ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 64 (4), 2012: 381-387

Morphometric Variation in Black Rat *Rattus rattus* (Rodentia: Muridae) from Tunisia

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Abstract: Black rat *Rattus rattus* is widely distributed in Tunisia. We examined for first time the morphological variation for 103 specimens as a function of their geographical origin, using univariate and multivariate statistics with traditional morphometrics. Our results supported the existence of two morphotypes of this species. The first one included individuals that populate Northern and Central part of Tunisia, while the second comprised specimens from the South. The probability of the correct classification of specimens was 96.8%, and indicated significant degrees of variation in morphometric characteristics between these two morphotypes. In addition, we tested the influence of age, sex, geography and some habitat variables (such as precipitation and vegetation) on the size of the skull. The results showed that the latitude and precipitations were significant effects on the skull size. The pattern of morphometric variation is probably correlated with local environmental factors.

Key words: *Rattus rattus*, classical morphometrics, geographic variation, Tunisia

Introduction

Black rat *Rattus rattus* (Linneaus 1758), is native to the Indian Peninsula and has since been introduced world-wide (Musser, Carleton 2005, Amori *et al.* 2008). This species has been identified as a potential carrier of diseases that can affect humans (Meehan 1984, Amori, Clout 2003, Millis 2006, Milena *et al.* 2003, Rahelinirina *et al.* 2010, Tollenaere *et al.* 2010a, Bastos *et al.* 2011). Despite the wide distribution of *R. rattus* and its detrimental impact on invaded ecosystems and human health, very few genetic studies have focused on natural populations of this species (Duplantier *et al*. 2003, Abdelkrim *et al.* 2005, Gilabert *et al.* 2007, Tollenaere *et al.* 2010b, Bsatos *et al.* 2011, Tollenaere *et al.* 2011). In addition, Ventura, Lopez-Fuster (2000) examined 23 skull mesureaments of *R. rattus* from Congreso Island in Spain, reported that these specimens are similar in size to Iberian individuals and in skull shape to Morocco animals. Considering this later fact and the geographical proximity between Chafarinas and Morocco coastline, it seems likely that Congreso black rats had their source in North-African populations. The size similarity between Iberian and Congreso rats does not appear to be the expression of a genetic relationship but a biometric convergence.

The measurement of shape has been classically derived from linear distance measurements sampling dimensions of length, height and width (Marcus 1990) and this framework has provided the methodological basis for the study of geographic variation in phenotypic morphological traits. Indeed,

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the morphometric analyses have been shown to be powerful to analyze skull variability among rodents (Chimimba 2001, Nicolas *et al.* 2008, Lalis *et al.* 2009, Bezerra, de Oliveira 2010). Up to date, no morphometric studies have ever been reported on the skull variability of *R. rattus* in Tunisia.

As geographically known, Tunisia occupies a central position in Maghreb region, between Moroccan and Algerian Atlas ends and Egyptian-Libyan depression of Nile valley, i.e., it represents the break zone between eastern and western parts of Maghreb region. In addition, Tunisia extends also to Sahara in the South, and thus offering a latitudinal climatic and vegetation gradient from Mediterranean shore to true desert.

Following this approach, the present study was undertaken to investigate the size variation of the skull in Black Rat *Rattus rattus* by using classical morphometric analyses of some craniodental parameters in order to analyze intra and inter-population variation in the size of the skull.

Material and Methods

A total of 103 adults individuals of Black rat *R. rattus* (50 males, 53 females) from 10 natural populations were included in the analyses (Fig. 1, Table 1).

Age classes were defined based upon molar wear stages and defined as: Stage C0: No upper M3 is erupted; Stage C1: Upper M3 erupted but not worn; Stage C2: Cusps still visible on all molars and link between first and second lobe of the upper M3 is very narrow; Stage C3: The longitudinal link between first and second lobe of upper M3 is larger and, in general, wider on the upper M12; Stage C4: the upper M3 displays nearly the total fusion of the first and second lobe of the longitudinal link that is very wide but it remains visible on the other molars cusps.

Thirteen cranial and dental measurements were taken on the 103 skulls using digital callipers (RUPAC, Italy) and values were rounded to the nearest 0.01mm. The skull distances were previously reported by (Ventura & Lopez-Fuster 2000) that include: total skull length (TSL), nasal length (NL), frontal length (FL), parietal length (PL), upper diastema length (UDL), incisive foramen length (IFL), nasal width (NW), interorbital width (IOW), interparietal width (IPW), occipital width (OW), mandible length (ML), articular width (AW) and zygomatic width (ZW).

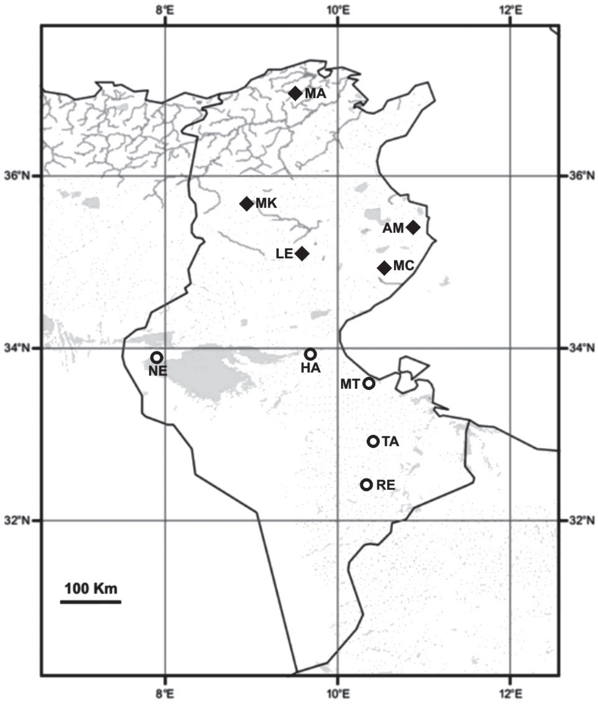

Fig. 1. A map showing the geographical localities from which the populations of *R. rattus* were collected in Tunisia. Different symbols indicated the two morphometric groups (Group 1 (Northern and Central Tunisia); Group 2 (Southern Tunisia; see Table1 for abbreviations of geographic locality).

Statistical analyses of morphometric parameters were performed using Past 1.81 (Hammer *et al.* 2001) and SPSS (16.0). Firstly, we performed analyses of variance (ANOVA) and multiple analyses of variance (MANOVA) to test the influence of sexual dimorphism, age, and geographical variables (habitat and vegetation) on size of the skull. The significance of differences among the clades for size-corrected values of measurements was tested by means of one-way ANOVAs. Afterwards, we performed a different set of analyses by using principal component analysis (PCA) for the log-transformed data of variance-covariance matrix for the specimens of clades.

Morphometric differentiation of the samples studied was also assessed by means of Canonical Variate Analysis (CVA) in which samples were taken as the clades. Correlations between adjusted measurements and canonical variates were computed to establish the contribution of the given variable for the total among specimens of clades variation explained by a given canonical variate.

Table 1. Localities and samples sizes (n) from which *R. rattus* populations were collected in Tunisia.

Region	Locality	Latitude	Longitude	Altitude (m)	Precipitation mean per year/mm	n
North	Mateur (MA)	37° 02'24"N	9° 39'54"E	65	600	12
	Maktheur (MK)	36°05'23"N.	9°22'44"E	900	500	11
Central	Amira (AM)	35° 30'08"N	11°02' 44"E	190	258	10
	Lessouda (LE)	35° 06'45"N	9° 31'67"E	700	234	9
	Menzel Chaker (MC)	34°56'92 "N	10°21'96 "E	105	200	10
South	Nefta (NE)	33°52'32"N	7°52' 49"E	58	15	10
	Hamma (HA)	33°53'56"N	9°47' 27"E	58	150	11
	Matmata (MT)	33°32' 97"N	9°57' 62"E	600	180	9
	Tataouine (TA)	32°55'14 "N	10°27'08"E	246	157	11
	Remada (RE)	32°19' 24"N	10°24' 10"E	300	25	10

Results

The 103 skulls measured, corresponding to individuals of age classes ranging from C2 to C5, were used in the statistical analyses. Multivariate analysis of variance showed that craniodental measurements were not significantly correlated either with age (Wilks' lambda = 0.74; $P = 0.50$) or with sex (Wilks' lambda = 0.89; $P = 0.68$), consequently, we continued to treat the whole data set as a pooled sample.

Analysis of variance, including geographic location as a factor for all the analysed variables, showed that all the morphometric variables differed significantly between specimens of the clades (Table 2, 3).

General trends of craniodental characters variation among samples of *R. rattus* were surveyed by using principal component analysis (PCA) of the 13 skull log-transformed distances of 103 individuals showing clearly two morphometric groups. The first clade comprised the specimens of North and Central while the second included the individuals of Southern Tunisia (Fig. 2). The Axis 1 and 2 displayed 71.65 % of the total variability. In this analysis, 63.24 % of the total variation was explained by the first component axis while 8.41 % was explained by the second axis. The correlations of each skull variable obtained from the Log PCA are provided in Table 2. PC1 was strongly correlated with TSL while PC2 and PC3 were negatively correlated with UDL and IOW respectively (Table 4).

We performed CVA analysis on the basis of the result of PCA which shown clearly two morphometric groups. The plot of the canonical variables CV1 and CV2 is shown in Fig. 3 and indicates a clear separation between the specimens of the two clades of *R. rattus* in Tunisia (Wilks' lambda = 0.013. $P < 0.0001$). The first canonical axis (CV1) accounted for 63.17% of the total variance and was strongly correlated with TSL, while CV2 which explained 9.44 % of variance and correlated with UDL and ZW (Table 5). The CVA analysis performed between the two morphometric groups or clades assigned correctly 96.8% of samples.

Regressions were performed based on log data versus latitude, longitude, altitude and precipitation.

Table 2. Results of one-way ANOVAs of *R. rattus* populations in Tunisia, showing differences in means of cranial variables among the two clades (*P<0.05).

Variables	F	P
TSL	6.023	0.0236*
NL	5.467	0.0034*
FL	8.951	0.0021*
PL	3.465	0.0128*
UDL	3.879	0.0013*
IFL	4.987	0.0042*
NW	3.658	0.0145*
IOW	6.456	0.0026*
IPW	2.768	0.0321*
OW	5.546	0.0061*
ML	6.145	0.0042*
AW	3.256	0.0002*
ZW	4.345	0.0045*

Table 3. Descriptive statistics for *R. rattus* populations in Tunisia (measurements in mm; SD: standard mean).

Region		TSL	NL	FL	PL	UDL	IFL	NW	IOW	IPW	OW	ML	AW	ZW
Northern	Mean	38.14	13.33	13.75	7.53	10.11	6.52	3.89	5.81	10.11	14.61	22.12	2.91	19.81
	SD of mean	0.95	0.87	0.67	0.89	0.71	1.12	0.55	0.57	0.99	1.23	0.93	0.73	1.34
Central	Mean	38.88	13.76	13.99	7.88	10.41	6.71	3.99	5.96	10.22	14.79	22.33	2.99	19.97
	SD of mean	0.39	0.91	0.96	0.69	0.69	1.18	0.69	0.66	0.68	1.09	0.72	0.84	1.22
Southern	Mean	40.22	14.89	14.78	8.25	11.44	7.53	4.25	6.14	11.18	15.03	23.80	3.24	20.12
	SD of mean	0.91	0.97	0.81	0.76	0.69	1.23	0.71	0.87	0.29	1.81	0.89	0.47	1.22

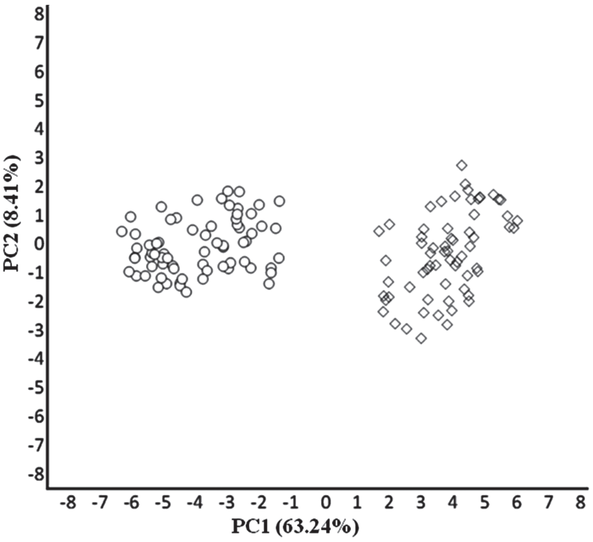

Fig. 2. Results of the first (PC1) and second (PC2) principal component analysis for thirteen cranial measurements in 103 individuals of *R. rattus* from Tunisia (individuals of Clade I; specimens of Clade II).

All of the skull size and shape parameters varied significantly with latitude and precipitation (Table 6).

Discussion

Our approach allowed for the first time to describe some of the size of skull variability in *R. rattus* populations in Tunisia. The patterns of variability were consistent with the variation in size of the skull amongst the two clades (Northern. and Central localities in one hand. and Southern populations in another hand). However, the observed variability among these populations was not related to age, although the present samples did not present skulls with initial age classe stages C0 and C1. The sex ratio was quite equilibrated (50 males versus 53 females). The absence of sexual dimorphism was also clearly established. Recently, it has been shown that some rodents sometimes can harbor sexual dimorphism (Lalis *et al.* 2009, Abdel-Rahman *et al.* 2008), but this remained the exception herein. Our results agrees with the general trends shown by western Mediterranean and Moroccan black rat populations (Granjon, Cheylan 1990, Ventura, Lopez-Fuster 2000).

Otherwise, general skull dimensions of *R. rattus* from Tunisia fall within the range given for the species in the Western Mediterranean and Moroccan regions (Cheylan 1986; Granjon, Cheylan 1990, 1993, Ventura, Lopez-Fuster 2000). However, Ventura, Lopez-Fuster (2000) reported that the skull dimensions of *R. rattus* originating in Congreso Island were significantly different (higher) than North African samples in Morocco. If so, dimensions of *R. rattus* from Congreso agree with the size increase that small mammals may show in particular insular conditions (Lomolino 1985). Literature data concerning skull size of *R. rattus* from Western Europe (Schwabe 1979, Granjon, Cheylan 1990) suggest that this convergence is not a function of a latitudinal variation.

Many hypotheses are cited to explain the body size variation by means of combination of climatic factors (James 1970) and metabolism rate, competition, mating success, and cost of transport (McNab 1971, Calder 1984, Schmidt-Nielsen 1984, Dayan *et al.* 1994) that cannot be tested here due to the absence of joint ecological studies on *R. rattus*. In Israel, which is also similar to Tunisia by having a strong climatic gradient, the small sized mammals, like rodents, greatly vary according to the temperature and precipitation (Nevo *et al.* 1986, Nevo 1989), but, none of these environmental variables produced obvious effect in *R.rattus* as declared by Yom-Tov, Geffen (2006). This is slightly inconsistent with the findings in the present study, where latitude, lon-

Table 4. Variable coefficients, Eigen values, and percentage variance explained by the first 3 components of a principal component analysis (PC1–PC3) performed on 13 ln-transformed skull measurements from R. rattus specimens. Asterisks (*) indicate variables that explained the greatest percentage of variation on each PC.

Variables	PC1	PC2	PC3
TSL	0.6039*	0.6961	0.3452
NL	0.1761	0.0741	0.0192
FL	0.2229	-0.1669	-0.060
PL	0.2842	0.0700	-0.2311
UDL	0.4639	-0.7222*	0.2319
IFL	0.1235	0.0331	0.0049
NW	0.1336	-0.0475	-0.0286
IOW	0.3803	0.3352	-0.6098*
IPW	0.0991	0.0223	-0.0056
OW	0.1500	0.1293	-0.0839
ML	0.3688	-0.1815	-0.2751
AW	0.1403	0.0103	-0.0871
ZW	0.0604	-0.0649	0.0259
Eigen values	8.2310	6.1150	3.798
% variance	63.24	8.41	5.24

Table 5. Results from 2 canonical variates analyses (CVA) performed for the 13 craniodental measurements of *R. rattus* populations.

Variables	CV1	CV2
TSL	0.7735	-0.2095
NL	0.2927	-0.0153
FL	0.1853	0.2530
PL	0.3592	-0.1515
UDL	0.3687	0.6136
IFL	0.2445	0.0983
NW	0.1824	0.2578
IOW	0.2798	-0.3370
IPW	0.3040	0.0409
OW	0.2296	-0.4102
ML	0.3220	-0.0083
AW	0.1996	-0.1238
ZW	0.1235	0.5679
% variance	63.17	9.44

gitude, altitude and precipitation variables exhibited a significant control on size parameters. As far as known, Black rat metabolism is well adapted to different conditions, but very few are known about their ecology or precise habitats in Tunisian localities. Other factors of intraspecific variability remain to be investigated in this species. However, the relative small size of our samples was also limited the geographical interpretations of the observed results.

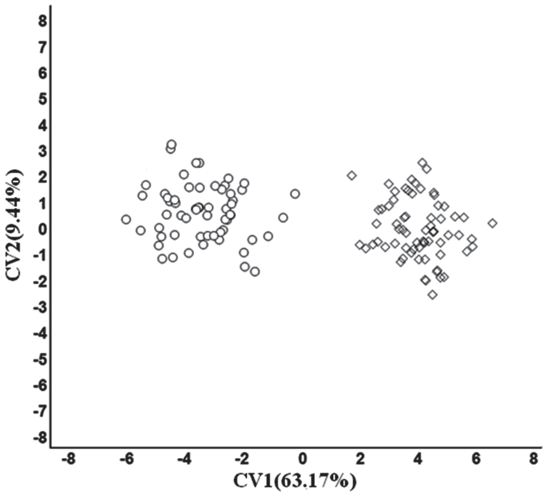

Fig. 3. Results of canonical variate analysis (CVA) of the 13 skull measurements examined between individuals of *R. rattus* (individuals of Clade I; specimens of Clade II).

Table 6. Regressions of the different skull size and shape parameters with environmental factors.

Factors	Log data
Latitude	3.456e-10 ***
Longitude	0.4327 NS
Altitude	0.08548 NS
Precipitation	4.456e-04 ***

*** P<0.001; NS: Not significant

Size variation is generally considered to be more labile to environmental gradients than shape (Thorpe 1976, Patton, Brylski 1987). However, our results indicated that for *R. rattus* populations, variation in skull size was associated with the environmental gradient. Other published results have shown that the shape and skull differences observed might not be attributed to static allometric differences among populations (Duarte *et al.* 2000, Reis *et al.* 2002).

Moreover, regressions for latitude and precipitations were significant for the whole data set. The major trend of shape variation observed was associated with a latitudinal gradient that has already been reported as a morphological discontinuity uncovered by canonical variates analysis (Reis *et al.* 2002).

In **conclusion**, our analyses demonstrated the existence of two morphotypes of *R. rattus* in Tunisia (Northern. Central and Southern localities). The results revealed an extensive variability of skull size among populations of the two morphotypes of this species collected from 10 localities in Tunisia, which could be explained in view of environmental and ecological variation. This finding was also reported in numerous rodent species (Macholán 2008; Nicolas *et al.* 2008; Ben Faleh *et al.* 2010). To this end, taking in account that the frequent karyotype of *R. rattus* in Africa was 2n=38 (Yosida 1980, Cheylan 1986, Denys *et al.* 2009, Bastos *et al.* 2011), it can be assumed probably that this morphometric divergence revealed between Tunisian populations is not accompanied by karyological or chromosomal variation but this hypothesis needs to be further verified in the future cytogenetic investigation.

Acknowledgements. The authors are grateful to all people who participated in the collection of samples.

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Received: 04.01.2012
Accepted: 26.09.2012