Kitchener 1999; Mazák 1967). Although later authors noticed the importance of skull (Hilzheimer 1905; Schwarz 1912; Pocock 1929; Brongersma 1935) or more detailed characters (Hilzheimer 1905) in the definition of subspecies, comparative quantitative analysis on more abundant specimens are obviously scarce, and it is unclear whether these characters are effective in distinguishing between putative subspecies (Kitchener 1999). It can thus be concluded that, of the eight traditionally accepted putative subspecies, most of them were based on very limited material and described on rather poor scientific evidence.

Later intraspecific taxonomy has derived more exclusively from the work of a Czech mammalogist, the late Vratislav Mazák. Based on his examinations on abundant samples from the major European collections, V. Mazák gave very extensive descriptions as well as size dimensions for each putative subspecies, and his detailed opinions on tiger subspecies (particularly in his book *Der Tiger*) formed a basic classification that became standard for 20 years and has widely been accepted and used in subsequent studies on tigers till recent times (Nowell and Jackson 1996; Seidensticker et al. 1999; Sunquist and Sunquist 2002).

Recently, the traditional eight putative tiger subspecies have been challenged by evaluations of morphology (Kitchener 1999) and molecular genetics (Wentzel et al. 1999). Kitchener (1999), based on his review of morphological variation and zoogeographical assessment, pointed out that there is more variation in body size, pelage marking and skull characters within putative subspecies than between them, and there is hardly any evidence to support significant geographic barriers to tiger gene flow (except deserts and sea isolation in the case of the Caspian and Sunda Island tigers) during the last two million years, and suggested that there is in fact little evidence to support so many discrete subspecies. Wentzel et al. (1999) also found comparatively little variation in mtDNA and MHC-DRB, and inferred that populations have probably undergone gene exchange until recently, suggesting a minimum of molecular genetic support for the current subspecies classification. Most recently, the traditional living tiger subspecies (with the description of a new subspecies *P.t. jacksoni*) were recognized by an extensive molecular survey using 134 voucher specimens (Luo et al. 2004, 2006).

Skull morphometrics in tigers: the aim of the present study

Cranial morphology is likely to be of major significance in reconstructions of tiger evolutionary history

and phylogeography. I here examine morphological diversity among tigers using multivariate analysis of craniometric characters, in particular to address the following specific aims:

1. To evaluate the degrees of similarities and differences among the major geographic populations, and patterns of diversity in cranial metric morphology.
2. To assess whether the eight putative tiger subspecies can be confirmed from a multivariate craniometric evaluation.

Material and methods

Samples

The major materials used in this study are the late Dr. Vratislav Mazák's original data set of measurements of tiger skulls from the major European collections (kindly communicated by Prof. Colin Groves), and supplementary data including specimens measured by myself or by colleagues from the major Chinese, Russian, Indian, several European and one U.S. museum collections. The complete data set consisted of 19 linear cranial, mandibular and dental measurements from 288 tiger skulls, from which I selected specimens measured only by V.M. and me with reliable locality records and certain origin in the wild for analysis. The samples contain young adult (basal suture half or nearly fused) and adult skulls (basal suture completely fused). The final total sample used in this study contains 273 skulls (Table 1). Only samples with complete data sets were included in subsequent multivariate analyses, reducing the total to 172 skulls consisting of 88 males and 84 females. Specimens with incomplete data were used for basic statistics (means, SD, and bivariate regression analysis). As tigers are very strongly sexually dimorphic in cranial-dental morphology (J. Mazák 2004), sexes were treated separately in all statistical analyses.

Measurements

Nineteen cranial, mandibular and dental measurements were taken. For testing measurement errors between observers (V.M. and J.H.M.), eight skulls of Indochinese tiger males

Table 1. Numbers of specimens used in this study by subspecies and sex of tigers

Putative tiger subspecies	Males	Females
<i>P.t. tigris</i> (Linnaeus, 1758)	143	130
<i>P.t. virgata</i> (Illiger, 1815)	59	33
<i>P.t. virgata</i> (Illiger, 1815)	6	12
<i>P.t. altaica</i> (Temminck, 1844)	18	15
<i>P.t. amoyensis</i> (Hilzheimer, 1905)	8	7
<i>P.t. corbetti</i> (Mazák, 1968)	19	27
<i>P.t. sumatrae</i> (Pocock, 1929)	8	20
<i>P.t. sondaica</i> (Temminck, 1844)	22	13
<i>P.t. balica</i> (Schwarz, 1912)	3	3

were selected randomly, and a paired *t*-test was undertaken to test whether there are any significant differences in measurements taken between V.M. and J.H.M. prior to further statistical analysis. The result indicated that there are no significant differences ($p < 0.05$) in measurements between the observers except basal length 2 (paired *t*-test, $n = 8$, d.f. = 3, $t = -1.056$, $p = 0.027$), so basal length 2 was removed from further analysis.

Craniodental variables used in this investigation are illustrated in Fig. 1 and their abbreviations are given as follows:

Greatest skull length (GLS), condylobasal length (CBL), basal length 1 (BL 1), infraorbital breadth (IFB), rostral breadth (RB), interorbital breadth (IOB), postorbital constriction (POC), bizygomatic breadth (BZB), mastoidal breadth (MB), supraoccipital breadth (SOB), occipital height (OH), greatest nasal length (GLN), upper carnassial length (P4L), C-P4 length (C-P4L), mandible length (ML), mandible height (MH), lower carnassial length (M1L), C-m1 length (C-m1L).

For measurement definitions see Appendix A.

Previous studies showed that size variation in tigers is apparently clinal (Hooijer 1947, Kitchener 1999), influenced by such factors as climate, size and availability of prey, intra- and inter-specific competition, and the annual productivity pulse of different habitats (Kitchener 1999). In order to determine whether there are any real craniometric differences in shape among the eight traditionally recognized putative tiger subspecies, analysis of “size-adjusted” data which was reported by Jungers et al. (1995), were applied. Size-adjusted variables (i.e. morphometric shape ratios) were created by

dividing all individual raw measurements by a generalized size factor [(GM, geometric mean), referred to as DM_RAW in 11 ATD reported by Jungers et al. 1995], which is calculated as the cube root of the product of Greatest skull length, Bizygomatic breadth and Mandible length (which best represent the size of cats craniometrically).

Data analysis

A number of multivariate analyses were carried out to evaluate the degree of similarity and dissimilarity in craniometric shape between the putative subspecies and to establish the pattern of craniometric variation in tigers. A one-way analysis of variance (ANOVA) was performed to test the differences among subspecies on all size-adjusted variables. Variables that differed significantly ($p < 0.01$) between subspecies were graphed in boxplots in order to show the means and interquartile ranges of each subspecies. In ANOVA, all size-adjusted ratios were angular transformed restore normality (Sokal and Rohlf 1995). Variables that can be used in distinguish between subspecies effectively for both sexes revealed by ANOVA were subsequently used for allometric analysis. To address allometry, ordinary least-squares (OLS) regression analysis was applied on \log_{10} -transformed raw individual measurements using the equation $\text{Log } Y = \text{Log } a + b \text{ Log } X$, where X is condylobasal length (CBL). The difference in slopes between sexes and subspecies was tested with pairwise *t*-test, where the *t* values can be calculated as $t = (b_1 - b_2)/(s_{b_1 - b_2})$.

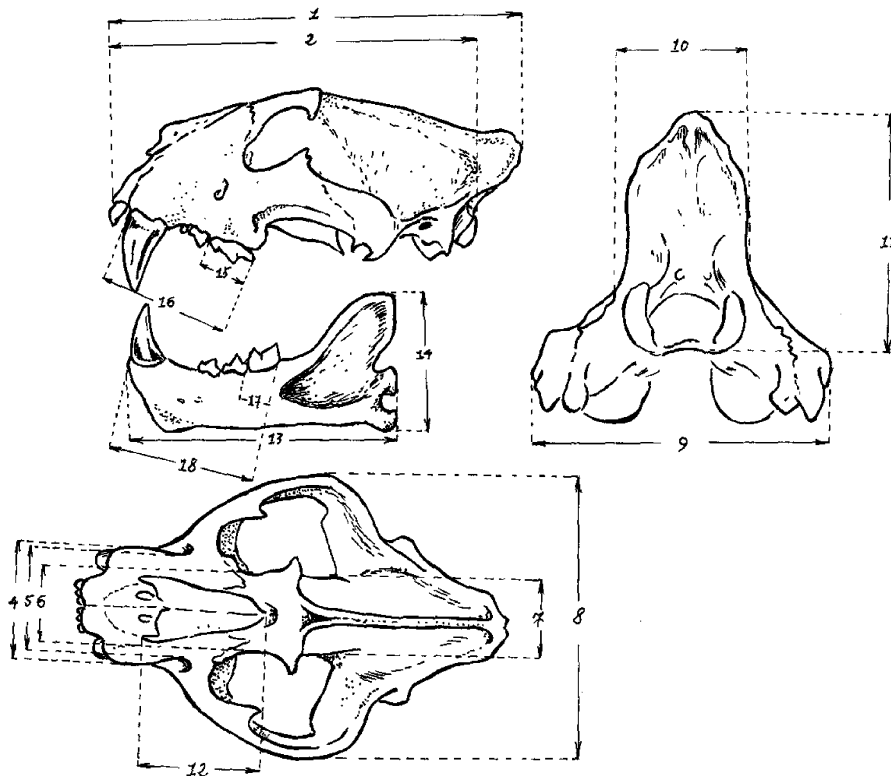


Fig. 1. Lateral and dorsal views of skull of an adult male Amur tiger (*P.t. altaica*) and occiput of an adult Java tiger (*P.t. sondaica*), showing 17 of the 18 craniometric variables used in this study (Basal length not shown).

Two multivariate statistical methods were used: principal component analysis (PCA) and canonical discriminant function analysis (DFA). Covariance-based PCA is a straightforward multivariate technique ideally suited for describing multivariate variation in graphical form and comparing within-group differences (Albrecht 1980; Albrecht et al. 2003). In this study, I use PCA to summarize graphically the craniometric relationships among the putative tiger subspecies and evaluate the degree of separation between them, in particular the two basic tiger forms (mainland group and island group), which were identified in previous studies (Cracraft et al. 1998; Kitchener 1999; Kitchener and Dugmore 2000; J. Mazák and Groves 2006). The overall morphological variation among 172 skulls is plotted in a multi-dimensional data space defined by 18 orthogonally constructed axes corresponding to the 18 size-adjusted craniometric variables. Only components with eigenvalues greater than 1 were extracted and thereafter they were rotated by the varimax criterion (Kaiser 1958) in order to rotate axes rigidly around the origin to a new position that displays information as efficiently as possible. Discriminant function analysis (DFA) uses correlation matrices to address the weighted combinations of variables, emphasizing between-group variation while minimizing within-group variation (Sokal and Rohlf 1995). Mahalanobis distances or squared generalized distances (D^2) based on step-wise DFA are effective in investigating morphological relationships among groups (Groves et al. 1992). In this study, step-wise DFA is applied in determining the craniometric differences among the eight traditionally recognized tiger subspecies. The step-wise DFA also uses group centroids (means of the groups on the DFs) to construct a matrix of Mahalanobis distances, which provides a phenetic distance between groups, and the F statistic was used to verify the degree of differentiation of pairwise distances between groups. Neighbor-joining (NJ) trees were constructed from the matrices of pairwise D^2 distance between the group centroids of each subspecies based on DFA for reveal the craniometric relationships among the putative tiger subspecies. Bivariate regression analysis was computed using SYSTAT (version 12.0), ANOVA, PCA and DFA were performed using SPSS (version 11.5), NJ trees were constructed using MEGA (version 2.1).

Results

A one-way ANOVA showed that, of a total of 18 size-adjusted variables, eight differ significantly ($p < 0.001$) among males and six are significantly different ($p < 0.001$) among females (Table 2). Significant differences among subspecies between the sexes differ slightly, but the most commonly significant inter-subspecific differences for both sexes relate to the shape of occiput (SOB, OH), nasal (GLN) and muzzle (RB, IFB). The highest F values for both sexes are in the shape of the supraoccipital bone, which separates the Java and Bali samples from those of mainland Asian and Sumatra completely (Fig. 2A). Nevertheless, boxplots show that size-adjusted variables which differ significantly ($p < 0.001$) among subspecies for both sexes also displayed some overlap in interquartile ranges among

Table 2. One-way ANOVA of size-adjusted measurements

Size-adjusted data (ratios)	Males		Females	
	F	p	F	p
GLS/GM	1.041	0.407	1.437	0.199
CBL/GM	1.147	0.340	2.585	0.017
BL/GM	1.363	0.229	2.711	0.013
RB/GM	6.381	0.000	2.802	0.010
IFB/GM	5.296	0.000	6.202	0.000
IOB/GM	2.377	0.027	2.456	0.023
POB/GM	5.082	0.000	4.553	0.000
BZB/GM	1.334	0.241	1.588	0.147
MB/GM	0.589	0.763	1.683	0.122
SOB/GM	40.719	0.000	22.552	0.000
OH/GM	4.502	0.000	4.399	0.000
GLN/GM	14.946	0.000	4.660	0.000
P4 L/GM	4.222	0.000	1.884	0.080
C-P4L/GM	2.847	0.009	5.569	0.000
ML/GM	1.335	0.241	0.598	0.756
MH/GM	5.248	0.000	4.057	0.001
M1 L/GM	3.732	0.001	3.816	0.001
C-M1L/GM	1.837	0.087	3.278	0.004

them (Fig. 2B, C), indicating that variation within subspecies is also marked, and the use of univariate shape variables to distinguish certain subspecies would not be possible, except for SOB/GM. Among the mainland Asian subspecies, however, relative nasal length gradually increases from north (*P.t. altaica*) to south (*P.t. tigris*), whilst muzzle breadth gradually decreases from north to south (which means that the northern subspecies possess relatively shorter nasals and wider muzzle, while in southern subspecies the nasal is long and the muzzle is relatively narrow), indicating that the variation in these characters is clinal. In addition, the boxplots also show that the cline is slightly more marked in males than in females, indicating that there is a variance dimorphism present here.

Basic allometric statistics for variables which significantly discriminate between putative subspecies revealed by ANOVA for both sexes are given in Appendix B. Bivariate regressions show low to moderate correlations (r) in all ratios among the putative subspecies, except Bali (which shows high r values except for OH/CBL in females; this is probably due to the small sample size), the values of the correlation coefficient varying with sex and subspecies. Slopes (b) are significantly different from zero ($p < 0.05$) for all putative subspecies. Significant differences in slopes between sexes ($p < 0.05$) were found in four variables and in five subspecies (see Appendix B), indicating that, in these subspecies, shape change is relatively greater with increased size in females than in males. The form of allometry varies in different variables from positive to negative (or even tending to isometry) in all subspecies. In SOB, most subspecies

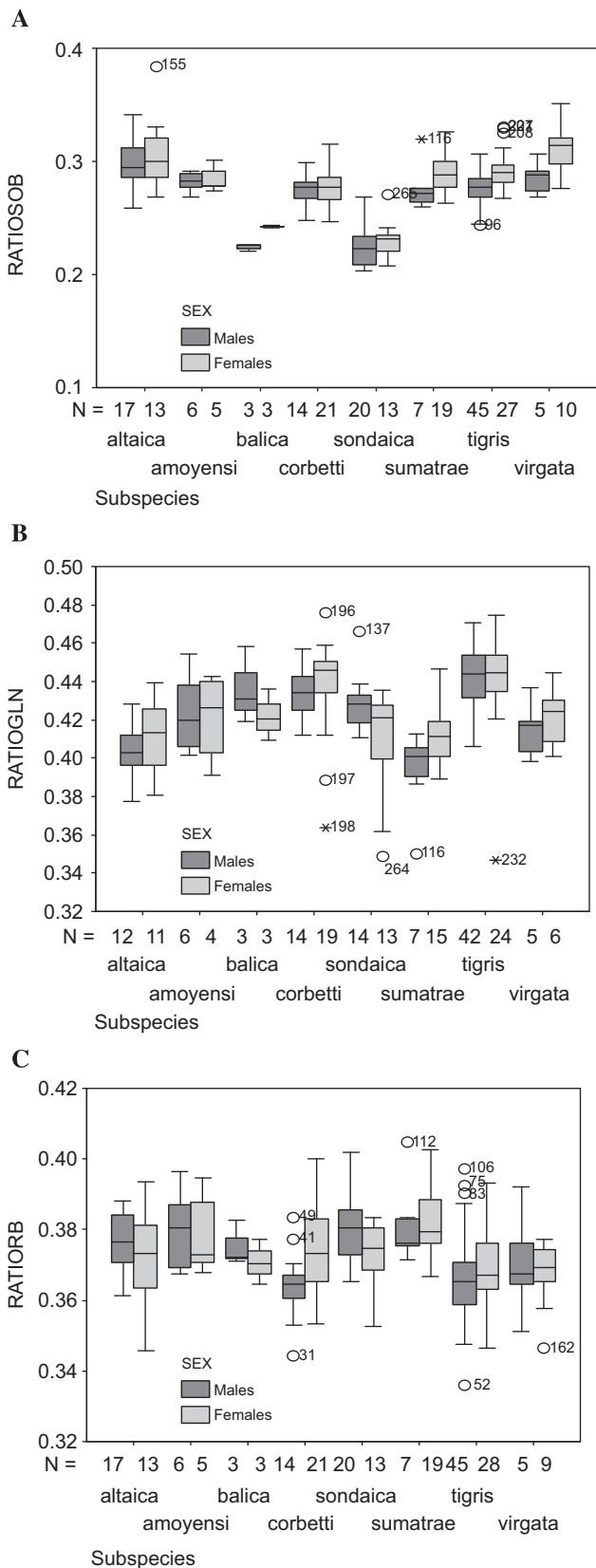


Fig. 2. (A) Boxplot of ratio SOB/GM; (B) boxplot of ratio GLN/GM; (C) boxplot of ratio RB/GM.

show negative allometry to CBL; Java is the most extreme, while Sumatran (both sexes) and Amur samples (female) are tend to be strongly positive to CBL. The Amur (both sexes) and Sumatran tigers (females) also show strong positive allometry of RB to CBL. Differences in slopes between subspecies also vary between sexes and variables (Table 3), but those in RB, GLN and OH tend to be more marked between the subspecies. Interestingly, significant differences in slope of SOB are not found between most subspecies, except between Java and Sumatra, Java and India (males only) and Java and Amur (females only). Overall, bivariate patterns in craniometric allometry vary with sex and subspecies, suggesting that sexual dimorphism may greatly influence the nature of geographic variation in tigers, from an allometric perspective.

The results of PCA based on covariance matrices using all 18 morphometric ratios (size-adjusted variables) for 88 males and 84 females are shown in Fig. 3(A, B) and Tables 4A and 4B, respectively. Four components were extracted in the PCA for males, which accounted for 62.44% of the total variance, and represent the overall intraspecific craniometric variation in males. PC 1, which accounts for 25.48% of the total variation, is largely a shape component that contrasts all length variables (GLS, CBL, BL) against skull breadth (BZB). PC 2 accounts for 18.44% of the total variance, and mainly contrasts anterior skull width (RB, IFB, IOB) against mandible height (MH). No differences were found between the putative subspecies in the first and second components, and samples overlapped extensively (result not shown), but specimens are separated clearly along the third component axis (Fig. 3A), with mainland and Sumatran samples to the right and Java/Bali samples to the left (note nevertheless that one Indian specimen was grouped with Java/Bali). The majority of separation on PC 3 (which contains 9.92% of the variance) is related to occiput shape (SOB, OH), suggesting that it is the form of the occiput that varies between these groups, in which the mainland and Sumatran samples have a relatively broad and high occiput while that in Java/Bali is considerably narrower. It is worth noting that the mainland samples, plus Sumatra, occupy much of the multivariate space while that of Java/ Bali is much smaller, suggesting that variations are greater on the mainland (relating to the fact that the mainland tigers cover a very large geographic area, whereas the Java/Bali specimens cover only a small area). PC 4, which accounts for 8.80% of the variance, is associated primarily with greatest nasal length (GLS). There is a clear trend for most Amur and Sumatran samples (together with some Chinese and Caspian specimens) to be separated from specimens of India, Indochina and Java/Bali, though the degree of separation is obviously not as strong as that displayed on PC 3, which means the nasal is proportionally

Table 3. Results (*t* value) of pairwise significant tests for differences in slopes between tiger subspecies

	<i>altaica</i>	<i>virgata</i>	<i>amoyensis</i>	<i>corbetti</i>	<i>tigris</i>	<i>sumatrae</i>	<i>sondaica</i>	<i>balica</i>
Males								
RB/CBL								
<i>altaica</i>	0.000							
<i>virgata</i>	−0.06	0.000						
<i>amoyensis</i>	−0.08	0.15	0.000					
<i>corbetti</i>	−1.65	−0.51	−1.81	0.000				
<i>tigris</i>	−3.49***	−0.56	−2.35*	−2.21*	0.000			
<i>sumatrae</i>	−1.92	−0.81	−2.17	−1.87	−2.54*	0.000		
<i>sondaica</i>	−2.41*	−0.60	−2.16	−1.94	−3.35**	−1.34	0.000	
<i>balica</i>	−1.54	−0.94	−2.04	−1.70	−2.04*	−1.32	−1.81	0.000
IFB/CBL								
<i>altaica</i>	0.000							
<i>virgata</i>	0.19	0.000						
<i>amoyensis</i>	0.27	−0.50	0.000					
<i>corbetti</i>	−1.88	−2.67*	−1.00	0.000				
<i>tigris</i>	−1.32	−2.12*	−0.39	−0.74	0.000			
<i>sumatrae</i>	−1.06	−1.85	−0.42	−0.57	−1.96	0.000		
<i>sondaica</i>	−0.42	−1.21	−0.08	−0.07	−1.30	−0.82	0.000	
<i>balica</i>	1.55	0.78	0.85	1.58	1.17	1.18	0.95	0.000
GLN/CBL								
<i>altaica</i>	0.000							
<i>virgata</i>	0.11	0.000						
<i>amoyensis</i>	−2.20*	−1.72	0.000					
<i>corbetti</i>	−4.57***	−2.15*	−1.30	0.000				
<i>tigris</i>	−4.19***	−1.62	−0.74	−3.36***	0.000			
<i>sumatrae</i>	−0.72	−0.64	0.42	0.01	−0.41	0.000		
<i>sondaica</i>	−4.53***	−2.56*	−1.72	−3.76***	−4.75***	−2.68*	0.000	
<i>balica</i>	−15.27***	−5.24***	−4.88***	−14.13***	−25.24***	−6.13***	−9.04***	0.000
SOB/CBL								
<i>balica</i>	0.000							
<i>sondaica</i>	1.05	0.000						
<i>sumatrae</i>	−1.36	−2.36*	0.000					
<i>tigris</i>	−1.53	−2.96**	0.23	0.000				
<i>corbetti</i>	−0.56	−1.59	0.74	0.76	0.000			
<i>amoyensis</i>	−0.69	−1.76	0.67	−1.02	−0.74	0.000		
<i>virgata</i>	1.08	0.24	1.97	1.45	1.11	1.16	0.000	
<i>altaica</i>	−0.54	−1.62	0.80	−0.95	−0.60	−0.63	−1.35	0.000
OH/CBL								
<i>balica</i>	0.000							
<i>sondaica</i>	1.25	0.000						
<i>sumatrae</i>	0.43	−1.92	0.000					
<i>tigris</i>	−0.80	−4.96***	−1.30	0.000				
<i>corbetti</i>	−0.54	−3.09**	−1.10	−2.27*	0.000			
<i>amoyensis</i>	0.57	−1.37	−0.05	−0.47	−0.08	0.000		
<i>virgata</i>	−0.34	−2.37*	−0.95	−1.48	−1.04	−1.48	0.000	
<i>altaica</i>	−0.77	−4.23***	−1.29	−3.59***	−1.81	−2.01	−0.78	0.000
Females								
RB/CBL								
<i>altaica</i>	0.000							
<i>virgata</i>	−0.28	0.000						
<i>amoyensis</i>	−0.14	−1.05	0.000					
<i>corbetti</i>	−1.40	−2.54*	−0.23	0.000				
<i>tigris</i>	−0.17	−1.17	0.27	−1.00	0.000			
<i>sumatrae</i>	−3.06**	−4.28***	−1.12	−4.36***	−4.10***	0.000		

Table 3. (continued)

	<i>altaica</i>	<i>virgata</i>	<i>amoyensis</i>	<i>corbetti</i>	<i>tigris</i>	<i>sumatrae</i>	<i>sondaica</i>	<i>balica</i>
<i>sondaica</i>	−1.29	−2.33	−0.39	−2.19*	−2.26*	−1.40	0.000	
<i>balica</i>	−4.70***	−6.48***	−1.29	−7.47***	−5.95***	−5.44**	−4.34**	0.000
IFB/CBL								
<i>altaica</i>	0.000							
<i>virgata</i>	−0.39	0.000						
<i>amoyensis</i>	−0.20	−0.75	0.000					
<i>corbetti</i>	−0.59	−0.91	0.06	0.000				
<i>tigris</i>	2.02	1.24	1.31	1.31	0.000			
<i>sumatrae</i>	−2.12*	−2.25*	−0.80	−2.37*	−3.38**	0.000		
<i>sondaica</i>	−0.61	−0.86	0.13	−0.94	−1.91	−0.19	0.000	
<i>balica</i>	−	−	−	−	−	−	−	0.000
GLN/CBL								
<i>altaica</i>	0.000							
<i>virgata</i>	−0.65	0.000						
<i>amoyensis</i>	−0.08	0.52	0.000					
<i>corbetti</i>	−2.21*	−0.29	−0.08	0.000				
<i>tigris</i>	−1.41	0.09	0.22	−1.00	0.000			
<i>sumatrae</i>	−1.32	0.24	0.35	−0.93	−1.14	0.000		
<i>sondaica</i>	0.71	1.23	1.09	1.17	0.84	0.66	0.000	
<i>balica</i>	−6.28***	−1.65	−1.04	−6.14***	−5.88***	−7.90***	−3.84**	0.000
SOB/CBL								
<i>balica</i>	0.000							
<i>sondaica</i>	1.76	0.000						
<i>sumatrae</i>	−0.84	−2.22*	0.000					
<i>tigris</i>	0.78	−0.75	1.02	0.000				
<i>corbetti</i>	0.23	−1.24	0.52	−0.94	0.000			
<i>amoyensis</i>	0.28	−1.25	0.52	−0.80	−0.39	0.000		
<i>virgata</i>	0.12	−1.33	0.42	−1.08	−0.58	−0.04	0.000	
<i>altaica</i>	−0.71	−2.15*	−0.40	−1.87	−1.40	−0.83	−1.36	0.000
OH/CBL								
<i>balica</i>	0.000							
<i>sondaica</i>	−0.18	0.000						
<i>sumatrae</i>	−1.44	−5.87***	0.000					
<i>tigris</i>	−1.28	−4.92***	−3.13**	0.000				
<i>corbetti</i>	0.01	−1.67	−0.11	−0.10	0.000			
<i>amoyensis</i>	0.91	1.16	2.29*	2.16*	1.06	0.000		
<i>virgata</i>	−0.45	−1.89	−0.54	−0.58	−1.99	−1.45	0.000	
<i>altaica</i>	−0.18	−0.82	0.39	0.29	−0.93	−1.18	−0.34	0.000

*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

shorter in Amur and Sumatran samples than in other samples.

Since some variables (IFB, IOB, POB, MH, M1L, C-m1L) were not taken on female Bali skulls, I reduced the original 18 size-adjusted variables to 12 to be entered into a PCA for females, in order to include all eight putative subspecies in the comparison. PCA produced three components, which encompassed only 49.75% of the total variance. The first component accounts for 26.37% of variance; as in males, it is again largely a shape component that contrasts all length variables (GLS, CBL, BL) against skull breadth (BZB). The second component accounts for 14.48% of the variance,

which is defined mainly by the shape of occiput (SOB, OH). Specimens are separated into two clusters, with all mainland and Sumatran samples ordered on the right and Java/Bali samples on the left, although it should be noted that overlaps are extensive among all samples and the separation between mainland/Sumatra and Java/Bali in females is obviously not as clear as that in males. The third component, which accounts for 8.89% of variance, has high negative loading on nasal length (GLS); no samples are distinguishable on PC 3.

Results of step-wise DFA using 18 size-adjusted craniometric ratios for males are shown in Fig. 4A, Table 5 and Appendix C. The DFA plot indicates that

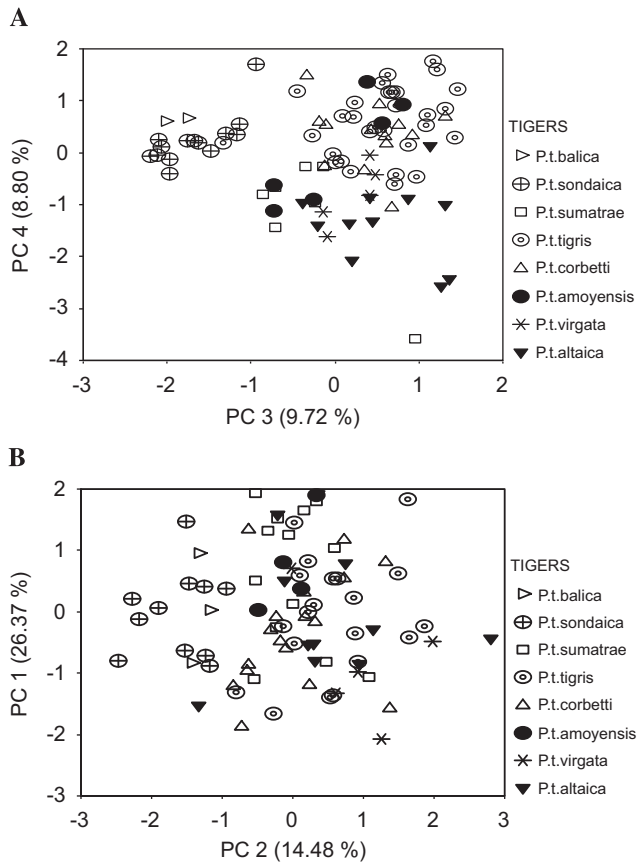


Fig. 3. (A) PCA plot of males of all eight putative tiger subspecies using 18 size-adjusted craniometric ratios, showing there is a clear separation between the mainland (including Sumatra) and Java/Bali samples on PC 3, and a trend for Amur/Sumatran and India, Indochinese and Java/ Bali samples to separate along PC 4 with some minor overlaps. (B) PCA plot of females of all eight putative tiger subspecies using 12 size-adjusted craniometric ratios, showing there is an extensive overlap between subspecies, but note nevertheless the substantial separation of Java/Bali samples from other samples on PC 1, though the separation is not very marked.

the major separation on DF1 is between mainland Asian samples and Java/Bali samples, the Sumatran samples being clearly classified with mainland Asian samples. DF1 (eigenvalue = 5.194) summarizes 50.2% of the total variance; correlations between individual craniometric ratios and discriminant functions (“structure coefficients”, Appendix C) indicate that it mainly contrasts occiput shape (SOB, OH) against skull length (GLS), in which the mainland samples (plus *sumatrae*) have a greater occipital breadth and long skull while that in Java/Bali samples is much narrower and shorter. The Sumatran, Amur/Caspian/South China, and India/Indochinese samples clearly form three distinguishable clusters on DF2, with some overlap. DF2 (eigenvalue = 3.192) contains 30.8% of variance, and shows high positive correlations with basal length

Table 4A. Factor loadings of PCA for comparing all eight putative males using 18 size-adjusted variables

Craniometric measurements	PC 1	PC 2	PC 3	PC 4
Greatest skull length	0.750	-0.092	0.278	0.181
Condylbasal length	0.888	0.151	0.066	0.121
Basal length	0.891	0.106	0.039	0.046
Rostral breadth	0.305	0.613	-0.242	-0.122
Infraorbital breadth	-0.138	0.627	-0.012	0.003
Interorbital breadth	-0.345	0.611	0.251	0.080
Postorbital constriction	-0.078	0.767	0.081	0.324
Bizygomatic breadth	-0.902	0.095	-0.126	-0.058
Mastoidal breadth	0.164	0.043	0.393	0.142
Supraoccipital breadth	-0.018	0.248	0.891	-0.312
Occipital height	-0.028	-0.287	0.688	0.102
Greatest nasal length	0.114	-0.064	0.151	0.914
Mandible length	0.646	-0.052	-0.081	-0.091
Mandible height	-0.241	-0.718	0.076	0.407
Upper carnassial length	0.440	0.209	-0.183	0.321
Lower carnassial length	0.344	0.220	-0.161	0.349
C-P4 length	0.481	0.652	-0.023	-0.024
C-m1 length	0.574	0.594	-0.065	0.206
Percentage of variance	25.48	18.44	9.72	8.80

Notes: Variables with high loading are marked with boldface.

Table 4B. Factor loadings of PCA for comparing all eight putative females using 12 size-adjusted variables

Craniometric measurements	PC 1	PC 2	PC 3
Greatest skull length	0.757	0.344	-0.188
Condylbasal length	0.913	0.009	0.127
Basal length	0.839	0.031	0.148
Rostral breadth	0.117	0.066	0.262
Bizygomatic breadth	-0.676	-0.063	0.053
Mastoidal breadth	0.364	0.341	0.127
Supraoccipital breadth	-0.095	0.937	0.299
Occipital height	0.210	0.590	-0.120
Greatest nasal length	0.212	0.399	-0.869
Mandible length	0.023	-0.286	0.139
Upper carnassial length	0.456	-0.075	-0.032
C-P4 length	0.380	0.144	0.161
Percentage of variance	26.37	14.48	8.89

Notes: Variables with high loading are marked with boldface.

(BL), infraorbital width (IFB) and negative correlation with nasal length (GLN).

Comparison of inter-group distance (Mahalanobis D^2 distances, Table 5) shows that most pairwise comparisons of D^2 are significant between subspecies, except *virgata/altaica*, *virgata/amoyensis*, *virgata/corbetti* and of course, *sondaica/balica*. The Caspian and Bali tiger shows relatively low distances to other subspecies, this probably being due to the small sample size. It is worthy of note that the D^2 value is on average high in Java,

indicating that the morphological distance is greater in particular between Java and all mainland subspecies than among the mainland subspecies.

A Jack-knifed classification (Appendix C) shows that only 67.8% of samples are correctly classified. It is worthy of note, however, that in the Jack-knifed classification, both Java and Bali samples are 100% distinguishable from all other samples.

Step-wise DFA of females using size-adjusted data yields a roughly similar major separation to that of

males; mainland Asia and Java/Bali samples are distinctly separated on DF1 (Fig. 4B), but there is a considerable overlap among mainland Asian samples, and Sumatran samples are mostly indistinguishable from those of the mainland, and this is also confirmed by the relative small D^2 distances among mainland samples and between mainland and Sumatran samples (Table 6). Structure coefficients (Appendix D) show that Supraoccipital breadth (SOB), Occipital height (OH) and basal length (BL) are highly positively correlated with DF 1 (eigenvalue = 3.905) while greatest skull length (GLS) is highly negatively correlated. DF2 (eigenvalue = 1.047) separates the Bali from the Java samples clearly; it does not positive correlate strongly with any of the variables, but moderately contrast nasal length (GLN), p4 length (p4L) and upper tooth row (C-p4L) against strongly with basal length (BL).

Most pairwise comparisons of D^2 are significant between subspecies, except *altaica/virgata*, *altaica/amoyensis*, *altaica/tigris*, *virgata/corbetti*, *virgata/tigris*, and *sondaica/balica*. As in males, D^2 values are again on average higher in Java than among all mainland subspecies. A Jack-knifed classification (Appendix D) shows that only 54.8% of samples are correctly classified, yet the Java sample compared to other analyzed samples shows a very high classification accuracy (over 90.9% of the sample are classified correctly, the remaining 9.1% being misclassified as Bali).

When the DFA is limited to mainland Asian samples only, the principal differentiation on DF 1 of male samples is between Amur and all others (Fig. 5A), although there is some minor overlap. The Amur sample is ordered on the left while all other samples are on the right, with the Caspian sample overlapping largely between the two; the Amur sample is the most distinct. DF1 (eigenvalue = 1.631) accounts for 68.6% of the variance; structure coefficients (Appendix E) show that it is mainly positively correlated with nasal length (GLN), postorbital constriction (POC) and negatively correlated with rostral breadth (RB). The separation on DF1 means that the Amur sample is characterized as a

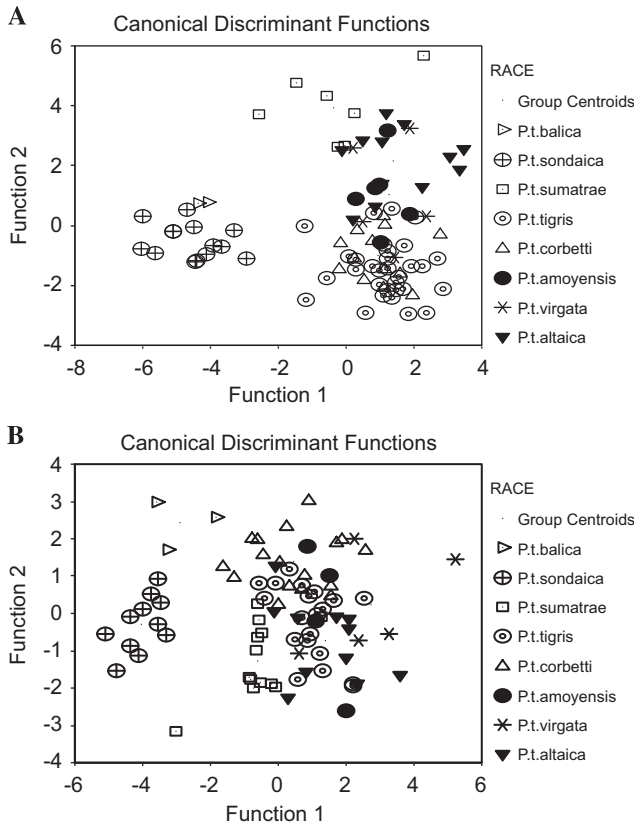


Fig. 4. (A) Step-wise DFA plot of males of eight putative tiger subspecies using size-adjusted measurements, (B) step-wise DFA plot of females of eight putative tiger subspecies using size-adjusted measurements.

Table 5. Mahalanobis D^2 distances between all eight putative male subspecies using size-adjusted data

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>	<i>P.t. sumatrae</i>	<i>P.t. sondaica</i>	<i>P.t. balica</i>
<i>P.t. altaica</i>	0.00							
<i>P.t. virgata</i>	2.145	0.00						
<i>P.t. amoyensis</i>	4.417	1.786	0.00					
<i>P.t. corbetti</i>	8.347	2.168	2.920	0.00				
<i>P.t. tigris</i>	11.161	3.447	5.076	2.963	0.00			
<i>P.t. sumatrae</i>	6.184	4.127	4.405	11.261	15.246	0.00		
<i>P.t. sondaica</i>	21.838	11.574	13.427	17.877	26.074	15.677	0.00	
<i>P.t. balica</i>	6.761	4.254	3.934	4.913	6.186	3.944	1.519	0.00

Notes: Group pairs with significant distances ($p < 0.001$) are marked with boldface.

Table 6. Mahalanobis D^2 distances between all eight putative female subspecies using size-adjusted data

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>	<i>P.t. sumatrae</i>	<i>P.t. sondaica</i>	<i>P.t. balica</i>
<i>P.t. altaica</i>	0.00							
<i>P.t. virgata</i>	1.306	0.00						
<i>P.t. amoyensis</i>	1.506	2.586	0.00					
<i>P.t. corbetti</i>	3.882	3.023	3.054	0.00				
<i>P.t. tigris</i>	1.981	2.198	3.431	3.354	0.00			
<i>P.t. sumatrae</i>	3.502	5.015	3.710	5.272	4.831	0.00		
<i>P.t. sondaica</i>	13.295	13.078	8.778	12.787	14.487	7.800	0.00	
<i>P.t. balica</i>	5.640	5.961	4.356	3.380	5.831	4.313	2.510	0.00

Notes: Group pairs with significant distances ($p < 0.001$) are marked with boldface.

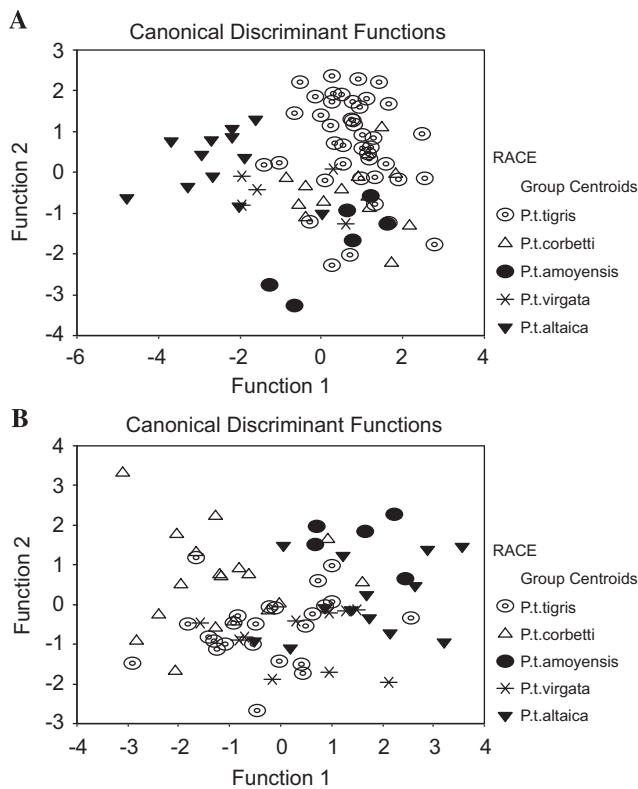


Fig. 5. (A) Step-wise DFA plot of males of mainland putative tiger subspecies using size-adjusted measurements, (B) step-wise DFA plot of females of mainland putative tiger subspecies using size-adjusted measurements.

form with shorter nasal, proportionally small width across postorbital constriction and broader muzzle, while the southern samples have proportionally longer nasal, greater width across postorbital constriction and narrower muzzle. Indian, Indochinese and South China samples are more or less distinguishable with clear overlap on DF2 (eigenvalue = 0.568). DF2 contains 23.9% of variance, which does not strongly positively correlate with any of the variables, but highly negatively with postorbital constriction (POC).

Comparison of D^2 values (Table 7) shows that the Indian, Indochinese and South China samples are very

distinct from Amur, while D^2 distance between Amur and Caspian, South China and Indochinese, and among Caspian, South China and Indochinese samples are statistically non-significant. Over 69.6% of samples are classified correctly, according to result of Jack-knifed classification (Appendix E).

DFA of female mainland Asian samples shows the main separation on DF 1 is between Amur, South China and Caspian samples on the one hand and India plus Indochina on the other. Again, overlap between the two groups is obviously great (Fig. 5B). DF1 (eigenvalue = 1.536) explains 67.7% of the variance, which strongly negatively contrasts mandible height (ML) and C-P4 length (C-P4L) and weakly positively with condylobasal length (CBL). DF2 accounts for 22.5% of the variance, which has high positive loading for C-P4 length (C-P4L), with no separation (Appendix F). D^2 distances (Table 8) are generally non-significant among most samples, except between Amur and Indochinese, South China and India and, India and Indochinese samples. A Jack-knifed classification shows only 50% of samples are classified correctly (Appendix F).

To further address the relationship between latitude and the overall craniometric variation (OCV, expressed as DF 1 of DFA) in mainland subspecies, regression analyses were carried out for both sexes separately. The results (Fig. 6A, B) show that the principal differences between mainland subspecies (males: shape of the nasal and muzzle; females: skull size, upper tooth row and mandible height) are moderately related with latitude [males: $\text{Log}_{10}(\text{OCV}) = 2.563 \pm 0.318 \text{Log}_{10}(\text{Latitude}) - 0.091 \pm 0.011$; $r = 0.691$; $\text{SE} = 1.121$; $F = 69.496$; $p < 0.001$; females: $\text{Log}_{10}(\text{OCV}) = -1.866 \pm 0.396 \text{Log}_{10}(\text{Latitude}) + 0.070 \pm 0.013$; $r = 0.569$; $\text{SE} = 1.238$; $F = 28.783$; $p < 0.001$], but clearly form a cline as increase or decrease with latitude.

The basic patterns of craniometric relationships among the putative subspecies are summarized in NJ trees (Fig. 7A, B), which indicate unambiguously that modern tigers rooted at the split between mainland and Sunda Island tigers. In a NJ dendrogram, the length of

Table 7. Mahalanobis D^2 distances between mainland putative male subspecies using size-adjusted data

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>
<i>P.t. altaica</i>	0.00				
<i>P.t. virgata</i>	2.742	0.00			
<i>P.t. amoyensis</i>	11.945	2.984	0.00		
<i>P.t. corbetti</i>	16.303	2.314	3.496	0.00	
<i>P.t. tigris</i>	20.584	4.212	7.051	4.106	0.00

Notes: Group pairs with significant distances ($p < 0.001$) are marked with boldface.

Table 8. Mahalanobis D^2 distances between mainland putative female subspecies using size-adjusted data

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>
<i>P.t. altaica</i>	0.000				
<i>P.t. virgata</i>	1.483	0.000			
<i>P.t. amoyensis</i>	1.682	3.787	0.000		
<i>P.t. corbetti</i>	11.434	4.719	7.451	0.000	
<i>P.t. tigris</i>	4.654	2.008	5.455	5.886	0.000

Notes: Group pairs with significant distances ($p < 0.001$) are marked with boldface.

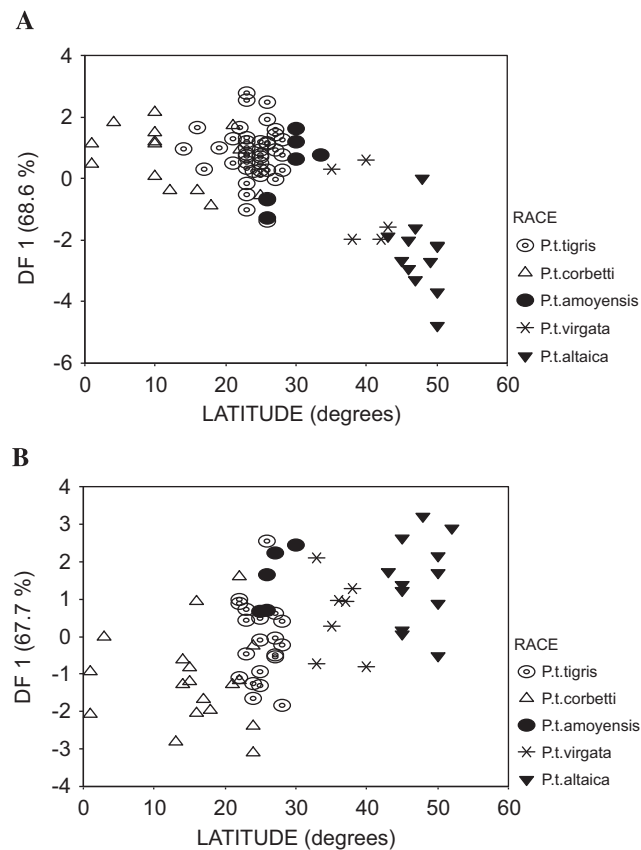


Fig. 6. (A) The relationship between overall skull morphology (DF1, using size-adjusted data) of males and latitude, showing the basic pattern in mainland populations is clinal. (B) The relationship between overall skull morphology (DF1, using size-adjusted data) of females and latitude, showing the basic pattern in mainland populations is clinal.

the branches are proportional to phenetic differences. The mainland tigers grouped closely together (especially marked in females), which suggests a closer relationships craniometrically between them, while the Java/Bali and Sumatran tigers show clearly a long branch to that of the mainland, suggesting a marked distinction in skull morphology between them and the mainland tigers. The NJ trees also show that relationships among mainland tigers differ slightly between the sexes, which indicates again that sexual dimorphism and subspeciation in the tiger are intimately interwoven.

Discussion

Results of this study in general accord with those reported in most recent studies on geographic variation of tigers both morphologically (Kitchener 1999; J. Mazák and Groves 2006) and genetically (Luo et al. 2004, 2006). Overall, tigers displayed a relatively high level of diversity in skull morphology (i.e. mainland Asia vs Sunda Island), comparable to other wide distributed pantherine cats (leopards: Meijarrd 2004; Miththapala 1992; lions: Christiansen 2007, but there are some differences from the present author's unpublished data, paper in preparation). The major population differentiation in tigers (Java/Bali, Sumatra, Northern Asia, Southern Asia) is well demonstrated by the present multivariate craniometric analysis. Establishing the pattern of cranial variation and relationships among subspecies, and evaluating species/subspecies level distinctions, can certainly shed light on the current issues of

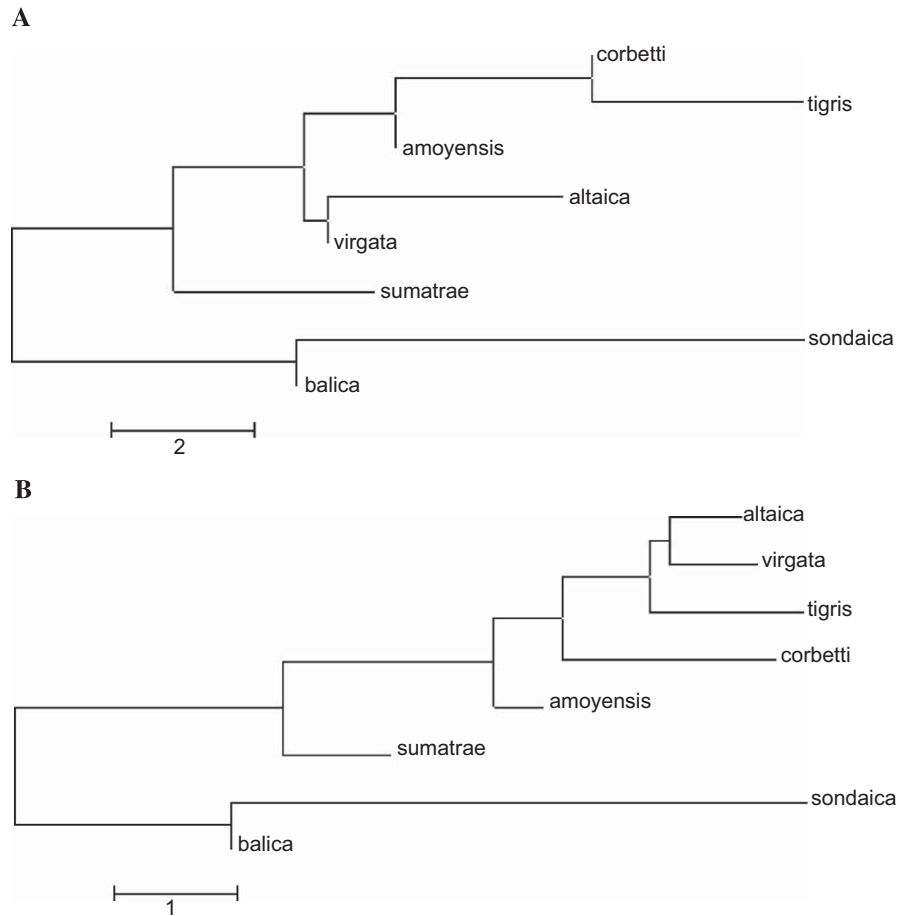


Fig. 7. (A) NJ tree constructed for males from the matrices of Mahalanobis distance between the group centroids of each subspecies based on DFA. (B) NJ tree constructed for females from the matrices of Mahalanobis distance between the group centroids of each subspecies based on DFA.

tiger geographic variation and classification, and has important implications for current tiger conservation strategies.

Patterns of cranial variation in tigers

Skull characters have long been used in distinguishing tiger subspecies (Brongersma 1935; Hemmer 1969, 1978, 1987; Hiltzheimer 1905; V. Mazák 1967, 1976, 1979; J. Mazák and Groves 2006; Pocock 1929, 1939; Schwarz 1912). Hemmer (1987) proposed that the marked cranial-dental distinctness among the putative tiger subspecies is due to a relatively long evolutionary history, which is even longer than in lions and leopards. It has however been urged that skull characters are much more variable within putative subspecies than between them, and some cranial differences observed are mainly size related (Kitchener 1999). For instance, Kitchener's analysis suggested that the shape of the occiput is unlikely to be an effective feature discriminating between subspecies when skull size is taken into

account. Therefore, the initial goal of this cranial metric analysis is to assess similarities and differences in skull characters among tiger subspecies after the effect of skull size is removed. Results of bivariate regression analysis indicated that patterns of craniometric variation in tigers are quite complex and are deeply influenced by allometric scaling and sexual dimorphism. Significant differences in slopes of some variables (i.e. GLN, OH, RB) between subspecies revealed in this study suggest that shape may change differently during cranial growth in some geographic populations, which has certain important evolutionary implications, and these are further confirmed by multivariate analysis (PCA and DFA). In such case, therefore, we could propose that the morphometric variables, which cause the major distinctions between tiger geographic populations are indeed shape related instead of size related.

Another important issue in tiger cranial variation patterns is variance dimorphism, giving evidence from multivariate analysis that males tend to be more variable than females, such that sexual dimorphism apparently plays one of the key roles in geographic variation in

tigers, as has already noticed in an earlier investigation (J. Mazák 2004). Differences in slope between sexes, at least in most of the putative subspecies, are well corroborated by the allometric analysis, indicating shape changes may be relatively greater with increased size in females than in males. There is potentially a difference in growth rate between the sexes (i.e. females grow at a relatively higher rate, probably reaching an asymptotic state at an earlier stage and changing little thereafter, while males grow more constantly throughout life), but these are needed to be confirmed from further allometric analysis focused on ontogenetic trajectory.

Results of this study (i.e. Mahalanobis D^2 distances, regression analysis on relationship between overall skull morphology and latitude) also confirmed that there are clinal variations within the mainland tiger group, as degrees of difference on the whole gradually increase from North to Southeast Asia and the Indian subcontinent (i.e. D^2 distances are high comparing Amur to East, Southeast Asia and Indian samples, while relatively lower between these latter and the Caspian sample). This pattern of variation has important implications for subspecies definition among the mainland tigers. The cline in mainland tigers may be due to direct adaptation to the local landscapes (i.e. primary cline), or may be the result of interbreeding of geographically neighboring populations that have developed different characters in isolation (i.e. secondary cline), or even some combination of both. Whatever type of cline it is, however, the most important proviso in determining subspecies is whether or not they can be distinguished in a relatively high percentage of cases (either the 75% rule in Mayr's criterion or the 90% rule in Corbet's criterion). In the case of the present study, the distinction between the Amur tiger and all others is fairly marked (i.e. D^2 distances, DFA plot), and the Chinese and India, Indochina and Indian samples differ on average (D^2 distances) as well. It also should be noted that the pattern of clinal variation revealed in this study is rather more complex than what has previously been found as far as sex is concerned (in bivariate regression the r values and F values are higher in males than in females, indicating that clinal variation is more marked in males than in females). Kitchener (1999) also noted that females generally show less marked clines in body size (represented by greatest length of skull and carnassial), while those in males are very marked.

It should be noted that divergence among major tiger populations can also be found in other cranial characters (shape of sagittal crest, degree of convexity of the frontal; V. Mazák 1967, 1979; Hemmer 1978), that can be used to distinguish some subspecies effectively (for example, Amur and Caspian tigers can be readily distinguished from those of India or South China by their prominent and strongly developed

sagittal crest; V. Mazák 1979 and author's observation). This can be hypothesized as the result of the combined effects of genetic drift in isolated populations and adaptations to rapidly changing landscapes and local prey fauna across their wide range during the Pleistocene glaciations. For instance, since big cats generally develop well-marked bony crests (sagittal crest, occipital crest) to enlarge the attachment area of temporalis and neck muscles (Ewer 1973; Kitchener 1991), the question why the Amur and Caspian tigers possess so much more developed sagittal crests could be tested by measuring the surface area of muscle attachment and, firstly, calculating the bite force of temporalis in Northern and Southern tiger populations and, secondly, determining whether there are any significant differences between them which might be connected with their preferred main prey species (supposedly wild boar for Northern Asian tigers, deer for Southern Asian tigers). Further investigations should pay particular attention to the evolutionary relationships between major tiger geographic populations and their prey species communities, as well as between tiger dispersal and major habitat changes and consequent effects on morphological variations.

Craniometric relationships among tigers

Throughout this study, the greatest craniometric distinction found in tigers is between the mainland of Asia and Java/Bali. The degree of the differentiation between the two is remarkably greater than that observed among any other subspecies, which is demonstrated unambiguously in all the statistical analyses applied in this study. The Java group in both sexes has the highest value of the Mahalanobis distance as well as the longest branch in NJ trees, much more so than that observed among other compared groups, and is most strongly distinctive in the DFA and somewhat distinct in PCA scatter plots. The Bali tiger is generally very similar to that of Java, and the distance between the two is very small. The Sumatran tiger seems to be somewhat intermediate between mainland Asia and Java/Bali groups, but largely retains its distinctive craniometric features (wider muzzle and proportionally shorter nasal), as revealed by the D^2 values, at least for males in DFA plot and a branch that is substantially distinct from mainland tigers in the NJ trees. These findings are well concordant with those of J. Mazák and Groves (2006). Within the mainland groups, based on Mahalanobis D^2 distances and specimen distributions in PCA and DFA scatter plots, the greatest distinction is between North Asia and the East/Southeast Asia/Indian subcontinent groups, which lie largely in skull length, nasal length and muzzle breadth. Degrees of differentiation between South China and India, Indochinese and

Indian tigers are also on average, but there are decidedly more overlaps among them than between them and North Asia. The Indian tiger appears relatively the most distinctive among these more southerly groups, while *corbetti* and *amoyensis* show moderate overlap. The Caspian tiger overlaps extensively with all other mainland samples.

Kitchener, who has proposed three very interesting models for the geographical variation among tigers, has provided a framework which can be tested by further studies with a variety of approaches (Kitchener 1999). In his Model 2, the isolation of tigers on the Sunda Islands will have resulted in the evolution of distinct subspecies there, while mainland tigers show a complex cline influenced by various environmental factors. Based on the present multivariate craniometric analysis, Kitchener's Model 2 is well supported, for the main part. Skulls of mainland Asia and Java/Bali are strongly differentiated mainly in the shape of occiput, that in the mainland forms being obviously broad while in Java and Bali it is remarkably narrow. Hemmer (1967, 1969, 1971) also noted that the lower carnassial (expressed as an index of M1 length/p4 length) in Java and Bali tigers is relatively longer than those in mainland Asia and Sumatran tigers. Biogeographically, the distinction between mainland Asia and Java/Bali tigers can clearly be attributed to the long period of isolation, as the increase in sea levels during the Pleistocene interglacials provided a significant barrier to gene flow between the mainland of southeast Asia and the Sunda Islands, which resulted in subsequent evolution of distinctive characters (specialized masticatory structure, smaller body size), a process known as insular dwarfism. This is also supported by the fossil evidence, as a cranium found in the Trinil fauna described as *Felis tigris soloensis* by Von Koenigswald in 1933 evidenced that the narrowness of the occiput was already present in middle Pleistocene Java tigers (Brongersma 1935; Hemmer 1969; von Koenigswald, 1933). Given the evidence from multivariate analysis that they are unambiguously distinguishable, by applying Genetic Conservation Units (GCU) the mainland Asia and Java/Bali tigers may be regarded as two basic forms in modern tiger phylogeny or they can even be regarded as two distinct but very closely related species if by applying Phylogenetic Species Concept (PSC). These hypotheses would be testable by use of molecular genetic techniques if mtDNA and nDNA could be extracted from preserved skeletons or skins of the extinct Java and Bali tigers.

As Kitchener suggested, Sumatran tigers likely represent a hybrid (mainland × Java), which originated from mainland Southeast Asian populations and colonized the Sunda Islands before the end of the last Ice Age (Kitchener 1999). This is well supported by the present analysis as well as by previous studies (Hemmer

1969, 1971; J. Mazák and Groves 2006). In a recent research J. Mazák and Groves (2006) have proposed that the Sumatran tiger should be regarded as a distinct species by applying the Phylogenetic Species Concept (J. Mazák and Groves 2006). Considering that Sumatran tigers retain both the typical mainland tigers' cranial character (wide occiput) on the one hand and the Sunda Island tigers' pelage markings on the other hand, and they are distinct from island form clearly and from mainland group craniometrically (J. Mazák and Groves 2006, present study) and genetically (Cracraft et al. 1998; Hendrickson et al. 2000; Luo et al. 2004), the unique taxonomic status of the Sumatran tiger must be recognized.

Relationships among the five traditionally recognized mainland subspecies now need to be discussed. As indicated by results of bivariate, multivariate analysis, mainland tigers generally show a relatively large overall variation in skull morphology and greater overlap among samples. Yet, there are still clear morphological separations among subspecies. Mahalanobis D^2 distances based step-wise DFA indicates that the greatest dichotomy among mainland Asian subspecies observed in this study is between North Asia and the most southerly subspecies (India, Indochina). Skulls of Amur tigers are characterized as relatively large sized, with broad and massive muzzle and proportionally shorter nasals. South China, Indochinese and Indian tigers are also somewhat separable, but with much overlap. In contrast, the Caspian tiger, which one would expect to be quite distinct from other subspecies, overlaps extensively with Siberian tigers, and partly with Southern Asian tigers, despite the likelihood that there was a significant biogeographic barrier between them (Kitchener 1999; Kitchener and Dugmore 2000).

It is worth noting that the evolutionary relationships among mainland subspecies, especially those between the Caspian tiger and Southern subspecies, are quite complex, a situation which is likely to be unresolved from the current investigation. Resolution of these complex relationships requires more available samples, especially for Caspian tiger and South China tigers as well as more variables used in multivariate craniodontal analysis, and other sources of non-metric skull traits (shape of sagittal crest, degree of convexity of the frontal, endocranial volume; Author's unpublished data) and molecular genetic analysis. The latest molecular survey (Luo et al. 2004, 2006) suggests that there are relatively low levels of genetic variation but nonetheless significant subspecific divisions in tigers, which can most likely be attributed to reduced gene flow and genetic drift in isolated populations over the last 72,000–108,000 years. This relatively young (compared with leopards, which are considered to have originated in Africa 470,000–825,000 years ago and have arrived in Asia 170,000–300,000 years ago: Uphyrkina et al. 2001)

history of subspeciation is consistent with the wide range of craniometric variation and the marked overlaps, but nonetheless the significant separations (on average) among mainland populations (except the Caspian population) observed from this study, suggest that the evolution of mainland subspecies is deeply influenced by the repeated geographic restrictions and expansions during Pleistocene stadials and interstadials.

Taxonomic status of the new Malay subspecies

P.t. jacksoni

In the latest molecular genetic survey a new tiger subspecies (*P.t. jacksoni*) of Southeast Asia has been proposed (Luo et al. 2004), characterized by three unique microsatellite alleles and five subspecific mtDNA haplotypes. In contrast, evidence from the recent morphological review of Southeast Asian tigers (J. Mazák and Groves 2006) did not support the subspecific distinctness of the Malay tiger. Clarification of the taxonomic affinities between the Malay tiger and its geographically neighboring populations would benefit further conservation strategies. In this section the scientific basis for the subspecific status of the Malay tiger is reviewed briefly.

To evaluate in particular the degree of difference between the Malayan and other mainland Asian subspecies, some supplementary DFA of size-adjusted data have been carried out. The results show that there is no significant difference between the Malayan and Indochinese/Indian subspecies as revealed by DFA plot and D^2 values for both males (Fig. 8) and females (results not shown) samples, which is consistent with J. Mazák and Groves' study. In the DFA plot, the Malayan samples overlap extensively with Indochinese and Indian samples; of three males, only one is classified correctly while the other two are misclassified with Indochina and India, respectively. The D^2 distances between Malayan samples and other mainland subspe-

cies are also obviously low and insignificant except, as before, for Amur (Appendix G).

Potentially the Isthmus of Kra could act as a biogeographic barrier restricting gene flow between the tigers of mainland Southeast Asia and the Malay Peninsula; it should be noted, however, that the catastrophic eruption of Toba at around 73,500 years ago in Sumatra would have largely destroyed vegetation across wide areas of North Sumatra and Malaya (Kitchener and Dugmore 2000), and consequently might have almost or entirely eliminated the Malayan tiger population. Depending on the rate of recovery of the vegetation, tigers could have followed their prey species as they re-colonized from the north (mainland Southeast Asia) or south (Sumatra) at in the last glaciation; evidently, colonization from the north rather than the south greatly predominated, as indicated by the larger values in size related craniometric variables observed in Malayan tigers (GLS of Malay males: mean 339, SD 21.69, $n = 4$; GLS of mainland Southeast Asia males: mean 330.27, SD 12.57 $n = 15$; see also Kitchener and Dugmore 2000). The inferred biogeographic evidence thus hardly supports the complete isolation of the new Malay subspecies from mainland Southeast Asia.

Of course, it should be noted that, because of the small sample size, results presented here are best regarded as preliminary, but they do form a morphological basis which might appear to provide little evidence to support the DNA-based separation of the Malayan population.

Conservation implications

This study has several implications for global tiger conservation. The effective conservation strategies for tigers in the wild and in captivity have greatly relied on our knowledge of patterns of their geographical variation and degrees of similarities and differences between traditionally recognized putative subspecies (Cracraft et al. 1998; Kitchener 1999; Kitchener and Dugmore 2000; J. Mazák and Groves 2006). My multivariate skull morphometric analyses suggest that there are probably two basic forms (mainland form and Java/Bali form) of tigers, which were isolated completely during the early or middle Pleistocene, and thereafter the distinctness of morphological characteristics (body size, diagnostic in skull characters, pelage coloration, stripe markings and fur structure) were developed. In modern formal taxonomy (Groves 2002), they would therefore be equivalent to two distinct species if applying the Phylogenetic Species Concept (PSC).

Unfortunately, the Java and Bali tigers were eradicated by the 1940s and 1980s, respectively. Among still extant taxa, it is certainly the Sumatran tiger that warrants the closest attention because of its unique

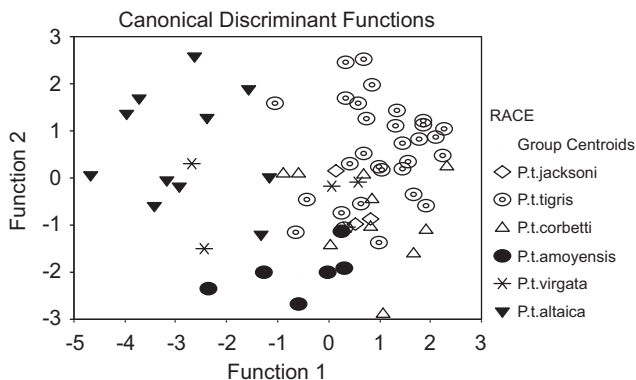


Fig. 8. Comparison of new Malay subspecies (*P.t. jacksoni*) and other putative mainland subspecies using size-adjusted measurements, noting that the Malayan samples are extensively overlapped with Indian and Indochinese samples.

systematic position; it is highly probable that it represents a form of hybrid origin (mainland \times Java) whose parental stocks colonised Sumatra from different directions and mixed, forming a homogeneous population and distinctive taxon, during the last Ice Age (Kitchener 1999; J. Mazák and Groves 2006). The subsequent more than 12,000 years' isolation allowed it to develop a series of distinct features (Cracraft et al. 1998), which are clearly consistent with morphological (J. Mazák and Groves 2006) and genetic (Hendrickson et al. 2000; Luo et al. 2004) analyses as well as the results of the present study. Therefore, the Sumatran tiger should receive conservation priority both in wild and in captive populations, since the current status of this particular population is extremely precarious, and it risks soon going extinct just like the Java tiger if effective measures are not taken in time (a maximum estimate of only 500 individuals alive in the wild today: Shepherd and Magnus 2004).

Among the five traditionally recognized mainland subspecies, one is already presumed extinct (the Caspian tiger, *P.t. virgata*) and another (the South China tiger, *P.t. amoyensis*) is critically endangered (Seidensticker et al. 1999, Tilson et al. 2004). The hope that the South China tiger can be preserved now fully depends on whether appropriate management is applied to captive populations, since it is believed that wild populations have become extinct (Tilson et al. 2004). Although the subspecific-level distinction of the South China tiger is somewhat less than that of the Siberian tiger, from this purely cranial metric analysis, this by no means implies that it is consubspecific with the Indochinese or Amur tiger, since it can well be distinguished from both in other cranial-dental characters (author's unpublished data, paper in preparation) and in molecular genetics (Luo et al. 2004, 2006). Therefore, ideas of genetic reinforcement from these geographically neighboring populations should be very cautiously approached and needs very serious consideration, since any inappropriate inbreeding programs would probably disrupt the co-adapted gene complexes of the South China tiger and compromise its last chance of survival.

This multivariate skull morphometric analysis also indicates that the Siberian tiger is the most distinctive in skull morphology of all mainland forms, and given the evidence that, in addition, they are isolated from other mainland populations by more than their maximum known dispersal distance (Kitchener and Dugmore 2000), this subspecies should likewise receive high conservation ranking.

Conclusions

In summary, the present study has demonstrated several major patterns of separation among the tigers

throughout their entire geographic ranges based on multivariate skull morphometric analysis. Patterns of cranial diversity in tigers may be influenced greatly by allometric scaling and sexual dimorphism. The greatest distinction observed in modern tigers is between mainland Asia and Java/Bali. Among the mainland tigers, the Siberian tiger is the most distinct, those of India, Indochina and South China are also differentiated on average. Variations within each mainland population are also marked, however, and there are extensive overlaps between them. In contrast, the biogeographically completely isolated Caspian tiger is indistinguishable from South or North populations craniometrically. Patterns of geographic variation in mainland tigers are generally clinal, which is deeply interwoven with sexual dimorphism.

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I am grateful to all who have helped me in various ways in my studies on tigers and eventually helped me to finish the present work. My most sincere thanks go to the late Dr. Vratislav Mazák (Prague) for his fundamental materials collected on tigers and to Prof. Colin P. Groves (Canberra) for his kind donation of V.M.'s data set of tiger skull measurements and original notes, and repeatedly arming me in my study and for reading a preliminary draft of this paper. My special thanks also go to Dr. Alexei Abramov (Saint Petersburg), Dr. Peter J. H. Van Bree (Amsterdam), Dr. Per Christiansen (Copenhagen), Dr. Géraldine Véron, and Dr. Philippe Gaubert (Paris), Dr. E.I. Zholnerovskaya (Novosibirsk), and Mr. Velizar H. Simeonovski (Chicago), for taking photographs or measurements of tiger skulls important to my study. I thank Drs. C.P. Groves, P. Christiansen, H. O'Regan, L. Werdelin and N. Yamaguchi for reading preliminary drafts of various versions of this paper and especially to Drs. P. Christiansen, C.P. Groves and L. Werdelin for their unusually perspicacious comments which improved the paper greatly. Very grateful thanks are due to the curators of various Chinese zoological collections where tiger specimens were examined and studied. I also thank Prof. Helmut Hemmer (Mainz), Prof. Mel Sunquist (Florida), Dr. Ronald Tilson (Minnesota), Dr. William Jungers (New York), Prof. Sheng Helin (Shanghai) and Dr. Luo Shujin (Maryland) for useful discussions or answers on some points raised in this study.

Appendix A

Definition of the 18 craniodental variables used in this study are shown in Table A1.

Table A1. Definition of the 18 craniodental variables used in this study

1	Greatest length: the greatest distance between prosthion and opisthocranion
2	Condylbasal length: length from prosthion to condylinon
3	Basal length: length from prosthion to basion
4	Rostral breadth: the greatest breadth across maxillae above canines
5	Infraorbital breadth: distance between inner edges of infraorbital foramina
6	Interorbital breadth: the smallest distance between inner edges of orbits
7	Postorbital constriction: the smallest breadth of postorbital bar
8	Bizygomatic breadth: the greatest distance between zygion-zygion
9	Mastoidal breadth: the greatest breadth across occipital crests above mastoidal process
10	Supraoccipital breadth: the smallest distance between notches of lateral margins of the occiput; the notch being situated approximately where sutura occipitoparietalis, sutura parietotemporalis and sutura occipitotemporalis meet
11	Occipital height: distance from basion to the tip of occiput
12	Greatest length of nasals: the greatest length of nasal bones measured in straight line
13	Mandible length: length from the most oral point of the lower jaw to condylinon mediale
14	Mandible height: distance from most inferior point of process angularis of the tip of processus muscularis
15	Upper carnassial length (Pm4): the greatest length of crown of upper carnassial
16	C-Pm4 length: the distance between anterior edge of canine alveolus and posterior edge of pm4 alveolus
17	Lower carnassial length (m1 length): the greatest length of the crown of lower carnassial
18	C-m1 length: distance between anterior edge of canine alveolus and posterior edge of m1 alveolus

Appendix B

Basic allometric regression statistic for subspecies and sexes of tigers, variables used for comparison are

selected from one-way ANOVA are shown in [Table B1](#). The results of pairwise significant tests for differences in slopes between the sexes are shown in [Table B2](#).

Table B1. Basic allometric regression statistic for subspecies and sexes of tigers, variables used for comparison are selected from one-way ANOVA

Males													
v.s	<i>altaica</i>				<i>virgata</i>				<i>amoyensis</i>				
CBL	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	
RB	0.902	-0.995 ± 0.360	1.202 ± 0.144	18	0.543	0.090 ± 1.463	0.764 ± 0.590	6	0.587	1.010 ± 0.675	0.397 ± 0.274	6	
IFB	0.755	-0.649 ± 0.566	1.040 ± 0.226	18	0.519	1.252 ± 0.550	0.270 ± 0.222	6	0.377	0.623 ± 1.619	0.534 ± 0.656	6	
SOB	0.483	0.184 ± 0.785	0.691 ± 0.313	18	0.208	2.434 ± 1.310	-0.225 ± 0.529	6	0.746	-0.141 ± 0.893	0.810 ± 0.362	6	
OH	0.866	-0.991 ± 0.526	1.211 ± 0.210	13	0.808	-1.472 ± 1.270	1.404 ± 0.512	6	0.657	0.296 ± 0.977	0.690 ± 0.396	6	
GLN	0.739	0.331 ± 0.471	0.685 ± 0.188	13	0.289	1.231 ± 1.337	0.326 ± 0.539	6	0.840	-1.583 ± 1.167	1.466 ± 0.473	6	
	<i>corbetti</i>				<i>tigris</i>				<i>sumatrae</i>				
	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	
RB	0.638	-0.035 ± 0.605	0.813 ± 0.246	18	0.786	0.026 ± 0.216	0.790 ± 0.087	53	0.817	-0.592 ± 0.741	1.046 ± 0.302	8	
IFB	0.684	-0.826 ± 0.755	1.111 ± 0.306	17	0.547	0.407 ± 0.329	0.617 ± 0.132	53	0.775	0.309 ± 0.541	0.663 ± 0.221	8	
SOB	0.435	-0.050 ± 0.980	0.769 ± 0.398	18	0.698	-0.726 ± 0.373	1.044 ± 0.150	53	0.714	-2.371 ± 1.683	1.714 ± 0.686	8	
OH	0.683	-1.081 ± 0.825	1.253 ± 0.335	18	0.759	-0.859 ± 0.358	1.164 ± 0.144	50	0.684	-0.651 ± 1.149	1.074 ± 0.468	8	
GLN	0.878	-1.445 ± 0.476	1.415 ± 0.193	18	0.810	-0.588 ± 0.285	1.071 ± 0.114	48	0.529	0.333 ± 1.083	0.674 ± 0.441	8	
	<i>sondaica</i>				<i>balica</i>								
	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>					
RB	0.715	-0.107 ± 0.453	0.846 ± 0.185	22	0.945	-1.103 ± 1.055	1.250 ± 0.433	3					
IFB	0.475	0.887 ± 0.420	0.415 ± 0.172	22	0.500	2.492 ± 1.055	-0.250 ± 0.433	3					
SOB	0.077	2.024 ± 0.821	-0.116 ± 0.335	22	0.866	-0.108 ± 1.055	0.750 ± 0.433	3					
OH	0.290	1.412 ± 0.421	0.233 ± 0.172	22	0.971	-2.338 ± 1.055	1.750 ± 0.433	3					
GLN	0.830	-2.262 ± 0.680	1.750 ± 0.278	16	1.000	-5.300 ± 0.000	3.000 ± 0.000	3					

Table B1. (continued)

Females																
v.s	<i>altaica</i>				<i>virgata</i>				<i>amoyensis</i>							
CBL	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>
RB	0.863	-1.595±0.594	1.448±0.244	14	0.659	0.560±0.512	0.560±0.213	11	0.700	-0.684±1.538	1.076±0.634	5				
IFB	0.868	-1.038±0.482	1.199±0.198	14	0.557	0.491±0.723	0.571±0.301	10	0.719	-0.373±1.541	0.930±0.636	4				
SOB	0.560	-1.906±1.601	1.542±0.658	14	0.542	0.244±0.927	0.658±0.386	9	0.629	-0.120±1.675	0.791±0.691	4				
OH	0.448	0.348±1.069	0.659±0.439	11	0.810	-0.172±0.763	0.875±0.317	6	0.500	3.150±2.102	-0.500±0.866	3				
GLN	0.732	-0.685±0.827	1.092±0.339	11	0.645	-1.635±1.908	1.500±0.794	7	0.775	-1.200±2.587	1.308±1.066	3				
	<i>corbetti</i>				<i>tigris</i>				<i>sumatrae</i>							
	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>
RB	0.715	-0.165±0.434	0.864±0.180	24	0.408	0.601±0.557	0.545±0.231	30	0.854	-1.619±0.520	1.473±0.217	19				
IFB	0.436	0.434±0.652	0.599±0.270	23	0.202	2.504±0.576	-0.255±0.238	29	0.701	-1.045±0.742	1.221±0.310	15				
SOB	0.310	0.333±0.973	0.602±0.403	23	0.154	1.214±0.743	0.249±0.308	29	0.561	-1.631±1.257	1.426±0.526	15				
OH	0.603	0.744±0.370	0.492±0.153	20	0.788	-1.923±0.594	1.603±0.246	28	0.898	-2.234±0.525	1.734±0.219	14				
GLN	0.738	-1.760±0.784	1.552±0.325	21	0.578	-0.961±0.851	1.223±0.352	26	0.740	-0.602±0.666	1.061±0.279	14				
	<i>sondaica</i>				<i>balica</i>											
	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	<i>a</i>	<i>b</i>	<i>n</i>								
RB	0.738	-0.537±0.672	1.017±0.280	12	1.000	-1.690±0.000	1.500±0.000	3								
IFB	0.608	0.569±0.531	0.536±0.221	12	-	-	-	1								
SOB	0.200	2.555±1.334	-0.359±0.556	12	0.945	-1.280±1.028	1.250±0.433	3								
OH	0.771	0.484±0.373	0.594±0.155	12	0.500	-0.670±2.055	0.500±0.866	3								
GLN	0.137	1.303±1.461	0.266±0.609	12	1.000	-4.010±0.000	2.500±0.000	3								

Boldfaced values indicate the slope is strongly positive or negative allometric to CBL.

Table B2. Results of pairwise significant tests for differences in slopes between the sexes

vs CBL	<i>altaica</i>		<i>virgata</i>		<i>amoyensis</i>		<i>corbetti</i>		<i>tigris</i>		<i>sumatrae</i>		<i>sondaica</i>		<i>balica</i>	
	M/F		M/F		M/F		M/F		M/F		M/F		M/F		M/F	
	<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>
RB	-3.91	***	-0.13	N.S	-1.16	N.S	-2.02	*	-1.42	N.S	-2.91	**	-2.18	*	-2.65	N.S
IFB	-2.95	**	-1.26	N.S	-0.48	N.S	-0.36	N.S	1.55	N.S	-2.54	*	-1.50	N.S	-	-
SOB	-1.43	N.S	-1.23	N.S	-0.20	N.S	-0.29	N.S	0.32	N.S	0.06	N.S	0.44	N.S	-1.29	N.S
OH	-0.14	N.S	-0.05	N.S	1.22	N.S	-0.08	N.S	-4.46	***	-2.28	*	-2.33	*	1.23	N.S
GLN	-2.13	*	-1.24	N.S	0.34	N.S	-2.69	*	-2.23	*	-1.36	N.S	1.35	N.S	-	-

p*<0.05; *p*<0.01; ****p*<0.001.

Appendix C

Function loadings of step-wise DFA for comparing all eight putative males using size-adjusted data are shown in Table C1. Jack-knifed classification matrix of step-wise DFA for comparing all eight putative males are shown in Table C2.

Appendix D

Function loadings of step-wise DFA for comparing all eight putative females using size-adjusted data are shown in Table D1. Jack-knifed classification matrix of step-wise DFA for comparing all eight putative female subspecies is shown in Table D2.

Table C1. Function loadings of step-wise DFA for comparing all eight putative males using size-adjusted data

Craniometric measurements	Function 1	Function 2
Greatest skull length	−0.664	0.198
Condylbasal length	−0.159	−0.532
Basal length	0.711	1.038
Rostral breadth	−0.633	−0.121
Infraorbital breadth	0.000	0.684
Postorbital constriction	0.380	−0.248
Supraoccipital breadth	0.889	0.133
Occipital height	0.644	−0.305
Greatest nasal length	0.154	−1.019
Upper carnassial length	−0.356	−0.086
C-P4 length	0.370	0.295
Percentage of variance	50.2	30.8

Notes: Variables with high loading are marked with boldface.

Table C2. Jack-knifed classification matrix of step-wise DFA for comparing all eight putative males

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>	<i>P.t. sumatrae</i>	<i>P.t. sondaica</i>	<i>P.t. balica</i>	Total	% correct
<i>P.t. altaica</i>	9	2	0	1	0	0	0	0	12	75
<i>P.t. virgata</i>	1	0	0	2	1	1	0	0	5	0
<i>P.t. amoyensis</i>	0	1	4	1	0	0	0	0	6	66.7
<i>P.t. corbetti</i>	0	2	2	6	3	0	0	0	13	46.2
<i>P.t. tigris</i>	1	1	3	5	21	0	0	0	31	67.7
<i>P.t. sumatrae</i>	1	0	1	0	0	5	0	0	7	71.4
<i>P.t. sondaica</i>	0	0	0	0	0	0	14	0	14	100
<i>P.t. balica</i>	0	0	0	0	0	0	0	2	2	100

Table D1. Function loadings of step-wise DFA for comparing all eight putative females using size-adjusted data

Craniometric measurements	Function 1	Function 2
Greatest skull length	−0.923	−0.109
Condylbasal length	−0.347	0.369
Basal length	0.629	−0.807
Rostral breadth	−0.412	−0.241
Bizygomatic breadth	−0.387	0.498
Mastoidal breadth	−0.663	−0.360
Supraoccipital breadth	1.141	−0.053
Occipital height	0.727	−0.286
Greatest nasal length	0.279	0.584
Upper carnassial length	0.187	0.523
C-P4 length	0.216	0.524
Percentage of variance	60.1	16.1

Notes: Variables with high loading are marked with boldface.

Appendix E

Function loadings of step-wise DFA for comparing mainland males using size-adjusted data are shown in Table E1. Jack-knifed classification matrix of step-wise DFA for comparing mainland putative male subspecies are shown in Table E2.

Appendix F

Function loadings of step-wise DFA for comparing mainland females using size-adjusted data are shown in Table F1. Jack-knifed classification matrix of step-wise DFA for comparing mainland putative female subspecies are shown in Table F2.

Table D2. Jack-knifed classification matrix of step-wise DFA for comparing all eight putative female subspecies

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>	<i>P.t. sumatrae</i>	<i>P.t. sondaica</i>	<i>P.t. balica</i>	Total	% correct
<i>P.t. altaica</i>	2	4	2	0	1	1	0	1	11	18.2
<i>P.t. virgata</i>	1	2	0	1	1	0	0	0	5	40
<i>P.t. amoyensis</i>	2	0	1	1	0	0	0	0	4	25
<i>P.t. corbetti</i>	2	0	2	6	4	1	0	2	17	35.3
<i>P.t. tigris</i>	2	1	0	2	13	2	0	0	20	65
<i>P.t. sumatrae</i>	0	1	0	1	1	10	0	0	13	76.9
<i>P.t. sondaica</i>	0	0	0	0	0	0	10	1	11	90.9
<i>P.t. balica</i>	0	0	0	1	0	0	0	2	3	66.7

Table E1. Function loadings of step-wise DFA for comparing mainland males using size-adjusted data

Craniometric measurements	Function 1	Function 2
Rostral breadth	−0.694	0.427
Postorbital constriction	0.653	−1.095
Bizygomatic breadth	0.271	0.423
Greatest nasal length	0.806	0.575
Percentage of variance	68.6	23.9

Notes: Variables with high loading are marked with boldface.

Table E2. Jack-knifed classification matrix of step-wise DFA for comparing mainland putative male subspecies

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>	Total	% correct
<i>P.t. altaica</i>	10	1	0	1	0	12	83.3
<i>P.t. virgata</i>	2	1	0	1	1	5	20
<i>P.t. amoyensis</i>	0	0	4	2	0	6	66.7
<i>P.t. corbetti</i>	0	4	2	5	3	14	35.7
<i>P.t. tigris</i>	0	2	5	4	31	42	73.8

Table F1. Function loadings of step-wise DFA for comparing mainland females using size-adjusted data

Craniometric measurements	Function 1	Function 2
Condylbasal length	0.685	0.188
Supraoccipital breadth	0.494	−0.401
C-P4 length	−0.796	0.775
Mandible height	−0.853	−0.281
C-m1 length	0.544	−0.007
Percentage of variance	67.7	22.5

Notes: Variables with high loading are marked with boldface.

Table F2. Jack-knifed classification matrix of step-wise DFA for comparing mainland putative female subspecies

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>	Total	% correct
<i>P.t. altaica</i>	4	1	5	0	3	13	30.8
<i>P.t. virgata</i>	2	4	0	1	3	10	40
<i>P.t. amoyensis</i>	1	0	4	0	0	5	80
<i>P.t. corbetti</i>	1	0	1	10	6	18	55.6
<i>P.t. tigris</i>	4	2	1	4	13	24	54.2

Table G1. Function loadings of DFA for comparing males of *P.t. jacksoni* and other putative mainland subspecies using size-adjusted data

Craniometric measurements	Function 1	Function 2
Greatest skull length	−0.442	−0.784
Condylbasal length	−0.294	0.663
Infraorbital breadth	−0.420	0.131
Postorbital constriction	0.561	−0.886
Occipital height	0.385	0.707
Greatest nasal length	0.926	0.242
Lower carnial length	0.461	−0.195
C-m1 length	−0.583	0.186
Percentage of variance	57.4	21.2

Notes: Variables with high loading are marked with boldface.

Table G2. Mahalanobis D^2 distances between males of *P.t. jacksoni* and other putative mainland subspecies using size-adjusted data

	<i>P.t. altaica</i>	<i>P.t. virgata</i>	<i>P.t. amoyensis</i>	<i>P.t. corbetti</i>	<i>P.t. tigris</i>	<i>P.t. jacksoni</i>
<i>P.t. altaica</i>	0.000					
<i>P.t. virgata</i>	3.033	0.000				
<i>P.t. amoyensis</i>	5.720	2.301	0.000			
<i>P.t. corbetti</i>	9.343	2.413	3.599	0.000		
<i>P.t. tigris</i>	12.955	3.270	5.586	3.436	0.000	
<i>P.t. jacksoni</i>	4.228	1.121	1.895	1.787	1.546	0.000

Notes: Group pairs with significant distances ($p < 0.001$) are marked with boldface.

Appendix G

Function loadings of DFA for comparing males of *P.t. jacksoni* and other putative mainland subspecies using size-adjusted data are shown in Table G1. Mahalanobis D^2 distances between males of *P.t. jacksoni* and other putative mainland subspecies using size-adjusted data are shown in Table G2.

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