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Three-dimensional geometric morphometrics of *Arvicanthis*: implications for systematics and taxonomy

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Abstract

Arvicanthis is an African murid, found throughout sub-Saharan Africa, Sudan and Egypt. Although in the past 10 years several studies have been carried out to assess its systematics, there is still a need for a general revision of the genus. In this study the morphometric relationships between 71 populations throughout the range were investigated. A three-dimensional geometric morphometric approach was used to assess differences in the size and shape of the skull. These were related to the different biogeographical domains characterizing the range of the genus and to molecular and karyotypic phylogenies. Results agree only in part with phylogeny, and show a close relationship with the environmental backgrounds of each species. It is therefore suggested that the adaptation of *Arvicanthis* to local environment has played an important role in the phenotypic evolution of the skull. This leads to problems in taxonomic definitions based on morphometrics, which should not be used without comparison with other independently derived characters such as the DNA and the karyotype.

Key words: *Arvicanthis* – Muridae – Africa – geometric morphometrics – geographic variation – phylogeography

Introduction

Arvicanthis Lesson 1842 is a widespread murid genus in Africa ranging from Egypt to Tanzania and from Senegal to Somalia. Although it is one of the most common and successful African rodents (Kingdon 1974), its systematics was partially unclear until the last decade (see, for example, Musser and Carleton 1993). In fact, *Arvicanthis* has been intensively studied during the last 10 years, with an exponential increase of information.

After Matthey's (1965) first description of the karyotype of *Arvicanthis* from the Central African Republic, chromosomal studies were the first to provide important insights into the systematics of the genus. As a result, four different karyotypes have been identified within *Arvicanthis niloticus* in western and central Africa, provisionally named ANI-1 ($2n = 62$; $AFn = 64$), ANI-2 ($2n = 58$; $AFn = 70$), ANI-3 ($2n = 62$; $AFn = 74$), ANI-4 ($2n = 62$; $AFn = 74$), which suggests a species complex (Volobouev et al. 1988; Granjon et al. 1992; Civitelli et al. 1995; Ducroz et al. 1997; Ducroz 1998) rather than the single species indicated by Musser and Carleton (1993). Regarding East Africa, of the Ethiopian endemics *Arvicanthis dembeensis* has the same karyotype as ANI-1 (Corti et al. 1995), *Arvicanthis abyssinicus* shows $2n = 62$ and $AFn = 64$, and *Arvicanthis blicki* has $2n = 48$ and $AFn = 64$. *Arvicanthis neumanni* (formerly *Arvicanthis somalicus*, according to Musser and Carleton 1993) was described in Tanzania by Ducroz (1998) and Fadda et al. (2001a) with $2n = 54$ and $AFn = 62$. Finally, a species with $2n = 62$ and $AFn = 62$ and related to ANI-3 was found close to the border with Kenya (Fadda et al. in preparation).

Together with cytogenetics, allozyme analyses (Kaminski et al. 1984, 1987; Capanna et al. 1996; Capula et al. 1997), DNA/DNA hybridization (Ducroz et al. 1997) and the entire sequence of the mitochondrial gene cytochrome *b* (Ducroz et al. 1998) indicated a pattern of phylogenetic relationships. Two main clades were identified. The first one included ANI-2, ANI-3, and ANI-4 and a population from western Uganda. The latter, included ANI-1, *A. niloticus* from *terra typica*, the

Ethiopian endemics and *A. neumanni* from Tanzania (Ducroz 1998; Ducroz et al. 1998).

Few published articles dealt with morphometrics, and these were limited to the Horn of Africa (Afework Bekele et al. 1993; Corti and Fadda 1996), and the Nile Valley (Fadda and Corti 1998). These studies led to the identification of morphometric distinction for some species, but also suggested that morphometric patterns reflect processes of adaptation to the different environments characterizing the species ranges.

In fact, species distributions follow the different biogeographical domains characterizing Africa, with ANI-1, ANI-3, ANI-2 and ANI-4 occurring in progressively moister habitats in the west and the ranges of the East African species being affected by the emergence of the Rift Valley.

The aim of this study was to describe the morphometric variation in the skull of 71 populations, covering most of the range of the genus, and to relate it to the geography, the environment, and the partially known phylogeny.

For this purpose, a three-dimensional geometric morphometrics approach was used. This method allows a recovery of the geometric properties of the shape of the skull in real three-dimensional space, thus avoiding the lack of information occurring when using traditional morphometrics (Marcus 1990) or a bi-dimensional geometric morphometrics approach (Rohlf and Marcus 1993). This approach is relatively new and only a few published articles have dealt with the three-dimensional geometric morphometrics of small mammals (Roth 1993; Corti et al. 1996; Reig 1996; Fadda et al. 1997; Fadda 1998; Corti et al. 2000; Fadda and Corti 2000; Hutchinson 2000).

Materials and methods

We analysed 659 specimens and 71 populations from the following areas: Benin (one population), Burkina Faso (two), Centrafrican Republic (two), Chad (two), Egypt (three), Ethiopia (15), Gambia (two), Ghana (one), Kenya (10), Mali (two), Niger (two), Senegal (four), Sierra Leone (one), Somalia (four), Sudan (six), Tanzania (five), Uganda (seven), Yemen (one) and Zaire (one) (Fig. 1; Appendix 1). Specimens representing 13 types from the British Museum of Natural

History were also included (Table 2). Each specimen was assigned to one of the five age classes detected according to molar tooth wear consumption (Delany 1971; Fadda and Corti 1998). All the specimens come from the collection of the following museums: British Museum of Natural History (London), Muséum National d'Histoire Naturelle (Paris) and Museo di Anatomia Comparata (University of Rome 'La Sapienza').

For each population the following geoclimatological data were collected: latitude, longitude, altitude, mean temperature, mean temperature of the coldest month, mean temperature of the hottest month, average rainfall, total radiation and potential evapotranspiration (from Agrimatological data, Africa; FAO 1984).

Landmarks and Procrustes analysis

Twenty-nine three-dimensional landmarks (L; Fig. 2) were collected on the skull of the 659 specimens using the following procedure.

Images were digitized using a Canon E700 (Canon Inc., Tokyo, Japan) camera and an OFG digitizing board (VP 1100-512-U-AT; Imaging Technology Inc., Woburn, MA, USA), with a resolution of 512 by 480 bit. Five images were recorded for each individual by rotating the skull at different angles, i.e. 0° (ventral side), 45°, 90° (lateral side), 120° and 180° (dorsal side), using the device described in Fadda et al. (1997). Sixty-five two-dimensional, x, y landmarks were then collected on these images (19, 11, 10, 15, 10, respectively) using the software TPSDIGW (Rohlf 1997). The five arrays of landmarks were translated to a common origin (L1), with landmarks 1, 2, 5, 7, 8, 16, 17, 18 and 19 (sagittal section) defining the x, y plane. Each array was then rotated around the x -axis (landmarks 1 and 5) for the number of degrees at which each image was recorded. The values of the 29 x, y, z three-dimensional landmarks was then obtained following the algorithm described in Fadda et al. (1997).

Error source was evaluated as digitizing error and device error. For this purpose, a subset of nine skulls (the original sample size is far too large) was recorded and digitized three times. The subset was chosen from specimens of the same population, age class and sex in order to be as homogeneous as possible. Landmarks were then collected at 1-week interval (1) from each set of digitized images and (2) from one set only. The first set includes both error sources, whereas the second includes the digitizing error only.

The percentage of incongruence (Hildebolt and Vannier 1988) was computed for both sets of repetitions. The precision of the device (the closeness of repeated measurements of the same quantity, Sokal and Rohlf 1981) was also evaluated. Results are explained in detail elsewhere (Fadda et al. 1997) and showed that the error is affected most by digitizing and that the device has a good precision. Nonetheless, digitizing error was not enough to perturb between-specimens assessment of shape and size differences. This made us confident to proceed with the analysis of data collected.

As the skull is symmetrical, the landmark co-ordinates of the two sides were averaged to avoid any asymmetry and duplication of information. The values of the y co-ordinate of the landmarks situated on the sagittal plane were then set to 0. Therefore, data analyses were performed on one side of the skull only, but visualized using the entire reconstructed skull.

The form variation was studied after decomposition into its shape and size components (see Rohlf and Marcus 1993), which were then analysed separately. The centroid size (the square root of the sum of the squared distances between each landmark and the centroid; Bookstein 1991) was considered as being representative of the size of each specimen.

Three-dimensional shape analysis was performed after Procrustes superimposition, computed through a generalized orthogonal least

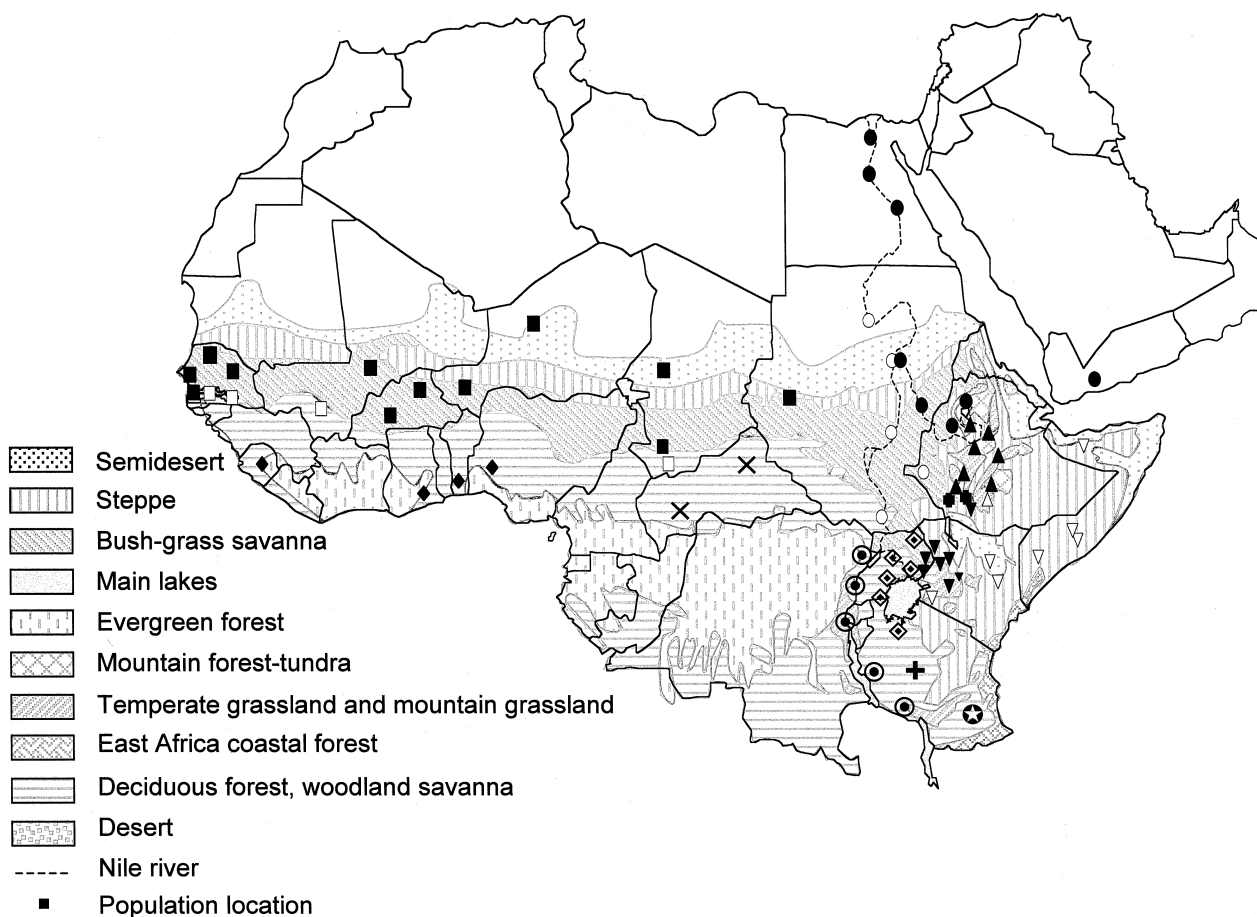


Fig. 1. Map showing the distribution of species and taxonomic units and the different biogeographical domains (from FAO 1984). Symbols of species are as follows: ■, ANI-1; ×, ANI-2; □, ANI-3; ■■, ANI-4; ●, *A. niloticus*; ○, *A. testicularis*; ▲, *A. abyssinicus*; △, *A. blicki*; ▽, *A. somalicus*; ■■, *A. sp.*; ■■, *A. sp1*; ▼, *A. sp2*; +, *A. sp3*; ■■, *A. sp4*

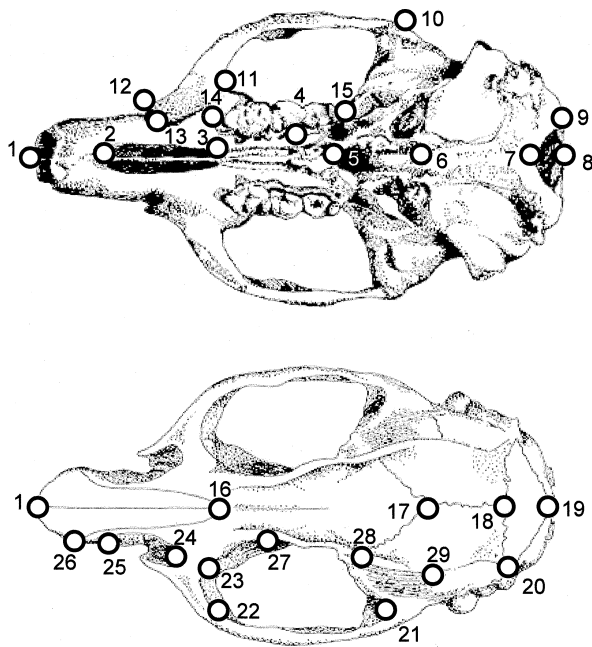


Fig. 2. The landmark locations in the skull of *Arvicanthis* (shown for the ventral and dorsal sides of the skull)

square procedure (GLS, Rohlf and Slice 1990). The reference or consensus configuration used for the fitting was the average of all specimens and defines the point of tangency of the tangent linear space to the shape spaces (Rohlf 1996).

All the specimens were aligned to the reference configuration and scaled using the shrunken ($1/\cos \rho$) option, i.e. the reciprocal of the cosine of the Procrustes distances (ρ) shrunk until the centroid size is equal to $\cos \rho$ (Dryden and Mardia 1998). Both Euclidean and Procrustes distances between each pair of individuals were calculated and a least-square regression and a correlation was performed to measure their deviation from a linear relationship (Rohlf 1998). This allows an evaluation of the approximation of tangent space to shape space.

As numerical analyses of three-dimensional landmarks imply a high number of variables (in this case the number of landmarks multiplied by 3, i.e. 87), a principal component (PC) analysis was performed to reduce the dimensionality. As a criterion for the number of PCs to retain, we followed Fadda and Corti (2000). We tested the approximation of the reduced space to the original one through a Mantel test between Procrustes distances and Euclidean distances computed on the retained PCs, until the correlation was high enough. The scores over the retained principal components were then analysed through uni- and multivariate statistics to estimate the differences between sexes, age classes, populations and taxonomic units and used to show ordination of the latter two.

Taxonomic units

Taxonomic units for all analyses were defined as follows. The four cytotoxic units, identified in West Africa were named as ANI-1, ANI-2, ANI-3 and ANI-4 according to Volobouev et al. (1988), Granjon et al. (1992), Ducroz et al. (1997). The population of Mopti (Mali) was ascribed to ANI-1 (Ducroz, personal comm.). A discriminant function previously defined for each of the known taxonomic units was used to classify the populations of Lawra (Ghana), Gemenyalla and Kontaur (Gambia), Nyala (Sierra Leone), Mao and N'Djamena (Chad), for which no information was available.

Regarding the 52 East African populations, a first analysis was run on those including a sufficient number (> 13) of specimens. An UPGMA based on Procrustes distances was used to identify clusters, which were then defined as operational units. Cophenetic values were calculated to test whether the topology of the obtained dendrogram matched the

original distances. Thereafter, a discriminant function was computed for each group and used to assign the populations with $n < 13$ specimens to one of the defined groups.

Statistical procedures

Differences in centroid size were estimated through one- and two-way analysis of variance (ANOVA), considering three major potential sources of variation: age, sex and species. Differences were also shown through box-plots. Multiple regression was used to test whether size differences between species are related to the climate and the geographic origin of populations.

A model II ANOVA was performed on each single principal component to identify the source of variation in relation to sex, age classes and species. Moreover, whenever there were two significant factors affecting a principal component, its variance was partitioned into its age, sex and species component, to assess the relative importance of each.

Shape differences were shown graphically by projecting selected eigenvectors into the aligned co-ordinate system and plotting the individuals with the negative and positive coefficients as a deviation from the consensus.

A partial least square (PLS) analysis (Streissguth et al. 1993; Rohlf and Corti 2000) was carried out to test whether the shape differences are related to different climatic regimes. This technique allows us to analyse the relationships between two sets of variables (in our case shape, represented by principal components, and geoclimatic variables) without being constrained by the necessity of orthogonality within each set as in canonical correlation analysis. PLS constructs pairs of vectors, to be multiplied by the columns of the two original matrices, respectively, representing linear combinations of the variables within each set. These linear combinations are such that the vectors account for as much of the covariation between the two original sets of variables as possible. The new variables describe what patterns, if any, of covariation exist between the two sets of original variables (see Rohlf and Corti, 2000 for further details and Fadda and Corti, 2000 and Corti et al. 1996 for applications).

The program GRF-ND (Slice 1993) was used to compute the centroid size and for the visualization of shape differences.

The software TPS-SMALL (version 1.11; Rohlf 1998) was used to calculate the relationships between Procrustes and Euclidean distances and the Procrustes residuals. All statistical analysis was carried out using the SAS package (SAS 1993; version. 6.12) and NT-sys (Rohlf 1997; version 2.02).

Results

Populations and species

Unknown individuals were assigned to a taxonomic unit as shown in Table 1. All the specimens from Sierra Leone and Ghana were assigned to the species ANI-4. This extended the range of the species from Sierra Leone to Nigeria (Fig. 1). The type of *A. mordax* from Nigeria also fell within the ANI-4 species.

Two specimens from N'Djamena (Chad) were assigned to ANI-3, thus supporting the hypothesis that the range of the species might be larger than expected, extending up to Chad (Fig. 1; Ducroz et al. 1997, 1998).

The UPGMA based upon Procrustes distances led to the identification of five clusters of East African populations, which are indicated as *A. sp.*, *A. sp1*, *A. sp2*, *A. sp3* and *A. sp4* (see Appendix and Fig. 1). Cophenetic correlation with Procrustes distances was 0.80, which suggests an optimal fit.

The attribution for the specimens belonging to the other populations is shown in Table 1 and Appendix 1. It should be noted that in some cases specimen from a population were attributed to different taxonomic units, i.e. Kisumu and Mumias (Kenya), Mbarara (Uganda), and Mwanza (Tanzania) (Table 1).

The attribution of the types to a given taxonomic unit is reported in Table 2.

Size

One- and two-way ANOVAS revealed that there were no differences between the sexes, whereas age classes and species differed significantly.

Differences between species evaluated for adults only were significant (Mean square = 3.59, d.f. 13; $F = 50.07$; $p = 0.0001$). The box plot in Fig. 3 shows that four different groups could be identified on the basis of size. *Arvicanthis blicki* and ANI-4 were the largest. The second largest group was formed by ANI-1, ANI-2, ANI-3 and *A. niloticus*; the third by *A. testicularis*, *A. sp.*, *A. sp1*, *A. sp2*, *A. sp3* and *A. abyssinicus*. The smallest group included *A. somalicus* and *A. sp4* (Fig. 3). A GT-2 test indicated that differences between these groups were always significant.

A multiple regression analysis showed that there was a significant correlation between the ecogeographical parameters and size ($r = 0.47$; $p = 0.0001$). In particular, size was mostly correlated with latitude ($r = 0.52$), longitude ($r = -0.52$), altitude ($r = -0.39$) and the temperature of the coldest month ($r = 0.44$). Thus, size generally decreased following a latitudinal gradient from north to south and increased longitudinally from east to west.

Shape

The correlation between Procrustes (Kendall shape space) and Euclidean (tangent space) distances was nearly 1, suggesting an almost perfect approximation of shape space through the tangent space.

Sixteen principal components were retained from the original 87. These principal components (PCs) represented 78.08% of the total variance, and the Mantel correlation between distances calculated on these components and the Procrustes distances was 0.98. This 16-dimension space therefore represented the tangent space adequately.

The results of MANOVA indicated that there were overall significant differences between populations and age classes (Table 3), but not between the sexes; thus, all subsequent analyses were performed irrespective of sex.

All ANOVAS performed over each PC showed significant differences between taxonomic units.

The third PC mainly accounted for variation in age class (Table 4). The general pattern characterizing growth is shown in Fig. 4 for each species as growth trajectories and as associated shape changes from the consensus, i.e. the average

Table 1. Specimen classification according to discriminant functions. The number of specimens included in each group is shown in brackets

Population	Country	Taxonomic unit
Mao (7)	Chad	ANI-1
N'Djamena (7)	Chad	ANI-1 (5) ANI-3 (2)
Dessie (4)	Ethiopia	<i>A. abyssinicus</i>
Entoto (5)	Ethiopia	<i>A. abyssinicus</i>
Gambela (5)	Ethiopia	<i>A. testicularis</i>
Sidamo (5)	Ethiopia	<i>A. sp2</i>
Gemenyalla (2)	Gambia	ANI-1 (1) ANI-3 (1)
Kontaur (2)	Gambia	ANI-1
Lawra (6)	Ghana	ANI-4
Cherangani (3)	Kenya	<i>A. sp2</i>
Kisumu (4 + type)	Kenya	<i>A. sp.</i> (2) <i>A. sp2</i> (2 + type) <i>A. somalicus</i>
Loita (4)	Kenya	<i>A. sp.</i> (1) <i>A. sp2</i> (2)
Mumias (3)	Kenya	<i>A. sp2</i>
Naivasha (type)	Kenya	ANI-4
Panyam (1)	Nigeria	ANI-4
Nyala (4)	Sierra Leone	ANI-4
Eggi (4)	Somalia	<i>A. testicularis</i>
Hargaisa (2)	Somalia	<i>A. somalicus</i>
Mwanza (10)	Tanzania	<i>A. sp.</i> (4) <i>A. sp2</i> (6)
Tabora (type)	Tanzania	<i>A. sp1</i>
Mbarara (5)	Uganda	<i>A. sp.</i> (3) <i>A. sp2</i> (2)
N'Dala (1)	Uganda	<i>A. sp.</i>
Serere (3)	Uganda	<i>A. sp.</i>
Lake Kimara (3)	Zaire	<i>A. sp1</i>

(excluding *A. sp4* due to small sample size). The allometric relationship between the neurocranium and the rostrum is evident. The neurocranium is larger and the rostrum shorter in younger specimens (PC3 -), and the inverse occurs in adults (PC 3 +); this is a common pattern in mammals. Other changes in shape affect the zygomatic arches, which become shorter during growth.

PC 1 and PC 4 clearly highlight differences between species (Table 4; Fig. 5). Although PC 2 displayed significant differences between taxonomic groups, the pattern depicted is difficult to interpret. Moreover, most of the variance of this PC is attributed to the error factor and it will not be discussed. The error factor in this case is represented by everything that is not taken account of by other mentioned factors (i.e. age classes and populations). In this respect it is not literally an error, but it is rather the statistical meaning of the error. This is also

Type	Geographic origin	Synonym	Taxonomic unit
<i>A. mordax</i>	Panyam (Nigeria)	<i>A. niloticus</i>	ANI-4
<i>A. abyssinicus praeceps</i>	Naivasha (Kenya)	<i>A. nairobae</i>	<i>A. sp2</i>
<i>A. pelliceus</i>	Lake Tana (Ethiopia)	<i>A. niloticus</i>	<i>A. niloticus</i>
<i>A. reptans</i>	Chander Falls (Kenya)	<i>A. somalicus</i>	<i>A. somalicus</i>
<i>A. rumruti</i>	Laikipia (Kenya)	<i>A. somalicus</i>	<i>A. sp2</i> ,
<i>A. abyssinicus rubescens</i>	Masindi (Uganda)	<i>A. niloticus</i>	<i>A. sp.</i>
<i>A. nubilans</i>	Kisumu (Kenya)	<i>A. niloticus</i>	<i>A. sp2</i>
<i>A. luctuosus</i>	Kaka (Sudan)	<i>A. niloticus</i>	<i>A. testicularis</i>
<i>A. testicularis centralis</i>	Chak Chak (Sudan)	<i>A. niloticus</i>	<i>A. testicularis</i>
<i>A. testicularis naso</i>	Lahej (Yemen)	<i>A. niloticus</i>	<i>A. niloticus</i>
<i>A. abyssinicus tenebrosus</i>	Tabora (Tanzania)	<i>A. niloticus</i>	<i>A. sp1</i>
<i>A. abyssinicus saturatus</i>	Didessa River (Ethiopia)	<i>A. abyssinicus</i>	<i>A. abyssinicus</i>
<i>A. rumruti pallescens</i>	Loita Plains (Kenya)	<i>A. nairobae</i>	<i>A. sp2</i>
<i>A. somalicus</i>	Chander Falls (Kenya)		<i>A. somalicus</i>

Table 2. The correspondence of the types with the assigned taxonomic unit, their geographic origin, and synonymy following Musser & Carleton (1993)

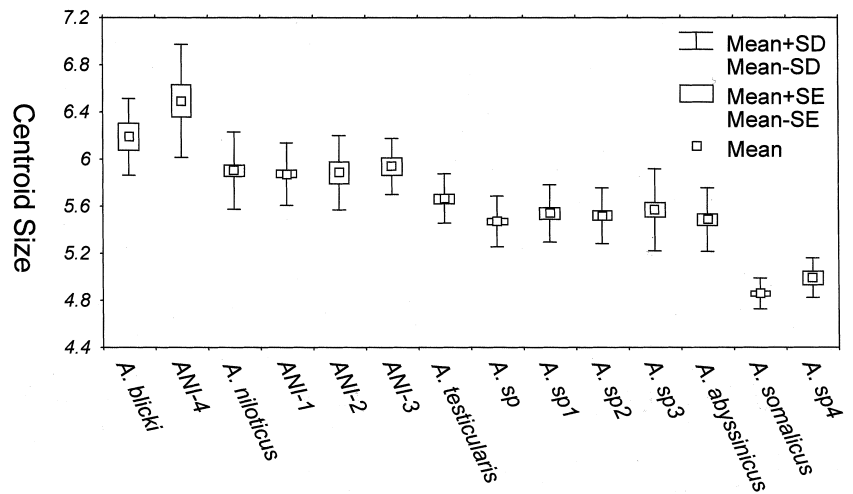


Fig. 3. Box plot of centroid size for all the taxonomic units, with the means, standard error and standard deviation

confirmed by the fact that differences between taxonomic units are significant, although the pattern shown is hardly meaningful. Therefore, this occurrence does not affect results. The percentages of variance explained by species, age classes and the error for the first eight PCs, including the second, are reported in Table 4.

The first PC (22.16% of the total variance) mainly shows the differences between East and West African species. *Arvicanthis blicki*, *A. abyssinicus* and *A. niloticus* had the highest scores, *A. testicularis*, *A. sp.*, *A. sp1*, *A. sp2*, ANI-4, and *A. somalicus* had intermediate scores, whereas ANI-1, ANI-2, ANI-3, *A. sp3*, and *A. sp4* had the lowest scores. The scatter plots and shape differences associated with these PCs are shown in Figs 5 and 6 (for taxonomic unit means only).

Species from East Africa (PC 1 +) were characterized by a shift forward and downward of the parietal and interparietal bones (Fig. 6). The processus squamosus of the zygomatic bar, in turn, has moved downward and modifications have also occurred in the frontal part of the orbital foramen and in the rostrum. All the West African species (PC -) show the opposite pattern of modifications.

The positive scores of the fourth PC mainly highlight the morphological features characterizing *A. blicki* (Figs 5 and 6). This species is characterized by extreme and unique shape changes, i.e. a contraction of the parietals and interparietals, a shortening and a downward twist of the rostrum, an enlargement of the orbit through a reduction of the inter-orbital constriction and a lateral shift of the zygomatic bar. Moreover, the foramen magnum has shifted forward. It must be remembered that this species is unique in the genus as it is confined to the Afro-Alpine habitats of the Ethiopian eastern plateau above 3000 m altitude.

Table 3. MANOVA. Significance tests for the difference between species (top) and age classes (bottom)

Test	F	df	p
Wilks'Λ	22.66	208	0.0001
	8.25	64	0.0001
Pillai's trace	16.17	208	0.0001
	7.03	64	0.0001
Hotelling-Lawley trace	30.49	208	0.0001
	9.69	64	0.0001
Roy's greatest root	187.10	16	0.0001
	32.45	16	0.0001

General morphometric relationships based on Procrustes distances are shown in Fig. 7 in the form of a UPGMA phenogram. Correlation between cophenetic values and the Procrustes distances was $r = 0.90$, suggesting a very good fit.

There are two main groups represented in the phenogram. One includes most of the East African species (excluding *A. sp3* and *A. sp4*) and ANI-4, and the other the West African ones. Interestingly, *A. blicki* has the highest Procrustes distances. In the former group, including the East African species, two clusters could be identified: one including *A. abyssinicus* and *A. niloticus*, and the other with *A. testicularis*, all of the species from Kenya, Uganda, ANI-4, and *A. neumanni*. In the West African group, there was a high similarity between ANI-1, ANI-3 and *A. sp3* from the Omo Valley (Ethiopia).

The PLS analysis indicated a strong relationship between shape and climatic and geoclimatic variables. The first pair of vectors, representing 64.64% of total covariation, were highly correlated ($r = 0.67$). In particular, there was a high correlation between shape and altitude ($r = -0.59$), mean annual temperature ($r = 0.65$) and temperature of the warmest month ($r = 0.69$).

The second pair of vectors suggest a latitudinal pattern (20.00% of covariance). The correlation between the geoclimatic and shape vectors was $r = 0.61$. The main correlation was with latitude ($r = 0.67$), and the temperature of the coldest month ($r = 0.63$).

The scatter plots of these first two pairs of vectors together with the taxonomic unit consensus are shown in Fig. 8. *Arvicanthis blicki* and *A. abyssinicus* have the extreme values,

Table 4. Principal component analysis on Procrustes residuals. Percentage of variance explained by the first eight PCs and the percentage of variance explained by species, age classes and the error for each PC

Principal components	Percentage of variance	Species (%)	Age classes (%)	Error (%)
PC 1	22.16	62.16	7.53	30.31
PC 2	13.94	17.96	0.00	82.04
PC 3	9.36	24.38	42.89	32.72
PC 4	7.22	49.91	11.02	39.07
PC 5	6.36	37.62	1.81	60.57
PC 6	5.78	16.41	15.92	67.65
PC 7	4.68	31.46	12.77	55.77
PC 8	4.00	25.30	14.98	59.72

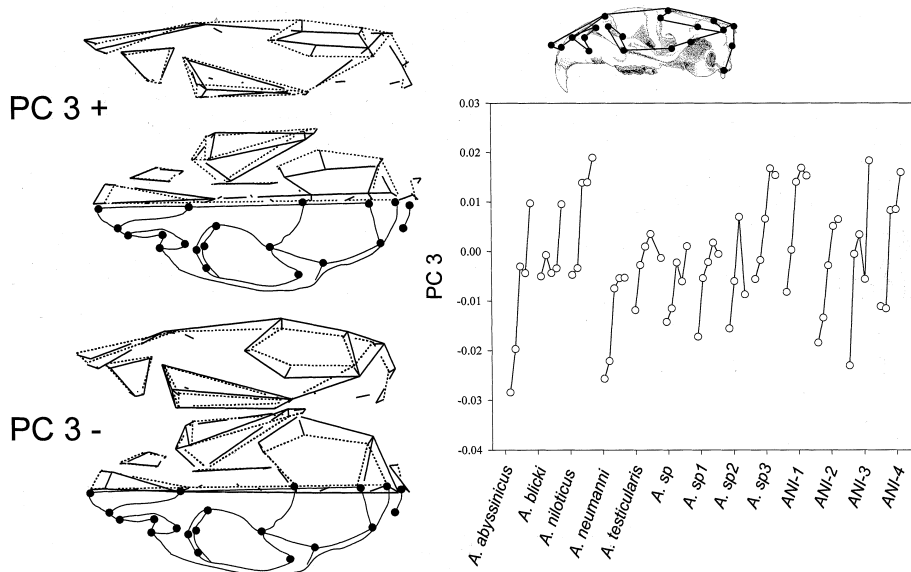


Fig. 4. Plot of the third PC showing the growth trajectory for each taxonomic unit. Shape differences associated with this vector are shown as deviations (filled line) from the reference (dotted line) for the juveniles (PC 3-) and adults (PC 3+). The reference form is represented as the average of all specimens

therefore suggesting that adaptation to altitude may have been an important cause in morphometric differentiation.

Discussion

Using geometric morphometrics most of the species in western, central and eastern Africa were identified. Nonetheless, there are still some taxonomic units that require further investigation in order to ascertain their specific status and to assess their phylogenetic relationships.

Although size does not allow a proper identification of a species, it was found that size increases according to altitude, latitude (from north to south), and longitude (from east to west). The positive and significant correlation with temperature in the coldest month suggests that *Arvicanthis* follows the Bergman rule, which states that species tend to become bigger in colder environments.

Morphometric similarities (Fig. 7) can be clustered into an East African and West African section, with few exceptions: the eastern *A. sp3* (Omo region) and *A. sp4* (central Tanzania), which are more similar to the West African species, and the western ANI-4, which is included in the eastern cluster.

Arvicanthis sp4 has the same small size as the Somali and Kenyan *A. neumanni* (the ‘small’ species according to Musser and Carleton 1993; Fig. 3), but a very different shape (Fig. 7). This can be interpreted in two ways: either *A. sp4* is a different species of small size included in the western cluster, or it is a geographic variant of *A. neumanni*.

There are two further clusters in East Africa: one including *A. niloticus* and *A. abyssinicus*, and the other *A. testicularis* and all the other taxonomic units. The maintenance of *A. testicularis* as a separate species has already been suggested by Fadda and Corti (1998). Moreover, it is impossible to establish whether some taxonomic units of this cluster (*A. sp.*, *A. sp1*, and *A. sp2*) are good species or morphological variants of *A. testicularis*. The distribution of *A. sp2* corresponds to that of the ‘small’ *A. nairobae*, following Musser and Carleton (1993), and that of *A. sp.* and *A. sp1* (from the western arch of the Rift Valley) corresponds to the ‘large’ *A. niloticus* (but see Fig. 3, where the sizes of these three taxa are seen to be as nearly identical). The ranges of *A. sp.* and *A. sp2* overlap within the Rift Valley, but the limited number of specimens does not allow any definitive taxonomic conclusion.

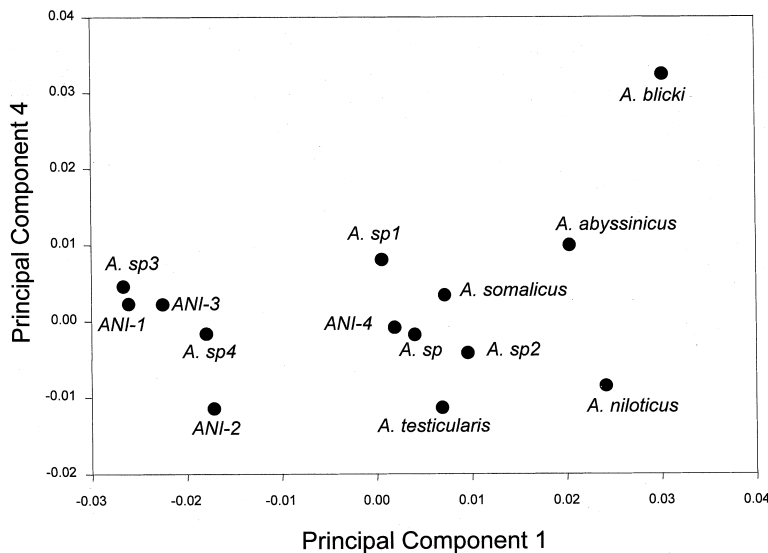


Fig. 5. Scatter plot of the first and fourth PCs showing the distribution of taxonomic units (means only)

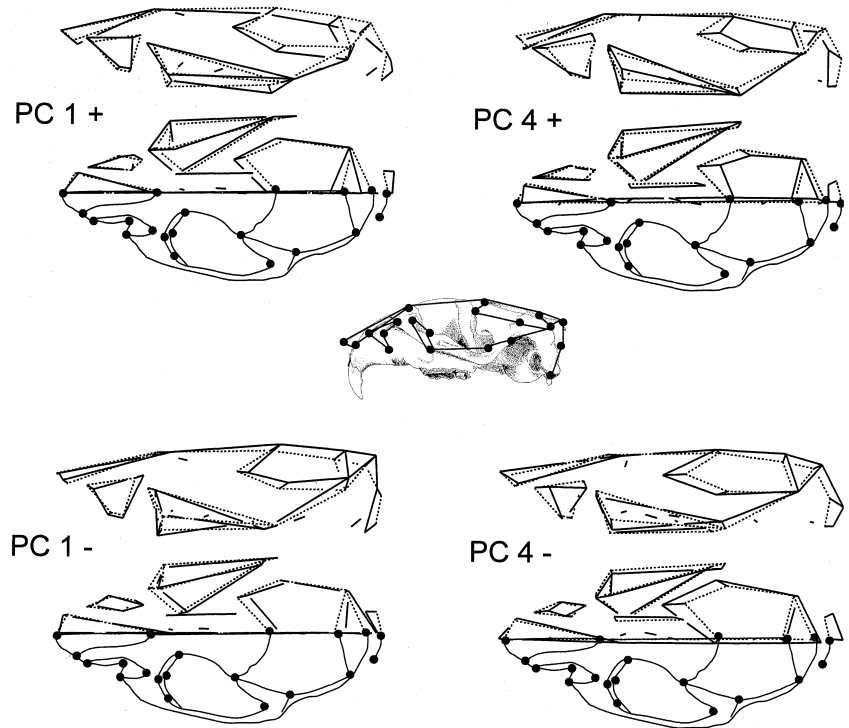


Fig. 6. Shape changes associated with the first and fourth principal components, for the dorsal and the lateral views of the skull. On the left are the shape changes for the first eigenvector; and on the right those for the fourth. The dotted lines represent the consensus. The plus and minus signs correspond to individuals with the highest and lowest scores for those vectors (see Fig. 5)

Phylogeography and environmental background

Because of the implications for taxonomy, size and shape patterns will be discussed in the light of the known phylogeny and of the different environments characterizing the range of the genus.

Different biogeographical domains characterize West Africa, which all overlap (Fig. 1): the Sahelian zone is characterized by grasses and shrub savannah, the Sudanian by shrub and woodland savannah and the Guineo-Congolese by moister habitats (including the tropical rain forest) (Stuart and Adams 1990).

The range of *Arvicanthis* reflects these biogeographical zones. In West Africa ANI-1 is prevalently distributed in the Sahelian, ANI-3 in the Sudanian zone, and ANI-2 and ANI-4 in a moister habitat (Ducroz et al. 1997; Ducroz 1998). In East Africa the emergence of the Rift Valley determined a dramatic alteration of the landscape and climate and a fragmentation of the open habitats (Downie and Wilkinson 1972; Werger 1978; Crossley 1979), which was typically preferred by *Arvicanthis*

(Kingdon 1974). This has favoured speciation and adaptation to very different habitats, from lowland savannah to afro-alpine moorland (e.g. *A. deembensis*, *A. abyssinicus* and *A. blicki*; Corti et al. 1996; Capanna et al. 1996).

Partial least squares analysis shows that morphometric variation reflects the different environments occurring over the range (Fig. 8). However, the geographic and climatic background is different in East and West Africa. Therefore, PLS analysis was repeated separately for the two regions. In West Africa, shape is mainly correlated with latitude ($r = 0.88$), rainfall ($r = -0.85$) and mean temperature ($r = 0.83$) (the first pair of vectors has a total correlation coefficient of $r = 0.90$). In East Africa, the main factors explaining morphological variation are altitude (first pair of vectors representing 63.4% of the total covariance; correlation coefficient $r = 0.68$) and latitude (second pair of vectors; 21.2% of the total covariance; correlation coefficient $r = 0.66$).

There is also a partial agreement with the phylogeny patterns from molecular systematics (Ducroz et al. 1998),

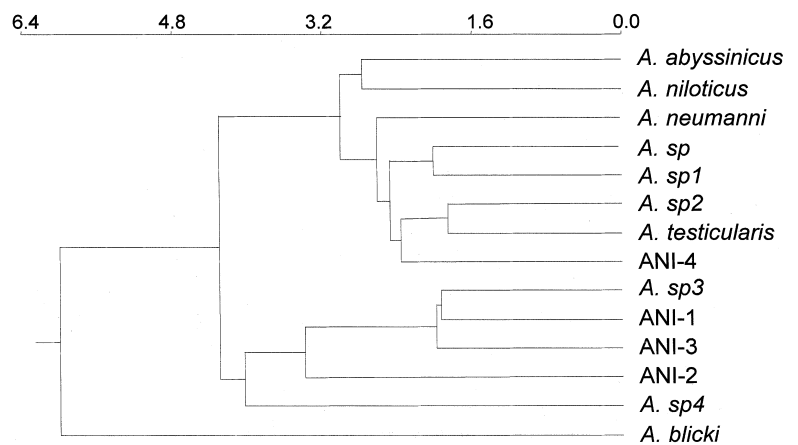


Fig. 7. UPGMA phenogram based on Procrustes distances between the taxa

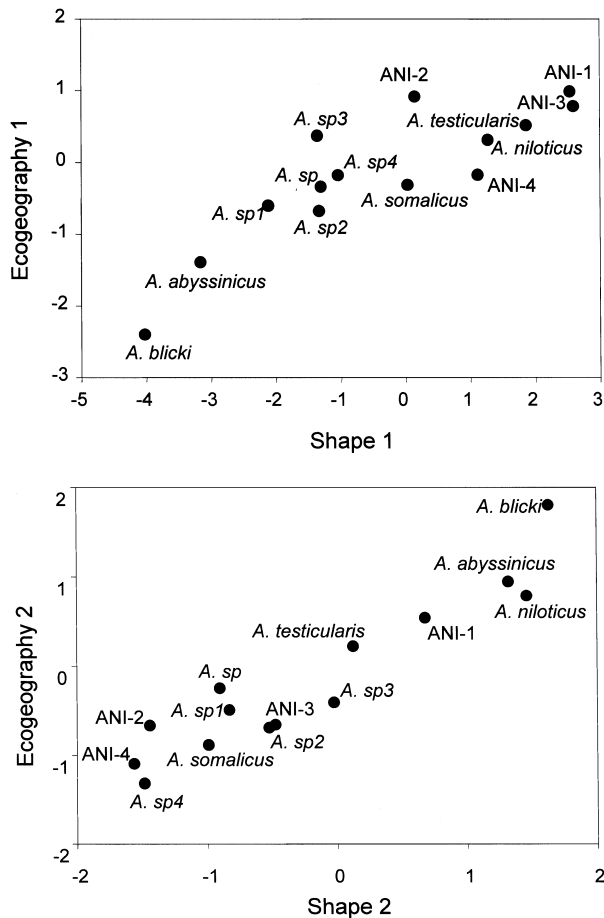


Fig. 8. Scatter plot of Partial Least Square of the first (top) and second (bottom) pairs of shape and ecogeographic vectors

multilocus protein electrophoresis (Capula et al. 1997), and cytogenetics (Civitelli et al. 1995; Corti et al. 1996; Ducroz et al. 1997; Volobouev et al. 1987, 1988). According to these phylogenetic hypotheses, there are two main clades, one including the East Africa species and the western ANI-1 and the other, the western, central and East African populations from Uganda and Tanzania (see the introduction for details). By contrast, population similarities based on Procrustes distances show a clear distinction between West African and East African populations (Fig. 7).

These contradictions can be explained by repeated, convergent evolution of similar morphology. The close relationships with the geo-climatic variables strongly suggest that morphometric variation in *Arvicanthis* can be more easily explained by an adaptation to local climatic conditions. However, changes in shape are still rather small, as usually found within genera (but see also Corti et al. 1996 for *Spalax*, and Fadda and Corti 2000 for *Myomys* – *Stenocephalemys*). For a functional interpretation, e.g. the rostrum or the zygomatic bar (implying a modification in the attachment of masseter muscles), an *ad hoc* experiment should be planned. At present, nothing is known on possible differences in the diet and feeding between species, as well as for other traits such as behaviour (Corti and Rohlf 2001), which could allow a direct interpretation of shape changes as functional adaptations. Therefore, even though the hypothesis of convergent evolution is plausible, the adaptive

significance of the shape changes described in the skull of these rats remains unclear.

Nonetheless, one of the possible causes may be the epigenetic factors occurring during post-natal growth. There is strong evidence suggesting that different rainfall across the years can play a remarkable role in affecting the size and shape of the skull during post-natal ontogeny in *Mastomys natalensis* (Leirs et al. 1993), even within the same population. This kind of effect might be present in *Arvicanthis* with different modes of shape changes during growth in different areas of the range. The distribution of age classes along growth trajectories (Fig. 4) is not homogeneous across species, i.e. those occurring in areas characterized by higher rainfall continue to change their shape during growth whereas in arid climates the shape of the skull does not change significantly from age 3 years onward.

Phenotypic evolution of the *Arvicanthis* skull as well as for other rodents is the product of different driving forces among which history (phylogeny) may not be the predominant factor. This has also been shown in other murid genera such as the *Myomys/Stenocephalemys* complex (Fadda and Corti 2000) and the *Spalax ehrenbergi* superspecies in the Middle East (Corti et al. 1996). This obviously results in problematic taxonomic definitions based upon linear measurements or landmarks typical of morphometrics. These should be used carefully and not without comparison with other independently derived characters such as DNA and the karyotype.

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Zusammenfassung

Dreidimensionale geometrische Morphometrie von Arvicanthis: Anwendung in Systematik und Taxonomie

Die Kusu-Grasratte *Arvicanthis*, ist eine Muride, die in Afrika südlich der Sahara, in Ägypten und im Sudan vorkommt. Obgleich in den letzten zehn Jahren mehrere Untersuchungen über ihre systematische Gliederung durchgeführt wurden, erscheint eine allgemeine Revision dieser Gattung weiterhin notwendig. In dieser Studie haben wir die morphometrischen Beziehungen zwischen 71 Populationen aus dem gesamten Verbreitungsgebiet untersucht. Ein 3-D geometrisches morphometrisches Verfahren wurde verwendet, um die Unterschiede in der Größe und der Form des Schädels zu erfassen. Diese wurden dann mit verschiedenen biogeographischen Domänen, die für das Verbreitungsgebiet der Gattung charakteristisch sind, und den molekularen und karyologischen Phylogenien in Beziehung gesetzt. Die Ergebnisse stimmen nur zum Teil mit der Phylogenien überein, zeigen aber einen engen Zusammenhang mit dem Hintergrund der Umweltbedingungen der einzelnen Arten. Es muß daher angenommen werden, daß bei *Arvicanthis* die Anpassung an die lokale Umwelt in der phänotypischen Evolution des Schädels eine große Rolle gespielt hat. Dies führt zum Problem einer taxonomischen Bestimmung, die nur auf Morphometrie beruht; eine solche sollte nicht ohne Vergleich mit anderen unabhängigen Merkmalen wie DNA-Sequenzen oder karyologischen Analysen angewandt werden.

References

- Afework Bekele; Capanna, E.; Corti, M.; Marcus, L. F.; Schlitter, D. A., 1993: Systematics and geographic variation of Ethiopian *Arvicanthis* (Rodentia, Muridae). *J. Zool. Lond.* **230**, 117–134.

- Bookstein, F. L.; 1991: Morphometric Tool for Landmark Data. Cambridge, UK: Cambridge University Press.
- Capanna, E.; Afework Bekele; Capula, M.; Castiglia, R.; Civitelli, M. V.; Codja, J.-Cl.; Corti, M.; Fadda, C., 1996: A multidisciplinary approach to the systematics of the genus *Arvicanthis*, Lesson 1842 (Rodentia, Muridae). *Mammalia* **60**, 677–696.
- Capula, M.; Civitelli, M. V.; Corti, M.; Afework Bekele; Capanna, E., 1997: Genetic divergence in the genus *Arvicanthis* (Rodentia, Murinae). *Biochem. Syst. Ecol.* **25**, 403–409.
- Civitelli, M. V.; Castiglia, R.; Codja, J.-Cl.; Capanna, E., 1995: Cytogenetics of the genus *Arvicanthis* (Rodentia, Murinae). 1. *Arvicanthis niloticus* from Republic of Benin (West Africa). *Z. Säugetierkunde* **60**, 215–225.
- Corti, M.; Fadda, C., 1996: Systematics of *Arvicanthis* (Rodentia, Muridae) from the Horn of Africa. a geometric morphometrics evaluation. *It. J. Zool.* **63**, 185–192.
- Corti, M.; Rohlf, F. J., 2001: Chromosomal speciation and phenotypic evolution in the house mouse. *Biol. J. Linnean. Soc.* **73**, in press.
- Corti, M.; Civitelli, M. V.; Castiglia, R.; Afework Bekele; Capanna, E., 1995: Cytogenetics of the genus *Arvicanthis* (Rodentia, Muridae). 2. The chromosomes of three species from Ethiopia. *A. abyssinicus*, *A. dembeensis* and *A. blicki*. *Z. Säugetierkunde* **61**, 339–351.
- Corti, M.; Di Giulio Maria, C.; Verheyen, W., 2000: Three-dimensional geometric morphometrics of the African rodents of the genus *Lophuromys*. *Hystrix-It. J. Mamm.* **11**, 145–154.
- Corti, M.; Fadda, C.; Simson, S.; Nevo, E., 1996: Size and shape variation in the mandible of the fossorial rodent *Spalax ehrenberehi*. A procrustes analysis of three dimensions. In: Marcus, L. F.; Corti, M.; Loy, A.; Naylor, G.; Slice, D. (eds), *Advances in Morphometrics*, NATO ASI Series. New York, USA: Plenum Press Co, pp. 303–320.
- Crossley, R., 1979: The Cenozoic stratigraphy and structure of the western part of the Rift Valley in Southern Kenya. *J. Geol. Soc.* **136**, 393–406.
- Delany, M. J., 1971: The biology of small rodents in Mayanja Forest, Uganda. *J. Zool. London* **165**, 85–129.
- Downie, C.; Wilkinson, P., 1972: The Geology of Kilimanjaro. Sheffield, UK: Geological Survey of Tanzania and Department of Geology Sheffield University.
- Dryden, I. L.; Mardia, K. V., 1998: Multivariate shape analysis. *Sankhya* **55**, 460–480.
- Ducroz, J. F.; 1998: Contribution des approches cytogénétique et moléculaire à l'étude systématique et évolutive de genres de rongeurs Murinae de la 'division' *Arvicanthis*. Ph.D. Dissertation. Paris, France: Muséum National d'Histoire Naturelle.
- Ducroz, J. F.; Granjon, L.; Chevret, P.; Duplantier, J. M.; Lombard, M.; Volobouev, V., 1997: Characterization of two distinct species of *Arvicanthis* (Rodentia, Muridae) in West Africa. cytogenetic, molecular and reproductive evidence. *J. Zool., Lond.* **241**, 709–723.
- Ducroz, J.-F.; Volobouev, V.; Granjon, L., 1998: A molecular perspective on the systematics and evolution of the genus *Arvicanthis* (Rodentia, Muridae). Inferences from complete cytochrome *b* gene sequences. *Mol. Phyl. Evol.* **10**, 104–117.
- Fadda, C., 1998: Sistematica e variazione geografica in roditori Africani. Morfometria geometrica e filogenesi molecolare. Ph.D. Dissertation. Rome, Italy: Università di Roma 'La Sapienza'.
- Fadda, C.; Corti, M., 1998: Geographic variation of *Arvicanthis* (Rodentia, Muridae) in the Nile Valley. *Z. Säugetierkunde* **63**, 104–113.
- Fadda, C.; Corti, M., 2000: Three dimensional geometric morphometric study of the Ethiopian *Myomys* – *Stenocephalemys* complex (Murinae, Rodentia). *Hystrix-It. J. Mamm.* **11**, 131–143.
- Fadda, C.; Faggiani, F.; Corti, M., 1997: A portable device for three dimensional landmark collection on skeletal elements. *Mammalia* **61**, 622–627.
- Fadda, C.; Corti, M.; Verheyen, E. 2001b: Molecular phylogeny of *Myomys/Stenocephalemys* complex and its relationships with closely related African genera (Murinae, Mammalia). *Biochem. Syst. Ecol.* **18**, 585–596.
- Fadda, C.; Castiglia, R.; Colangelo, P.; Corti, M.; Machang'u, R.; Makundi, R.; Scanzani, A.; Tesha, P.; Verheyen, W.; Capanna, E., 2001a: The rodent fauna of Tanzania 1: a cytotoxic report from the Maasai Steppe. *Rend. Fis. Acc. Lincei* in press.
- FAO, 1984: Agroclimatological Data for Africa, 2. Countries South of Equator. Rome, Italy.
- Granjon, L.; Duplantier, J. M.; Catalan, J.; Britton-Davidian, J., 1992: Karyotypic data on rodents from Senegal. *Isr. J. Zool.* **38**, 106–111.
- Hildebolt, C. F.; Vannier, M. W., 1988: Three-dimensional measurement accuracy of skull surface landmarks. *Am. J. Ph Anthropol.* **76**, 497–503.
- Hutchinson, J. M. C., 2000: Three into two doesn't go. Two-dimensional models of bird eggs, snail shells and plant roots. *Biol. J. Linnean Soc.* **70**, 161–187.
- Kaminski, M.; Rousseau, M.; Petter, F., 1984: Electrophoretic studies on blood proteins of *Arvicanthis niloticus*. *Biochem. Syst. Ecol.* **12**, 215–224.
- Kaminski, M.; Sykiotis, M.; Duplantier, J. M.; Poulet, A., 1987: Electrophoretic variability of blood proteins among populations of two genera of African rodents. *Arvicanthis* and *Mastomys* from Senegal. Genetic polymorphism and geographic differences. *Biochem. Syst. Ecol.* **15**, 149–165.
- Kingdon, J., 1974: East African Mammals. an Atlas of Evolution in Africa, Vol. 2: Hares and Rodents. London, UK: Academic Press.
- Leirs, H.; Verheyen, W.; Verhagen, R.; Wendelen, W.; De Bruyn, L., 1993: Morphometric problems due to variable growth seasonality in *Mastomys* rats. 250. Fourth Cong. European Soc. Evol. Biol. **250**
- Marcus, L. F., 1990: Traditional morphometrics. In: Rohlf, F. J.; Bookstein, F. L. (eds), *Proceedings of the Michigan Morphometric Workshop*. University of Michigan Museum of Zoology, Special Publication 2. Ann Arbor, MI, USA, pp. 95–130.
- Matthey, R., 1965: Etude de Cytogénétique sur des murinae Africains appartenant aux genres *Arvicanthis*, *Praomys*, *Acomys* et *Mastomys* (Rodentia). *Mammalia* **29**, 228–249.
- Musser, G. G.; Carleton, M. D., 1993: Family Muridae. In: Wilson, D. E.; Reeder, D. H. (eds), *Mammal Species of the World. A Taxonomic and Geographic Reference*. Washington DC: Smithsonian Institution Press, pp. 501–755.
- Reig, S., 1996: Correspondence between interlandmark distances and caliper measurements. In: Marcus, L. F.; Corti, M.; Loy, A.; Naylor, G.; Slice, D. (eds), *Advances in Morphometrics*, NATO ASI Series. New York, USA: Plenum Press Co., pp. 371–385.
- Rohlf, F. J., 1996: Morphometric spaces, shape comparison component and the effect of linear transformations. In: Marcus, L. F.; Corti, M.; Loy, A.; Naylor, G.; Slice, D. (eds), *Advances in Morphometrics*, NATO ASI Series. New York, USA: Plenum Press Co., pp. 117–129.
- Rohlf, F. J., 1997: NTSYS-PC. Numerical Taxonomy and Multivariate Analysis System, Version 2.01. Setauket, NY, USA: Exeter Publications.
- Rohlf, F. J., 1998: TpsSmall (Version 1.11). Stony Brook, NY, USA: Ecology and Evolution.
- Rohlf, F. J.; Corti, M., 2000: The use of two-block partial least-squares to study covariation in shape. *Syst. Biol.* **49**, 740–753.
- Rohlf, F. J.; Marcus, L. F., 1993: A revolution in morphometrics. *Trends Ecol. Evol.* **8**, 129–132.
- Rohlf, F. J.; Slice, D., 1990: Extension of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* **39**, 40–59.
- Roth, V. L., 1993: On three-dimensional morphometrics, and on the identification of landmark points. In: Marcus, L. F.; Bello, E.; Garcia-Valdecasas, A. (eds), *Contributions to Morphometrics*. Madrid, Spain: M.N.C.N., pp. 41–61.
- SAS, 1993: SAS software, Version 6.12. Cary, NC, USA: SAS Institute.
- Slice, D., 1993: GRF-ND. Generalized Rotational Fitting of n-dimensional Landmark Data. <http://life.bio.sunysb.edu/morph/>.
- Sokal, R. R.; Rohlf, F. J., 1981: *Biometry*. 3rd edn. New York, USA: Freeman.
- Streissguth, A. P.; Bookstein, F. L.; Sampson, P. D.; Barr, H. M., 1993: The Enduring Effects of Prenatal Alcohol Exposure on Child Development. Ann. Arbor, MI, USA: University of Michigan Press.

Stuart, S. N.; Adams, R. J., 1990: Biodiversity in Sub-Saharan Africa and its Islands: Conservation, Management and Sustainable Use. Gland, Switzerland: IUCN.

Volobouev, V.; Viegas-Péquignot, E.; Petter, F.; Dutrillax, B., 1987: Karyotypic diversity and taxonomic problems in the genus *Arvicanthis*. *Genetica* **72**, 147–150.

Volobouev, V.; Viegas-Péquignot, E.; Lombard, M.; Petter, F.; Duplantier, J. M.; Dutrillaux, B., 1988: Chromosomal evidence for a polytypic structure of *Arvicanthis niloticus* (Rodentia, Muridae). *J. Zool. Syst. Evol. Res.* **26**, 276–285.

Werger, M. J. A., 1978: The Karoo-Namib region. In: Werger, M. J. A. (ed.), *Biogeography and Ecology of Southern Africa*. The Hague, The Netherlands: Junk, pp. 231–299.

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Appendix 1

Country and locality origin of the samples, with the latitude, longitude and altitude, and the names used for the taxonomic units.

Country	Locality	Latitude	Longitude	Altitude (m)	Taxonomic unit	
Burkina Faso	Ougadougou (2)	12°22' N	01°31' W	306	ANI-3	
	Oursi (8)	14°41' N	00°27' W	277	ANI-1	
Benin	Attogon (18)	06°30' N	02°00' E	9	ANI-4	
Gambia	Gemenyalla (2)	13°19' N	14°13' W	14	ANI-1* ANI-3*	
	Kontaur (2)	13°34' N	15°56' W	2	ANI-1*	
Ghana	Lawra (6)	5°00' N	3°30' W	322	ANI-4*	
Mali	Bamako (5)	12°40' N	7°59' W	332	ANI-3	
	Mopti (9)	14°29' N	4°10' W	272	ANI-1	
Niger	Air (11)	19°00' N	08°10' E	503	ANI-1	
	Niamey (9)	13°32' N	2°05' E	224	ANI-1	
Nigeria CAR	Panyam (1)	7°23' N	3°56' E	228	ANI-4*	
	Bangui (20)	4°23' N	18°37' E	510	ANI-2	
	Gordil (7)	10°00' N	22°30' E	336	ANI-2	
Senegal	Dakar (6)	14°38' N	17°27' W	24	ANI-1	
	Fété Orlé (30)	15°00' N	14°20' W	17	ANI-1	
	Kedougou (15)	12°33' N	12°11' W	167	ANI-3	
	Richard-Toll (16)	16°28' N	15°45' W	7	ANI-1	
Sierra Leone	Nyala (4)	7°32' N	12°30' W	3	ANI-4	
Chad	Mao (7)	14°06' N	15°17' E	356	ANI-1*	
	N'Djamena (7)	12°10' N	14°59' E	295	ANI-1* ANI-3*	
	Aswan (6)	24°02' N	32°53' E	200	<i>A. niloticus</i>	
Egypt	Asyut (7)	27°03' N	31°01' E	70	<i>A. niloticus</i>	
	Cairo (15)	30°03' N	31°13' E	19	<i>A. niloticus</i>	
	Alila (6)	7°50' N	39°32' E	2800	<i>A. abyssinicus</i>	
	Bahrdar (10)	11°36' N	37°24' E	1840	<i>A. niloticus</i>	
Ethiopia	Debre Markos (10)	10°20' N	37°50' E	2440	<i>A. abyssinicus</i>	
	Dangila (13)	11°18' N	36°56' E	2180	<i>A. abyssinicus</i>	
	Dessie (8)	11°10' N	39°40' E	2540	<i>A. abyssinicus</i>	
	Didessa (1)	7°40' N	36°50' E	1577	<i>A. abyssinicus</i>	
	Dinshu (17)	7°01' N	40°03' E	2700	<i>A. blicki</i>	
	Entoto (18)	9°38' N	39°38' E	2880	<i>A. abyssinicus</i>	
	Gambela (8)	8°15' N	34°35' E	480	<i>A. sp3</i> <i>A. testicularis</i>	
	Lake Shala (1)	7°15' N	38°40' E	2010	<i>A. testicularis</i>	
	Lake Tana (1)	12°32' N	37°26' E	2270	<i>A. niloticus</i>	
	Lake Zwai (1)	8°00' N	38°45' E	1640	<i>A. abyssinicus</i>	
	Lake Abaja (10)	6°20' N	37°50' E	1290	<i>A. sp3</i>	
	Omo (31)	7°44' N	37°14' E	1870	<i>A. sp3</i>	
	Sidamo (8)	6°25' N	38°18' E	1670	<i>A. sp2</i>	
	Lake Baringo (10)	0°30' N	36°02' E	100	<i>A. sp2</i>	
	Kenya	Chander Falls (7)	0°08' N	38°05' E	1104	<i>A. somalicus</i>
		Cherangani (4)	1°14' N	35°06' E	2134	<i>A. sp2</i>
Kisumu (7)		0°08' S	34°47' E	1146	<i>A. sp.</i> <i>A. sp2</i>	

Appendix 1**Continued**

Country	Locality	Latitude	Longitude	Altitude (m)	Taxonomic unit
	Laikipia (13)	0°16' S	36°33' E	1770	<i>A. sp2</i>
	Loita (5)	1°35' S	35°10' E	1200	<i>A. somalicus</i>
	Mumias (5)	0°20' N	34°29' E	2133	<i>A. sp2</i>
	Nairobi (8)	1°18' S	36°55' E	1624	<i>A. sp2</i>
	Naivasha (1)	0°43' S	36°26' E	1900	<i>A. sp2</i>
	Nyango (13)	2°18' N	37°54' E	1345	<i>A. somalicus</i>
Yemen	Lahej (9)	13°01' N	44°54' E	200	<i>A. niloticus</i>
	Afmedù (22)	0°31' N	42°05' E	29	<i>A. somalicus</i>
Somalia	Eggi (6)	3°15' N	45°32' E	158	<i>A. sp3</i>
	Giobar (1)	2°48' N	45°30' E	108	<i>A. somalicus</i>
	Hargaisa (6)	9°12' N	44°00' E	1326	<i>A. somalicus</i>
	Blue Nile (17)	11°47' N	34°23' E	470	<i>A. niloticus</i>
Sudan	Darfur (12)	13°37' N	25°20' E	730	<i>A. testicularis</i>
	Kaka (11)	11°45' N	32°47' N	382	<i>A. testicularis</i>
	Khartoum (18)	15°36' N	32°33' E	380	<i>A. testicularis</i>
	Merowe (3)	19°10' N	20°29' E	226	<i>A. testicularis</i>
	Yuba (11)	4°52' N	31°36' E	457	<i>A. testicularis</i>
	Dodoma (9)	6°10' S	35°40' E	1120	<i>A. sp4</i>
Tanzania	Kigoma (7)	4°55' S	29°48' E	885	<i>A. sp1</i>
	Mwanza (10)	2°31' S	32°56' E	1140	<i>A. sp.</i>
	Rukwa (8)	7°57' N	31°36' E	1710	<i>A. sp1</i>
	Tabora (2)	5°05' N	32°50' E	1190	<i>A. sp.</i>
	Kampala (23)	0°19' N	32°35' E	1140	<i>A. sp.</i>
Uganda	Kotido (13)	2°32' N	34°41' E	1347	<i>A. sp.</i>
	Masindi (1)	1°41' N	31°43' E	1147	<i>A. sp.</i>
	Mbarara (6)	0°37' S	30°39' E	1413	<i>A. sp.</i>
	Nambumali (19)	0°59' N	34°13' E	3411	<i>A. sp.</i>
	Q. Elisabeth N.P. (7)	1°15' S	29°59' E	1869	<i>A. sp1</i>
	Serere (5)	1°43' N	33°37' E	1123	<i>A. sp.</i>
Zaire	Lake Kimara (4)	1°41' S	29°14' E	1552	<i>A. sp1</i>