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Evolutionary history of the Karoo bush rat, *Myotomys unisulcatus* (Rodentia: Muridae): disconcordance between morphology and genetics

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Morphological characters have historically been used as the basis for mammalian taxonomic designations and, in a geographic context, subspecies descriptions. Geographic genetic structuring of a species, however, often reflects a contrasting classification for sampled populations. To investigate morphological and genetic congruence, geometric morphometrics and phylogeographic mitochondrial DNA sequence analyses of a South African plains-dwelling species, *Myotomys unisulcatus*, the Karoo bush rat, was performed across its range. A Bayesian population structure analysis identified two closely-related distinct genetic assemblages: the first contains populations from both the eastern, southern, and western parts of the species range (coastal lowland group), and the second comprises individuals from the Little Karoo (central interior group). Areas of sharp elevation (the Great Escarpment), coupled to vegetational differences, appeared to be the main factor limiting gene flow between these two groups. Geometric morphometric analyses on the ventral and dorsal views of the crania of *M. unisulcatus* failed to support the genetic groupings. Instead environmental factors in the respective biomes appeared to play a more important role in shaping the crania of both genders. The contrasting patterns obtained between morphology and genetics in *M. unisulcatus* is probably indicative of phenotypic plasticity throughout the range of the species, and it is hypothesized that regional environmental factors play a prominent role in explaining geographic morphological variation within the species. *Biological Journal of the Linnean Society*, 2011, **102**, 510–526.

ADDITIONAL KEYWORDS: cranium – cytochrome *b* – geometric morphometrics – mammal – mitochondrial DNA – phenotypic plasticity – phylogeography – South Africa.

INTRODUCTION

Discordance between phenotypic traits and genetic data sets may be driven by elevated molecular lineage diversification, phenotypic plasticity or rapid morphological divergence (Endler, 1980; Bromham *et al*., 2002; Wiens *et al*., 2006). Because of these differences, morphological similarities may not always reflect the common ancestry of taxa (D'Anatro & D'Elia, 2010). The taxonomy of southern African rodents (especially subspecies and ecotypes) are based mainly on morphological characteristics (De Graaff, 1981) and it is known that body size, for example, can be environmentally influenced (Cardini, Jansson & Elton, 2007). Individuals in dryer environments are under strong selective pressures to be larger to accumulate more resources (Armitage, 1999). In addition, cranial shape, in particular, can be influenced by a multitude of

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factors, including selective forces acting on the sensory organs (Rae *et al*., 2006), behavioural aspects (Byrne & Bates, 2007), and environmental factors (Cardini *et al*., 2007), as well as the genetic components of the species (Jamniczky & Hallgrimsson, 2009). Given this scenario, it is imperative to use data sets derived from multiple marker systems to understand intraspecific variation.

The Karoo bush rat, *Myotomys unisulcatus* (F. Cuvier, 1829), is a terrestrial plains-dwelling murid rodent species endemic to the semi-arid regions of South Africa. It has a distribution that spans the south, western, and interior regions of South Africa (Fig. 1A), and occupies a range that exhibits much variation in environmental conditions (Mucina & Rutherford, 2006). At the microhabitat level, they prefer regions with high plant cover and dense foliage and often occur near rocky outcrops (Skinner & Chimimba, 2005). Unlike some of the other species in the Otomyini group, *M. unisulcatus* does not require suitable habitat for burrowing because they construct extensive above-ground stick lodges, usually one nest per bush (Jackson *et al*., 2002). Throughout the range, geographic isolates of the species differ in nesting habits and habitats, and they show variation in body coloration, body size, and dietary intakes (De Graaff, 1981). Because of these differences, five subspecies were previously described within *M. unisulcatus* (De Graaff, 1981; Skinner & Chimimba, 2005), with the body dimensions and coloration ranging from larger, lighter coloured individuals in the western more arid regions, to smaller, darker coloured individuals in the eastern more mesic regions.

No clear differentiation was previously found between regional *M. unisulcatus* populations sampled using multivariate analyses of skulls, as well as karyotypic analyses (Van Dyk *et al*., 1991). Although Van Dyk *et al*. (1991) questioned the subspecies boundaries, it is possible that the techniques used were simply not sensitive enough to show recent genetic differentiation. A number of shallow and pronounced mitochondrial DNA (mtDNA) genetic provinces have recently been identified in various taxa in the southern African sub-region. Contemporary barriers to gene flow included, amongst others, vegetation and rainfall differences (Kryger, Robinson & Bloomer, 2004; Swart, Tolley & Matthee, 2009; Tolley *et al*., 2009) and plains separating mountainous outcrops (Matthee & Robinson, 1996; Smit, Robinson & Jansen van Vuuren, 2007; Swart *et al*., 2009). Most of the above-mentioned genetic signatures are linked to taxa occupying mountainous habitats, and the few studies conducted on plains-dwelling organisms showed little congruence with respect to genetic structure (Jansen van Vuuren & Robinson, 1997; Matthee & Robinson, 1997; Rambau, Robinson & Stanyon, 2003; Kryger *et al*., 2004; Russo, Chimimba & Bloomer, 2006).

The present study aimed to use more sensitive techniques to re-investigate the phylogeographic structure and evolutionary history of *M. unisulcatus* at both the morphological and genetic level. First, we investigated whether genetic variation from mtDNA is geographically structured in *M. unisulcatus*. Second, we investigated the cranial morphological variation present in the species. The two data sets were evaluated for congruency, and environmental factors (e.g. rainfall, temperature, altitude) that may influence skull shape in this species were also investigated.

MATERIAL AND METHODS

Sampling

A total of 49 *M. unisulcatus* individuals were sampled in the field and an additional 156 specimens were obtained from the Transvaal, Durban, South African, and Amathole Museums' collections. A total of 121 individuals from 17 localities were analyzed for morphometrics and 111 individuals from 15 localities were sequenced (Fig. 1A, Table 1).

Genetic methods

A DNeasy Kit (Qiagen) was used to extract DNA from museum skins and fresh muscle tissue. For the museum samples, a three-step wash procedure was performed (100% ethanol, 75% ethanol, and 100% distilled water) to remove surface contamination before extraction. Because of the degraded nature of museum samples, the amplification of a larger portion of a gene was problematic and Otomyini specific cytochrome *b* primers were designed (forward primer OtoF1-5′-ACAGCATTCTCATCAGTAAC-3′ and reverse primer OtoR1-5′-GCGTCTGAGTTTAGTCCT-3′), corresponding to the *Mus musculus* gene (position L14325 to H14788). These primers generated 463-bp fragments that were produced via the polymerase chain reaction. Sequences were obtained using methods *sensu* Smit *et al*. (2007) and they were aligned manually (by eye) in BIOEDIT Sequence Alignment Editor, version 7.0.5.2 (Hall, 1999).

To reveal relationships among maternal haplotypes, a median-joining network was constructed in NETWORK, version 4.5.1.6 (http://www.fluxus-engineering.com). The optimum number of potential posterior geographic groupings '*K*' was identified using stochastic optimizations in a bayesian analysis of population structure in BAPS, version 5.2. (Corander *et al*., 2008). A nonspatial mixture analysis of individuals was performed, the results of which

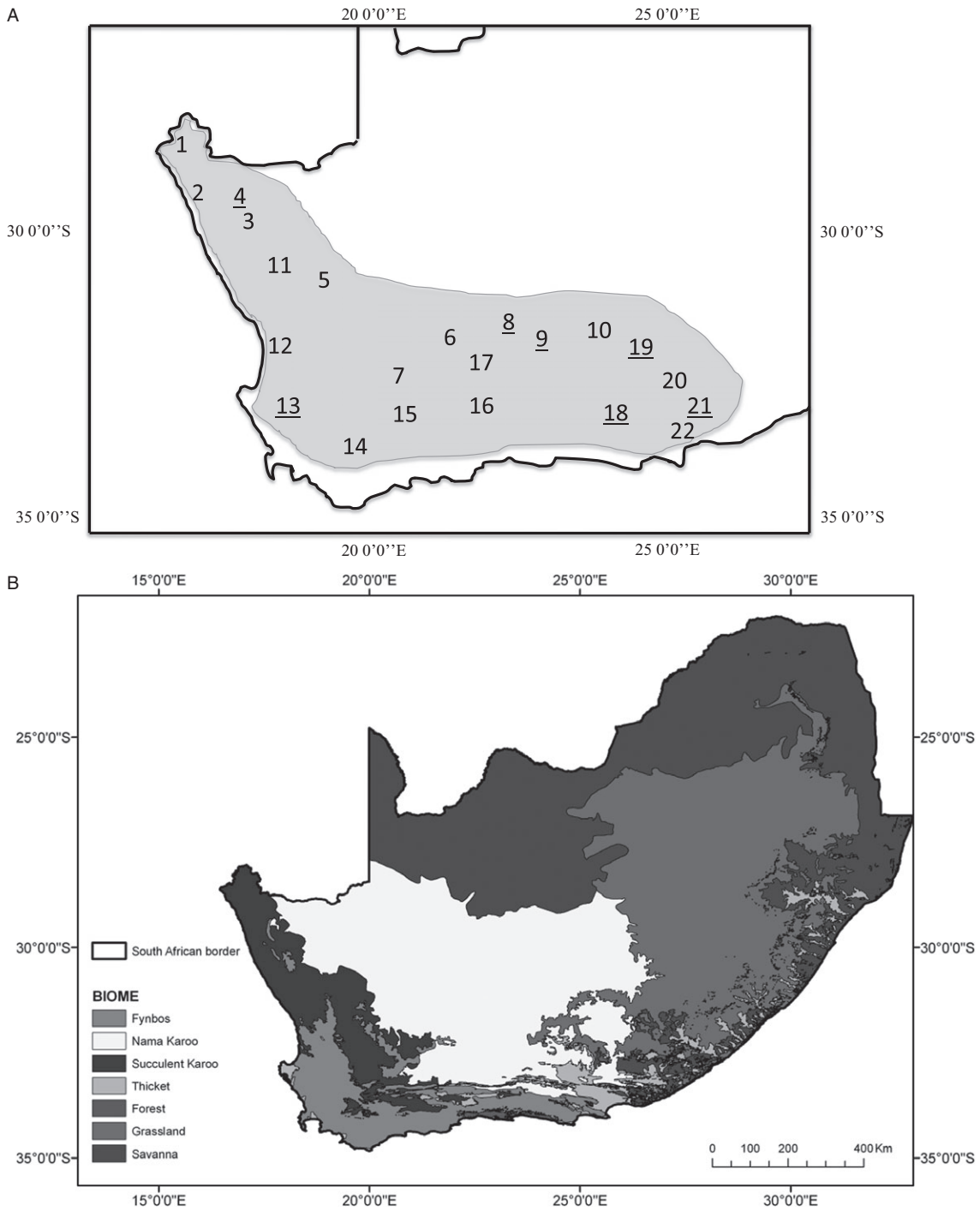

Figure 1. A, approximate distribution of *Myotomys unisulcatus* (Roberts, 1951; shaded area), as well as localities sampled (Table 1). Locality numbers that are underlined indicate those localities included in the morphological analyses only. B, approximate biome boundaries in South Africa (biomes adapted from Mucina & Rutherford, 2006). The map was obtained and adapted from the South African National Biodiversity (SANBI) vegetation map (http://www.SANBI.org.za).

were used in the admixture analysis (100 iterations, with five reference individuals iterated ten times).

Mean sequence diversity for the entire dataset and within groups was calculated using the uncorrected *p*-distance matrix in MEGA, version 4 (Tamura *et al*., 2007). ARLEQUIN, version 3.01 (Excoffier & Lischer, 2010) was used to calculate nucleotide and haplotypic diversity, and to perform an analysis of molecular

Table 1. Environmental variables of sampling localities in each province, Global Positioning System (GPS) coordinates (decimal degrees), biome and rainfall seasonality, and the number of individuals (*N*) sampled at each locality

Locality	Name	Province*	Biome†	Subspecies‡	GPS–South	GPS–East	Altitude (m)	Rainfall (mm/year)	Rainfall season§	Maximum temperature (°C)	Minimum temperature (°C)	Temperature stability	Mitochondrial DNA (*N*)	Morphology (*N*)
1	Richtersveld	NC	SK	BRO	–28.21	17.06	380	48.1	WRZ	22.62	12.08	10.54	9	4
2	Port Nolloth	NC	SK	BRO	–29.16	16.52	10	66.5	WRZ	19.08	10.89	8.19	6	8
3	Springbok	NC	SK	BRO	–29.41	18.01	950	181.1	WRZ	24.11	12.07	12.04	14	0
4	Steinkopf	NC	SK	BRO	–29.12	17.49	914	135.6	WRZ	24.11	12.07	12.04	0	3
5	Calvinia	NC	SK	–	–31.26	19.49	1049	212.9	WRZ	24.50	8.49	16.01	9	11
6	Fraserburg	NC	NK	–	–31.92	21.51	1006	203.7	SRZ	24.74	9.29	15.45	5	0
7	Sutherland	NC	SK	–	–32.34	20.40	1550	261.4	WRZ	20.36	3.32	17.04	13	0
8	Carnarvon	NC	NK	GRA	–30.21	21.49	1000	219.4	SRZ	23.87	8.27	15.60	0	7
9	Victoria West	NC	NK	GRA	–31.27	23.09	1219	266.7	SRZ	24.74	9.29	15.45	0	11
10	Richmond	NC	NK	GRA	–31.02	23.46	1359	333.5	SRZ	24.74	9.29	15.45	4	0
11	Van Rhynsdorp	WC	SK	BRO	–31.35	18.59	66	200.0	WRZ	26.23	11.21	15.02	8	4
12	Lamberts Bay	WC	SK	BER	–32.07	18.27	100	142.4	WRZ	22.35	11.25	10.53	11	9
13	Piquetburg	WC	FB	–	–32.36	18.18	12	320.1	WRZ	25.90	11.51	14.39	0	5
14	Cape Floristic Region	WC	FB	–	–33.46	20.05	365	289.3	WRZ	24.98	11.01	13.97	4	9
15	Laingsburg	WC	FB	UNI	–33.20	20.86	650	128.6	WRZ	25.54	11.24	14.30	6	4
16	Oudtshoorn	WC	NK	GRA	–33.58	22.20	332	238.6	WRZ	23.31	9.16	14.15	5	0
17	Beaufort West	WC	NK	GRA	–32.14	21.37	1040	371.1	SRZ	24.99	10.72	14.27	6	10
18	Steytlerville	EC	NK	GRA	–33.14	24.22	570	240.3	SRZ	25.13	10.14	14.99	0	5
19	Middelburg	EC	NK	GRA	–31.36	25.00	1248	274.7	SRZ	25.13	10.14	14.99	0	4
20	Cradock	EC	NK	ALB	–32.10	25.37	927	304.7	SRZ	25.13	10.14	14.99	4	6
21	Bedford	EC	FB	ALB	–32.41	26.06	800	595.9	SRZ	25.13	10.14	14.99	0	4
22	Albany	EC	TB	ALB	–33.11	26.45	120	503.1	YRZ	25.13	10.14	14.99	7	17

*NC, Northern Cape Province; WC, Western Cape Province; EC, Eastern Cape Province.
†FB, Fynbos Biome; NK, Nama Karoo Biome; SK, Succulent Karoo Biome; TB, Thicket Bushveld Biome (*sensu* Mucina & Rutherford, 2006).
‡ALB, *M. u. albiensis*; BER, *M. u. bergensis*; BRO, *M. u. broomi*; GRA, *M. u. grantii*; UNI, *M. u. unisulcatus*.
§SRZ, Summer rainfall zone; WRZ, Winter rainfall zone; YRZ, Year-round rainfall zone (*sensu* Chase & Meadows, 2007).

variance (AMOVA) on two alternative grouping scenarios: (1) using the groups obtained in BAPS and (2) using the previously described subspecies. To test for demographic changes in the population, we utilized Fu's F_S statistic (Fu, 1997) in ARLEQUIN.

Morphological methods

The 107 (55 females and 51 males) ventral and 119 (68 females and 51 males) dorsal views of *M. unisulcatus* adult crania (adult age classes 4 and 5) were photographed using a Panasonic DMC-LC40 digital camera. Age classes were determined using premolar wear *sensu* Taylor, Meester & Kearney (1993). The skulls were placed on a horizontal plane, in the middle of the focal area, with the camera 0.3 m above the cranium, and a spirit level was used to ensure that the lens and the specimen were parallel. Homologous landmarks were digitized using tpsDig (Rohlf, 1998) (Fig. 2), and imported into the R, version 2.11.1 (R Development Core Team, 2010) for all further analyses. Geometric morphometric procedures and codes were employed *sensu* Claude (2008). Landmarks were superimposed using partial generalized Procrustes analysis and the superimposed landmark data were then projected into the Kendal tangent space by orthogonal projection. The cranial sizes were retained in the centroid size, defined as the 'square root of the summed squared distances from each landmark to the configuration centroid (Bookstein, 1991).

To investigate the differences in the means of the centroid sizes between the sexes, an analysis of variance (ANOVA) was performed. A multivariate analysis of variance (MANOVA, using the Hotelling–Lawley test statistic) was performed between the principal components of the orthogonal projections of the two genders to test for sexual dimorphism.

The mean shapes of individuals from each locality were calculated, and these configurations were used in further analyses. ANOVAs and MANOVAs of the centroid sizes and the shape variables, respectively, were performed for females and males separately to investigate possible congruency with the genetic

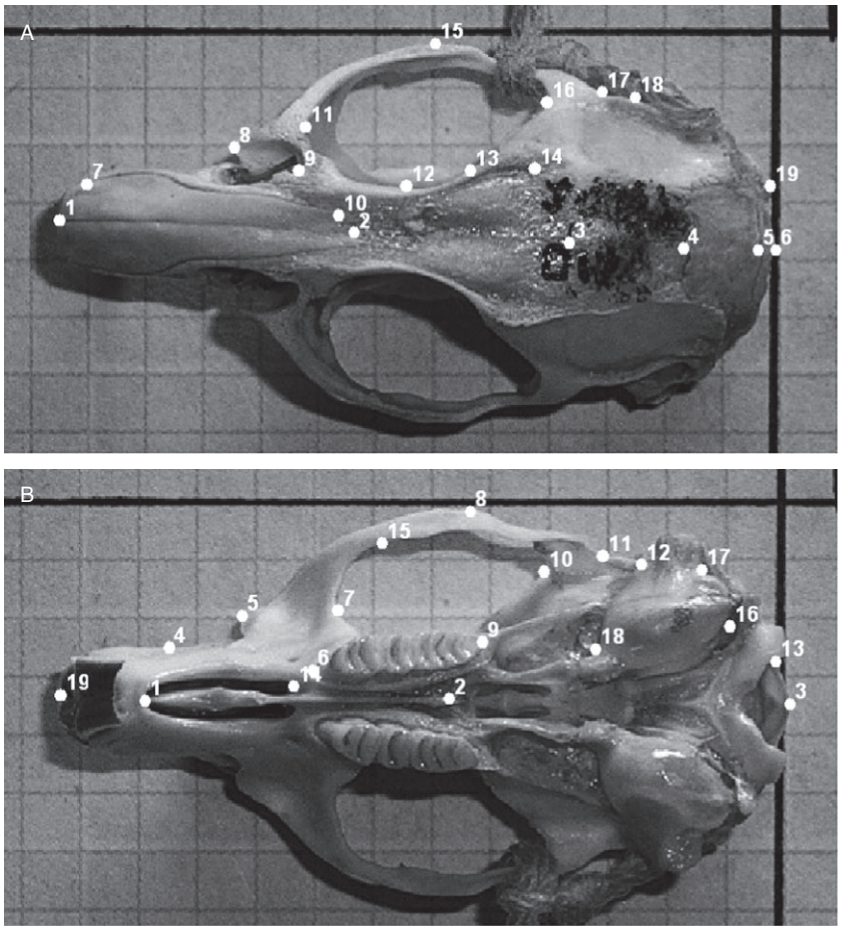

Figure 2. Homologous landmarks chosen for dorsal (A) and ventral (B) views of *Myotomys unisulcatus* crania.

assemblages. Principal components of the orthogonal projections of the mean shapes were obtained and a linear discriminant analysis was performed to test how well the predetermined genetic groupings (found in the BAPS analysis) could be used to classify individual morphometric cranial shapes. Only individuals from those localities used in the genetic analyses were included in the linear discriminant analysis.

ANOVAs (first and second principal components and centroid sizes) and MANOVAs (all principal components) were performed to investigate shape and size changes between the discrete environmental variables groupings (rainfall seasonality and biome boundaries). Pearson product moment correlations were used to investigate the correlation between the principal components and centroid size with continuous environmental variables [rainfall, altitudinal data, minimum temperature, maximum temperature and Global Positioning System (GPS) coordinates]. A scatterplot of the first two principal components were also constructed, as well as thin-plate splines to visualize shape change along the principal component axes.

Environmental variables

Environmental variables, such as mean rainfall per year (mm/annum), mean maximum and minimum annual temperature (°C), were obtained from the South African Weather Service (Table 1). Altitudinal data (a.s.l.) were obtained from the locality information linked to the museum specimens or from the field. Biome boundaries (Mucina & Rutherford, 2006) and rainfall seasonality data (Chase & Meadows, 2007) were classified as discrete variables.

Subspecies delimitations

The historical description of the subspecies boundaries was obtained from De Graaff (1981). Only those specimens that had been clearly identified to the subspecies level were included in the analyses (Table 1). As noted above, AMOVA was used for the molecular comparisons, whereas, in the morphological analyses, a MANOVA was performed in R, version 2.7.0 (R Development Core Team, 2010) to determine the validity of the subspecies boundaries using the cranial shape residual values in both sexes. ANOVAs were performed to determine the significance of the variances of the centroid sizes between the previously described subspecies.

RESULTS

Genetic analysis

Fifty-three unique haplotypes were identified for the 111 individuals analyzed and genetic variability was relatively high for the species (Table 2). Bayesian population structure analysis (BAPS) indicated that two geographic assemblages exist, with probabilities of individuals belonging to one of two groups $\geq 95\%$ (Fig. 3A). The first group comprise an assemblage referred to here as the central assemblage from Sutherland (population 7), Laingsburg (population 15), Oudtshoorn (population 16), and Beaufort West (population 17). The central assemblage is mainly situated inland in the Little Karoo region (Nama Karoo Biome), whereas the remaining closely-related haplotypes belonging to the second BAPS group are scattered mostly along the coastal plains (Succulent Karoo, Fynbos, and Thicket Bushveld biomes), as well as inland to Bushmanland and Namaqualand (Nama Karoo biome). The latter assemblage is predominantly but not exclusively represented by lower altitude populations and is referred to here as the main assemblage. When the posterior distribution of the two BAPS assemblages are overlaid on the haplotype network, the same two main assemblage are present and separated by one mutational step (Fig. 3B). The mean sequence divergence between the populations of the central assemblage was higher ($1.16\% \pm 0.30\%$) than that between populations within the main assemblage ($0.77\% \pm 0.18\%$; Table 2). Nucleotide diversity (π) and haplotype diversity within the central assemblage was slightly higher than in the main assemblage (Table 2). Significant deviations from neutrality were detected with Fu's test for both assemblages (main assemblage: $F_S = -23.09$, $P < 0.0001$; central assemblage: $F_S = -7.69$, $P < 0.01$).

Despite the differentiation found within the network and the BAPS analyses, the AMOVA

Table 2. Genetic variability within *Myotomys unisulcatus* and between the two genetic assemblages

	Sample Size (N)	Number of haplotypes	Nucleotide diversity ($\%\pi \pm$ SD)	Haplotype diversity ($h \pm$ SD)	Net sequence diversity ($\% \pm$ SD)
Main assemblage	78	33	0.77 ± 0.44	0.91 ± 1.00	0.77 ± 0.18
Central assemblage	33	20	1.16 ± 0.64	0.94 ± 1.00	1.16 ± 0.31
Combined	111	53	0.97 ± 0.54	0.82 ± 0.97	1.20 ± 0.24

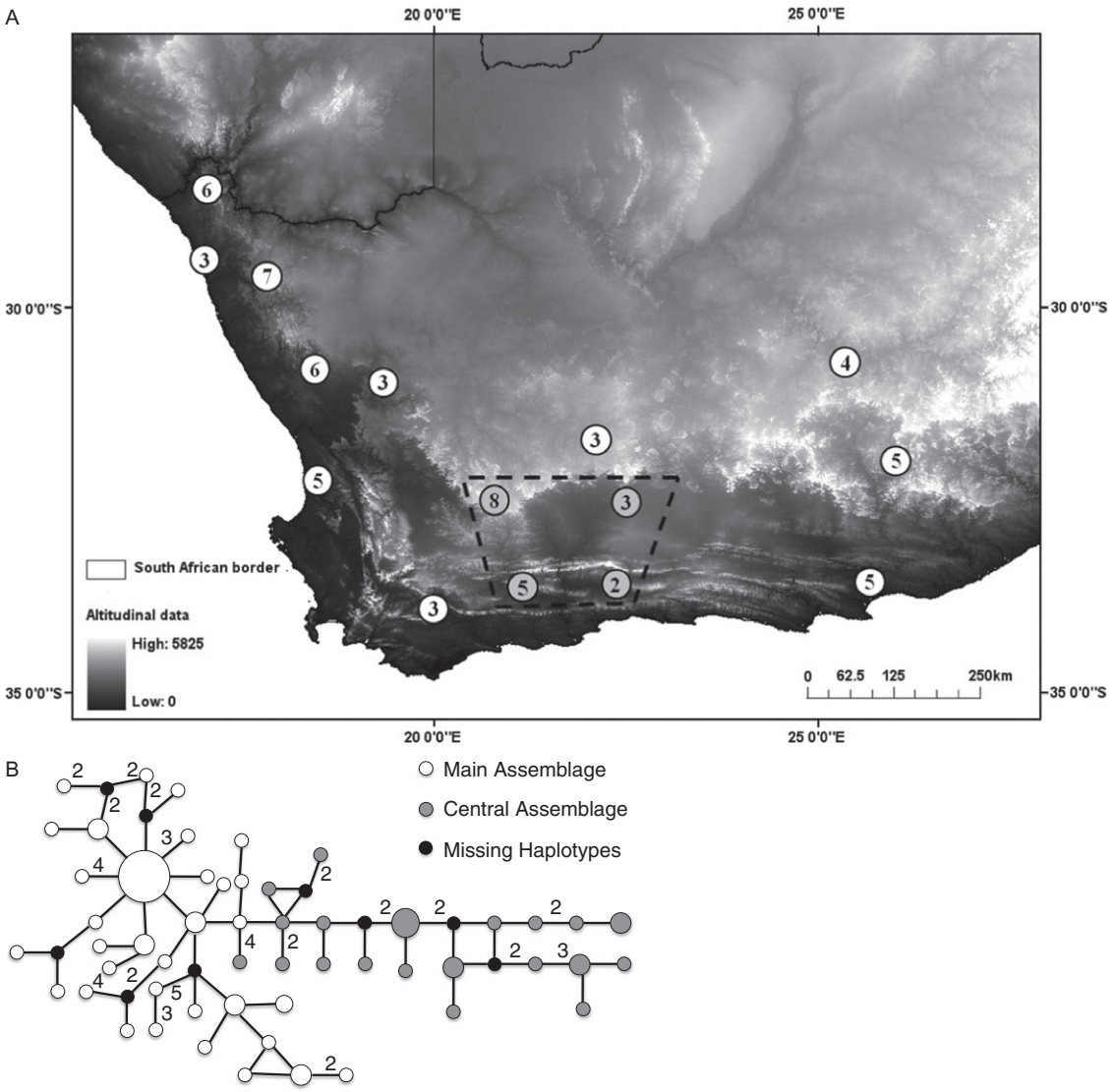

Figure 3. A, haplotype distributions overlaid on an elevation map of South Africa showing number of haplotypes present at each locality. For both figures, those haplotypes in (A) and localities in (B) that are shaded white belong to the main BAPS assemblage, whereas those shaded in grey belong to the central BAPS assemblage (maps obtained and adapted from the South African National Biodiversity (SANBI) vegetation map; http://www.SANBI.org.za). B, median joining network (scale reflects the number of specimens possessing a particular haplotype and the number of mutational steps among haplotypes are indicated when it is larger than one).

suggested that the major source of genetic variation was the within-populations variance component when the two BAPS genetic assemblages were used as the a priori (Table 3). When analysing the genetic structure between populations that fall into the varying rainfall zones and biomes, the between-group variances once again did not contribute the largest percentage to the overall variation (Table 3). However, significant pairwise Φ_{ST} values were found consistently between individual populations belonging to the two genetic assemblages, with values in the range 0.27–0.91 (average = 0.63 ± 0.15; Table 4). Generally, lower pairwise Φ_{ST} values were found within assemblages, with the main assemblage in the range 0.00–0.52 compared to the central assemblage, which possessed higher values in the range 0.53–0.81 (Table 4).

Morphological analysis

Sexual dimorphism

Although this species exhibits sexual dimorphism in general body size and mass, there was no significant

Table 3. Results of the analysis of molecular variance reflecting the distribution of genetic variation among the genetic assemblages obtained in BAPS, among subspecies, rainfall zones, and biome boundaries

	Percentage contributed to overall variation			Fixation indices		
	Between groups	Within populations	Among-populations/ within-groups	Φ_{SC}	Φ_{ST}	Φ_{CT}
Genetic assemblages	37.34	39.10	23.56	*0.38*	*0.61*	*0.37*
Subspecies	13.74	46.99	39.28	*0.46*	*0.53*	*0.14*
Rainfall zones	0.00	57.48	62.74	*0.52*	*0.47*	0.00
Biomes	10.96	48.95	40.10	*0.45*	*0.51*	0.11

Fixation indices, as well as the percentage of variation contributed by each variance component, is presented, with significant values ($P < 0.05$) shown in italics.

dimorphism in centroid size (ventral: $F = 1.20$, $P = 0.28$; dorsal view: $F = 2.38$, $P = 0.13$). There were significant differences in the shape variables between the sexes for both the ventral ($F = 10.23$, $P < 0.01$) and dorsal views ($F = 4.83$, $P < 0.01$), and males tended to have larger tympanic bullae, as suggested by the ventral view principal component analysis (landmark 16 was more posterior in females relative to the other posterior landmarks), as well as laterally flattened zygomatic arches (landmark 8 was more posterior) (Fig. 4).

Congruence with genetic assemblages

There were no significant differences in the variances of the centroid sizes between the two genetic assemblages (Tables 5, 6), and therefore no further analyses were performed on the centroid sizes in all datasets. There were also no significant differences in cranial shapes of females between the two genetic assemblages (Tables 5, 6). Even though there was a significant difference between the male dorsal cranial shapes, the linear discriminant analyses were not able to distinguish these, given the a priori genetic groups in either view, for either sex (Fig. 5).

Environmental determinants of cranial dimensions

The shape and size variables in any of the datasets were not significantly correlated with either altitudinal data or maximum temperature values, nor were they significantly correlated with north to south geographic coordinates (GPS-South) (Table 7). Minimum temperature may be playing a role in shaping male crania (the first and second principal components were significantly correlated with minimum temperature in the ventral and dorsal views, respectively).

Rainfall appears to be affecting cranial dimensions. The first principal component of the female ventral cranial shapes significantly correlate negatively with east-to-west GPS coordinates (Table 7). The second principal component and the centroid sizes are significantly negatively correlated with mean annual rainfall levels. These significant values indicate that females in the more westerly populations, which fall into the Winter Rainfall Zone (WRZ), exhibit larger tympanic bullae (more posterior position of landmark 16), in contrast to the smaller bullae of the more easterly populations within the Summer Rainfall Zone (SRZ) (Fig. 6). Male dorsal cranial shape exhibited a significant difference between the WRZ and the Year-Round Rainfall Zone (YRZ), which may be the reason for the significant difference between the genetic assemblages in this dataset. The dorsal view of females did not show any significant relationships with either the annual rainfall levels or rainfall seasonality; however, there may be differences between the cranial shapes of the easterly and westerly populations (i.e. the first principal component is significantly correlated with the east-to-west geographic coordinates) that do not show a significant relationship.

Subspecies verification

The recognition of subspecies at the genetic level within this species may not be warranted because BAPS only recovered two groups that were not congruent with the five previously described subspecies. This was corroborated by the AMOVA between the subspecies, which indicated that the largest source of genetic variation was found within subspecies and between populations within subspecies, whereas the least variation was found between the subspecies (Table 3).

In the morphological analyses, neither the centroid sizes, nor the cranial shapes, exhibited significant differences between all of the previously described subspecies (Tables 5, 6). When analyzed separately, a

Table 4. Sequence divergences, sequence diversities (with standard errors in brackets) and pairwise Φ_{ST} values among sampling populations

Locality	*n*	ID	1	2	3	5	6	10	11	12	14	20	22	7	15	16	17
Richtersveld	9	1	1.00 (0.3)	*0.43*	*0.46*	*0.34*	0.09	*0.29*	*0.35*	*0.35*	*0.28*	*0.33*	*0.34*	*0.57*	*0.61*	*0.77*	*0.58*
Port Nolloth	6	2	0.60 (0.3)	0.40 (0.2)	0.06	0.05	*0.40*	*0.21*	*0.39*	*0.31*	0.10	0.02	0.03	*0.58*	*0.64*	*0.88*	*0.69*
Springbok	14	3	0.50 (0.2)	0.00 (0.0)	0.40 (0.2)	*0.11*	*0.33*	*0.26*	*0.35*	*0.35*	0.14	*0.10*	0.07	*0.59*	*0.67*	*0.85*	*0.66*
Calvinia	9	5	0.40 (0.2)	0.00 (0.0)	0.10 (0.0)	0.70 (0.2)	0.18	*0.25*	*0.38*	0.04	0.13	0.02	0.04	*0.57*	*0.60*	*0.81*	*0.63*
Fraserburg	5	6	0.10 (0.1)	0.30 (0.1)	0.20 (0.1)	0.10 (0.1)	0.30 (0.2)	0.20	*0.32*	0.22	0.21	0.23	*0.21*	*0.58*	*0.65*	*0.91*	*0.70*
Richmond	4	10	0.40 (0.2)	0.20 (0.1)	0.10 (0.1)	0.20 (0.1)	0.20 (0.1)	1.60 (0.4)	0.11	*0.41*	0.00	0.07	0.12	*0.27*	*0.36*	*0.62*	*0.30*
Van Rhynsdorp	8	11	0.40 (0.2)	0.40 (0.2)	0.30 (0.1)	0.40 (0.2)	0.30 (0.1)	0.00 (0.1)	0.60 (0.2)	*0.52*	0.10	*0.26*	*0.32*	*0.45*	*0.56*	*0.80*	*0.58*
Lamberts Bay	11	12	0.30 (0.2)	0.20 (0.1)	0.20 (0.1)	0.00 (0.0)	0.10 (0.1)	0.40 (0.1)	0.50 (0.2)	0.40 (0.2)	*0.34*	*0.27*	0.21	*0.66*	*0.69*	*0.88*	*0.74*
Cape Floristic Region	4	14	0.40 (0.2)	0.00 (0.1)	0.00 (0.0)	0.10 (0.1)	0.10 (0.1)	0.00 (0.1)	0.00 (0.0)	0.20 (0.1	0.90 (0.3)	0.00	0.00	*0.41*	*0.44*	*0.83*	*0.57*
Cradock	4	20	0.50 (0.2)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.20 (0.1)	0.10 (0.1)	0.20 (0.1)	0.10 (0.1)	0.00 (0.0)	1.10 (0.3)	0.02	*0.52*	*0.54*	*0.80*	*0.57*
Albany	7	22	0.50 (0.2)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.20 (0.1)	0.10 (0.1)	0.30 (0.2)	0.10 (0.1)	0.00 (0.0)	0.00 (0.0)	0.70 (0.2)	*0.55*	*0.59*	*0.81*	*0.60*
Sutherland	13	7	1.10 (0.4)	0.80 (0.3)	0.80 (0.3)	0.90 (0.3)	0.80 (0.3)	0.20 (0.1)	0.50 (0.2)	1.00 (0.3)	0.40 (0.2)	0.80 (0.3)	0.80 (0.3)	0.70 (0.2)	*0.58*	*0.59*	*0.53*
Laingsburg	6	15	1.40 (0.4)	1.10 (0.4)	1.10 (0.4)	1.10 (0.4)	1.10 (0.4)	0.50 (0.3)	0.90 (0.3)	1.20 (0.4)	0.60 (0.3)	1.00 (0.4)	1.10 (0.4)	1.00 (0.4)	0.80 (0.2)	*0.66*	*0.62*
Oudtshoorn	5	16	2.30 (0.7)	2.10 (0.7)	2.00 (0.7)	2.10 (0.7)	2.10 (0.7)	1.10 (0.4)	1.70 (0.6)	2.30 (0.7)	1.60 (0.6)	2.00 (0.7)	2.10 (0.7)	0.80 (0.4)	1.00 (0.4)	0.10 (0.1)	*0.81*
Beaufort West	6	17	1.00 (0.4)	0.90 (0.4)	0.80 (0.4)	0.90 (0.4)	0.80 (0.4)	0.30 (0.2)	0.70 (0.4)	1.00 (0.4)	0.60 (0.4)	0.80 (0.4)	0.80 (0.4)	0.60 (0.3)	0.90 (0.3)	0.90 (0.4)	0.30 (0.2)

Sequence divergences (as percentages below diagonal), intrapopulation sequence diversity (diagonal elements as percentages shaded in dark grey) and pairwise Φ_{ST} values (above diagonal). Light grey shading indicates populations classified into the central assemblage, and significant values ($P < 0.05$) are indicated in italics. Number of samples (*n*) and locality ID is as in Table 1.

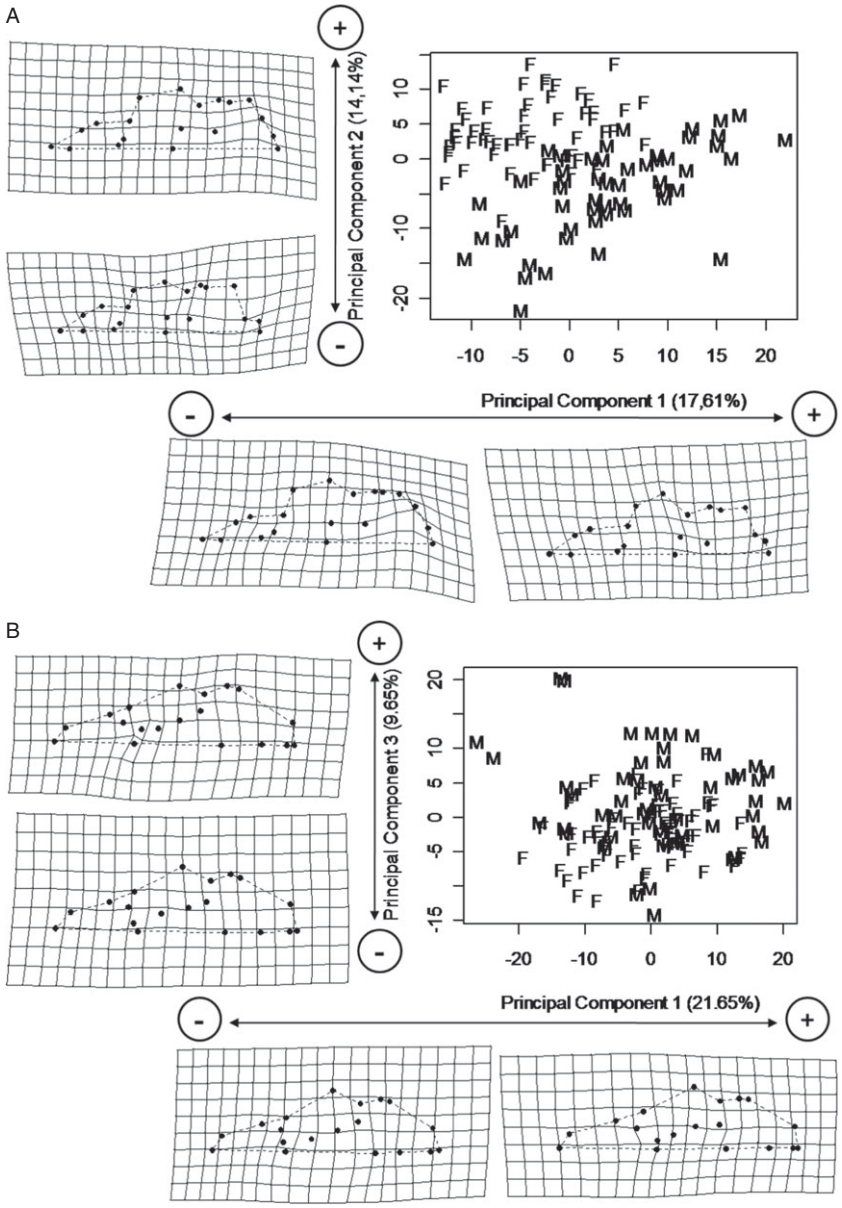

Figure 4. Scatterplot showing the distribution of females (F) and males (M) along the first two, and the first and third, principal components (PCs) for the ventral (A) and dorsal (B) views, respectively. TPS grids are shown for the positive (+) and negative (–) ends of the respective axes, aiming to visualize the shape change along each axis. Percentages in the axes labels indicate the proportion that each axis contributed to the overall variation.

few differences in cranial shapes between the subspecies were found to either be significant (e.g. between ventral cranial shapes of *M. u. albiensis* and *M. u. unisulcatus* in males) or approach significance (e.g. between dorsal cranial shapes of *M. u. albiensis* and *M. u. unisulcatus*, and ventral cranial shapes of *M. u. grantii* and *M. u. unisulcatus* in males) (Tables 5, 6). The differences between the centroid sizes of female *M. u. albiensis* and *M. u. grantii* approached significance in both views. Because the subspecies boundaries range across differing environments, for example, in rainfall levels, temperature, vegetation, soil substrate and altitude (Mucina & Rutherford, 2006), these significant differences between some of the subspecies in some of the datasets may be a result of environmental factors acting upon the cranial dimensions, and not a result of reproductive isolation between thesubspecies. The relationship between the male cranial shapes and minimum temperature was the only one found to be significant. This relationship

Table 5. Differences between the ventral shape variables (principal components) and centroid sizes of the mean shapes of populations, using multivariate analysis of variance (MANOVA) [with Hotelling–Lawley test statistic (H–L)] and analysis of variance (ANOVA), respectively

		Females								Males							
		Shape (MANOVA)				Centroid Size (ANOVA)				Shape (MANOVA)				Centroid Size (ANOVA)			
		d.f.	H–L	d.f.	*F*	*P*	SS	MSS	d.f.	*F*	*P*	d.f.	H–L	d.f.	*F*	*P*	SS	MSS	d.f.	*F*	*P*
Genetic groups	Main/Central	1	14.80	9	1.64	0.54	7.66×10^{-04}	7.66×10^{-04}	1	0.05	0.83	1	5.58	9	0.62	0.76	7.93×10^{-04}	7.93×10^{-04}	1	0.02	0.88
Subspecies†	ALB/BER	1	0.62	2	0.17	0.72	3.86×10^{-02}	3.86×10^{-02}	1	4.85	0.16	1	112.40	2	56.20	0.09	1.53×10^{-02}	1.53×10^{-02}	1	3.49	0.20
	ALB/BRO	1	162.50	4	40.62	0.12	3.84×10^{-02}	3.84×10^{-02}	1	3.21	0.15	1	50.76	4	12.69	0.21	7.09×10^{-03}	7.09×10^{-03}	1	0.09	0.78
	ALB/GRA	1	4.70	5	0.94	0.65	3.54×10^{-02}	3.54×10^{-02}	1	5.32	0.07	1	11.36	5	2.27	0.46	4.16×10^{-02}	4.16×10^{-02}	1	1.99	0.22
	ALB/UNI	1	0.53	2	0.12	0.76	2.70×10^{-02}	2.70×10^{-02}	1	3.39	0.21	1	750.09	2	*375.04*	*0.04*	1.00×10^{-07}	1.00×10^{-07}	1	0.00	1.00
	BER/BRO	1	113.50	2	56.75	0.09	3.34×10^{-03}	3.34×10^{-03}	1	0.21	0.69	1	0.11	2	0.05	0.95	4.11×10^{-03}	4.11×10^{-03}	1	0.03	0.89
	BER/GRA	1	112.75	3	37.58	0.12	5.51×10^{-03}	5.51×10^{-03}	1	0.95	0.40	1	40.88	3	13.63	0.20	1.37×10^{-04}	1.37×10^{-04}	1	0.00	0.95
	BER/UNI	NA	NA	NA	NA	NA	6.88×10^{-04}	6.88×10^{-04}	1	NA	NA	NA	NA	NA	NA	NA	1.02×10^{-02}	1.02×10^{-02}	1	NA	NA
	BRO/GRA	1	6.45	5	1.29	0.58	4.54×10^{-04}	4.54×10^{-04}	1	0.05	0.84	1	147.30	5	29.46	0.14	1.30×10^{-02}	1.30×10^{-02}	1	0.16	0.71
	BRO/UNI	1	27.04	2	13.52	0.19	6.57×10^{-04}	6.57×10^{-04}	1	0.04	0.86	1	2.99	2	1.49	0.50	3.57×10^{-03}	3.57×10^{-03}	1	0.02	0.89
	GRA/UNI	1	17.56	3	5.85	0.29	1.68×10^{-03}	1.68×10^{-03}	1	0.29	0.63	1	348.69	3	116.23	0.07	1.95×10^{-02}	1.95×10^{-02}	1	0.61	0.49
Rainfall season‡	SRZ/WRZ	1	255.22	9	28.36	0.14	2.63×10^{-04}	2.63×10^{-04}	1	0.02	0.89	1	22.81	9	2.53	0.45	4.77×10^{-03}	4.77×10^{-03}	1	0.10	0.76
	SRZ/YRZ	1	3.90	7	0.56	0.78	2.59×10^{-02}	2.59×10^{-02}	1	2.29	0.17	1	61.01	7	8.72	0.26	3.09×10^{-03}	3.08×10^{-03}	1	0.12	0.74
	WRZ/YRZ	1	34.57	12	2.88	0.43	3.63×10^{-02}	3.63×10^{-02}	1	2.31	0.15	1	425.50	12	35.46	0.13	1.90×10^{-04}	1.91×10^{-04}	1	0.00	0.95
Biome*	FB/NK	1	76.73	7	10.96	0.23	4.00×10^{-05}	3.99×10^{-05}	1	0.00	0.96	1	9.77	7	1.40	0.57	9.62×10^{-03}	9.62×10^{-03}	1	0.46	0.52
	FB/SK	1	36.95	8	4.62	0.35	1.05×10^{-02}	1.05×10^{-02}	1	0.77	0.41	1	4.03	8	0.50	0.80	2.18×10^{-02}	2.18×10^{-02}	1	0.41	0.54
	FB/TB	1	0.87	3	0.29	0.84	1.87×10^{-02}	1.87×10^{-02}	1	1.01	0.39	1	0.15	3	0.05	0.98	2.82×10^{-02}	2.82×10^{-02}	1	1.43	0.32
	NK/SK	1	48647.00	9	*5405.20*	*0.01*	1.36×10^{-02}	1.36×10^{-02}	1	1.48	0.25	1	254.08	9	28.23	0.15	2.39×10^{-03}	2.39×10^{-03}	1	0.05	0.83
	NK/TB	1	1.81	4	0.45	0.79	1.84×10^{-02}	1.84×10^{-02}	1	2.60	0.18	1	72.36	4	18.09	0.17	1.24×10^{-02}	1.24×10^{-02}	1	0.58	0.49
	SK/TB	1	8.06	5	1.61	0.53	4.12×10^{-02}	4.12×10^{-02}	1	3.82	0.11	1	0.47	5	0.09	0.98	7.32×10^{-03}	7.32×10^{-03}	1	0.10	0.76

Differences between the genetic assemblages obtained in BAPS, subspecies delimitations, rainfall seasonality, and biome boundaries were investigated. The sexes were investigated separately. *F*-values are shown, with significant values ($P < 0.05$) shown in italics. For the ANOVA, the sum of squares (SS) and the mean sum of squares (MSS) are also presented. ANOVAs and MANOVAs could not be performed between *Myotomys unisulcatus bergensis* (BER) and *Myotomys u. unisulcatus* (UNI) because both of these subspecies only contained one population each.

†ALB, *Myotomys u. albiensis*; BER, *M. u. bergensis*; BRO, *Myotomys u. broomi*; GRA, *Myotomys u. grantii*; UNI, *M. u. unisulcatus*.

‡SRZ, Summer rainfall zone; WRZ, Winter rainfall zone; YRZ, Year-round rainfall zone (*sensu* Chase & Meadows, 2007).

*FB, Fynbos Biome; NK, Nama Karoo Biome; SK, Succulent Karoo Biome; TB, Thicket Bushveld Biome (*sensu* Mucina & Rutherford, 2006).

Table 6. Differences between the dorsal shape variables (principal components) and centroid sizes of the mean shapes of populations, using multivariate analysis of variance (MANOVA) [with Hotelling–Lawley test statistic (H–L)] and analysis of variance (ANOVA), respectively

		Females										Males									
		Shape (MANOVA)					Centroid Size (ANOVA)					Shape (MANOVA)					Centroid Size (ANOVA)				
		d.f.	H–L	d.f.	*F*	*P*	SS	MSS	d.f.	*F*	*P*	d.f.	H–L	d.f.	*F*	*P*	SS	MSS	d.f.	*F*	*P*
Genetic groups	Main/Central	1	5.61	9	0.62	0.76	2.50×10^{-03}	2.50×10^{-03}	1	0.09	0.78	1	8298.00	9	***921.99***	***0.03***	1.23×10^{-03}	1.23×10^{-03}	1	0.01	0.92
Subspecies†	ALB/BER	1	3.70	2	1.85	0.46	7.87×10^{-03}	7.87×10^{-03}	1	0.61	0.52	1	9.81	2	4.90	0.30	5.56×10^{-03}	5.56×10^{-03}	1	0.03	0.88
	ALB/BRO	1	178.12	4	44.53	0.11	0.01	0.01	1	0.60	0.48	1	3.31	4	0.83	0.67	0.05	0.05	1	0.17	0.70
	ALB/GRA	1	5.96	5	1.19	0.60	0.09	0.09	1	5.61	0.06	1	27.95	5	5.59	0.31	3.01×10^{-03}	3.01×10^{-03}	1	0.02	0.89
	ALB/UNI	1	9.55	2	4.78	0.31	0.03	0.03	1	2.49	0.26	1	182.93	2	91.47	0.07	9.50×10^{-04}	9.48×10^{-04}	1	0.01	0.95
	BER/BRO	1	5.00	2	2.50	0.41	8.60×10^{-05}	8.60×10^{-05}	1	0.00	0.96	1	0.15	2	0.08	0.93	7.39×10^{-03}	7.39×10^{-03}	1	0.02	0.91
	BER/GRA	1	5.99	3	2.00	0.47	0.01	0.01	1	0.70	0.47	1	1.36	3	0.45	0.77	1.56×10^{-03}	1.56×10^{-03}	1	0.02	0.91
	BER/UNI	NA	NA	NA	NA	NA	5.44×10^{-03}	5.44×10^{-03}	1	NA	NA	NA	NA	NA	NA	NA	1.28×10^{-03}	1.28×10^{-03}	1	NA	NA
	BRO/GRA	1	137.88	5	27.58	0.14	0.03	0.03	1	1.37	0.30	1	1.55	5	0.31	0.87	0.03	0.04	1	0.15	0.71
	BRO/UNI	1	0.08	2	0.04	0.96	9.92×10^{-03}	9.92×10^{-03}	1	0.34	0.62	1	5.39	2	2.70	0.40	0.02	0.02	1	0.04	0.86
	GRA/UNI	1	2.35	3	0.78	0.66	2.14×10^{-04}	2.14×10^{-04}	1	0.01	0.92	1	10.54	3	3.51	0.37	3.20×10^{-05}	3.20×10^{-05}	1	0.00	0.99
Rainfall season‡	SRZ/WRZ	1	5.33	9	0.59	0.77	0.01	0.01	1	0.55	0.48	1	566.71	9	62.97	0.10	0.12	0.12	1	1.08	0.33
	SRZ/YRZ	1	31.65	7	4.52	0.35	0.03	0.03	1	1.36	0.28	1	26.02	7	3.72	0.38	0.02	0.02	1	0.24	0.64
	WRZ/YRZ	1	35.76	12	2.98	0.43	6.64×10^{-03}	6.64×10^{-03}	1	0.23	0.64	1	3918.90	12	***326.57***	***0.04***	0.06	0.06	1	0.43	0.53
Biome*	FB/NK	1	9.81	7	1.40	0.57	0.07	0.07	1	3.79	0.09	1	9.92	7	1.42	0.57	2.73×10^{-03}	2.73×10^{-03}	1	0.04	0.84
	FB/SK	1	20.22	8	2.53	0.45	0.09	0.09	1	4.44	0.07	1	2.55	8	0.32	0.89	0.01	0.01	1	0.09	0.77
	FB/TB	1	890.24	3	***296.75***	***0.04***	0.04	0.04	1	7.75	0.07	1	0.34	3	0.11	0.94	0.10	0.11	1	1.43	0.32
	NK/SK	1	7.95	9	0.88	0.69	1.26×10^{-03}	1.26×10^{-03}	1	0.05	0.84	1	3.68	9	0.41	0.85	0.03	0.03	1	0.25	0.63
	NK/TB	1	1.06	4	0.27	0.88	2.55×10^{-03}	2.55×10^{-03}	1	0.10	0.77	1	1.31	4	0.33	0.84	0.13	0.13	1	2.14	0.22
	SK/TB	1	0.53	5	0.11	0.97	9.81×10^{-04}	9.81×10^{-04}	1	0.03	0.86	1	1.29	5	0.26	0.89	0.07	0.07	1	0.41	0.55

Differences between the genetic assemblages obtained in BAPS, subspecies delimitations, rainfall seasonality, and biome boundaries were investigated. The sexes were investigated separately. *F*-values are shown, with significant values ($P < 0.05$) shown in bold italics. For the ANOVA, the sum of squares (SS) and the mean sum of squares (MSS) are also presented. ANOVAs and MANOVAs could not be performed between *M. u. bergensis* (BER) and *M. u. unisulcatus* (UNI) because both of these subspecies only contained one population each.

†ALB, *M. u. albiensis*; BER, *M. u. bergensis*; BRO, *M. u. broomi*; GRA, *M. u. grantii*; UNI, *M. u. unisulcatus*.

‡SRZ, Summer rainfall zone; WRZ, Winter rainfall zone; YRZ, Year-round rainfall zone (*sensu* Chase & Meadows, 2007).

*FB, Fynbos Biome; NK, Nama Karoo Biome; SK, Succulent Karoo Biome; TB, Thicket Bushveld Biome (*sensu* Mucina & Rutherford, 2006).

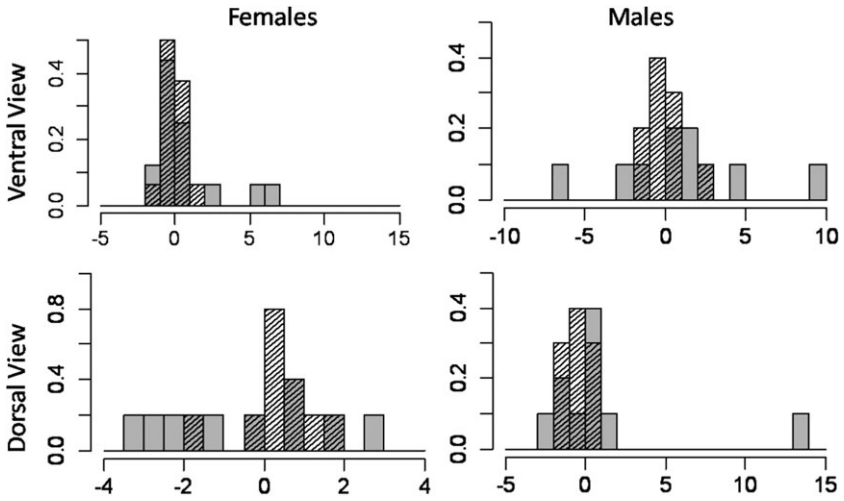

Figure 5. Density histograms of the linear discriminant analyses results (first discriminant axis) using the genetic assemblages as a priori groups. The main assemblage is shown by hatched bars, whereas the central assemblage is represented by grey bars. The ventral and dorsal views of the *M. unisulcatus* crania for males and females were analyzed separately.

does not explain the significant relationship between *M. u. albiensis* and the other subspecies, and so other factors than those investigated in the present study, may play a role in shaping male crania.

DISCUSSION

The most striking result to emerge from the present study is the existence of two mtDNA assemblages in *M. unisulcatus*. What caused the shallow genetic structure between the central assemblage and the main assemblage is not readily apparent. For a plains-dwelling species, elevated areas, such as mountain ranges, may play a role in creating genetic breaks in the species. A few areas of increased elevation separate the two genetic groups found in the present study. First, separating the Little Karoo in the south and the Great Karoo in the south-central regions of South Africa is the Grootswartberge. Between the Grootswartberge and the Nuweveldberge (forming the natural border of the Western Cape and Northern Cape provinces) is an area of lower elevation (Linder, 2001), where the central assemblage is located. The Nuweveldberge forms part of the Great Escarpment and north of this is the Nama Karoo, and the beginning of the African Plateau (McCarthy & Rubidge, 2005). It is possible therefore that sharp altitudinal gradients, probably provided by the Great Escarpment in this case, may be a factor in limiting current gene flow between populations (Matthee & Robinson, 1996).

In broad terms, the phylogeographic pattern of *M. unisulcatus* mirrors that of four species with differing life histories including the rock hyrax, *Procavia capensis* (Prinsloo & Robinson, 1992), the southern African scrub hare, *Lepus saxatilis* (Kryger *et al*., 2004), the southern rock agama, *Agama atra* (Swart *et al*., 2009), and the rock elephant shrew, *Elephantulus edwardii* (Smit *et al*., 2007). Within all four species, a genetic break was found between the Great Karoo, and the coastal plains of the Little Karoo. It was suggested that the Great Karoo clade in *P. capensis* was the ancestral population, and that recent dispersal had occurred from this region into the rest of South Africa, distributing a common haplotype throughout geographically distant populations (Prinsloo & Robinson, 1992). No barrier was suggested to have existed between the Western Cape clade and the Central clade in *L. saxatilis* (Kryger *et al*., 2004); however, because this species may prefer more open habitat (scrub or savanna woodland), sharp elevations may similarly limit gene flow within the species. Climatic shifts were considered to be the driving force of the genetic differentiation between sub-clades within *A. atra* (Swart *et al*., 2009). *Elephantulus edwardii* individuals from the central parts of the Karoo (Beaufort West and Williston) were genetically and morphologically different enough from *Elephantulus edwardii s.s.* to warrant the description of a new species (Smit *et al*., 2008).

The shape and size of the crania appear not to reflect the same phylogeographic structure obtained from the analysis of the cytochrome *b* gene. However, environmental factors may also be playing a role in shaping Karoo bush rat crania, but not cranial size, which may be affected by local conditions experienced

Table 7. Results of the Pearson product-moment correlations between the shape [first and second principal components (PC1 and PC2, respectively)] and centroid sizes (CS), and environmental variables [annual rainfall, altitudinal data, minimum and maximum annual temperature, and geographic coordinates (GPS)]

		Females								Males							
		PC1			PC2			CS		PC1			PC2			CS	
		%	*t*	*r*	%	*t*	*r*	*t*	*r*	%	*t*	*r*	%	*t*	*r*	*t*	*r*
Rainfall (mm)	Ventral	22.8	–0.51	–0.13	21.7	*–2.50*	*–0.54*	*–2.33*	*–0.51*	33.5	–1.48	–0.36	19.7	0.18	0.05	0.20	0.05
Altitude (m)	view		–0.97	–0.24		0.94	0.24	0.16	0.04		–0.34	–0.09		–0.92	–0.23	1.10	0.27
Temperature – maximum (°C)			–1.42	–0.34		–1.03	–0.26	0.44	0.11		–0.72	–0.18		0.46	0.12	1.22	0.30
Temperature – minimum (°C)			2.20	0.49		–0.24	–0.06	0.06	0.02		*2.71*	*0.57*		1.66	0.39	0.93	0.23
GPS–South			1.17	0.29		1.91	0.44	1.29	0.32		0.75	0.19		–0.26	–0.07	–0.04	–0.01
GPS—East			*–2.36*	*–0.52*		–1.76	–0.41	–1.87	–0.44		–1.71	–0.40		–0.90	–0.23	0.38	0.10
Rainfall (mm)	Dorsal	28.2	–2.07	–0.47	17.7	–0.66	–0.17	–0.17	–0.04	28.6	–0.90	–0.23	15.2	–0.63	–0.16	0.76	0.19
Altitude (m)	view		–1.73	–0.41		1.23	0.30	–0.44	–0.11		–0.02	–0.01		0.22	0.06	1.91	0.44
Temperature – maximum (°C)			–0.54	–0.14		0.03	0.01	1.93	0.45		0.02	0.01		–0.63	–0.16	1.88	0.44
Temperature – minimum (°C)			1.16	0.29		–0.18	–0.05	0.77	0.20		1.46	0.35		*2.27*	*0.51*	–0.13	–0.03
GPS–South			0.63	0.16		0.73	0.19	–0.81	–0.20		0.23	0.06		0.69	0.18	0.09	0.02
GPS–East			*–2.63*	*–0.56*		–0.44	–0.11	–0.37	–0.10		–1.05	–0.26		–0.14	–0.04	1.22	0.30

The ventral and dorsal views of the sexes were investigated separately. The *t*-values are shown, with those in italics indicating statitical significance ($0.05 < P < 0.01$). Correlations (*r*-values) are also shown.

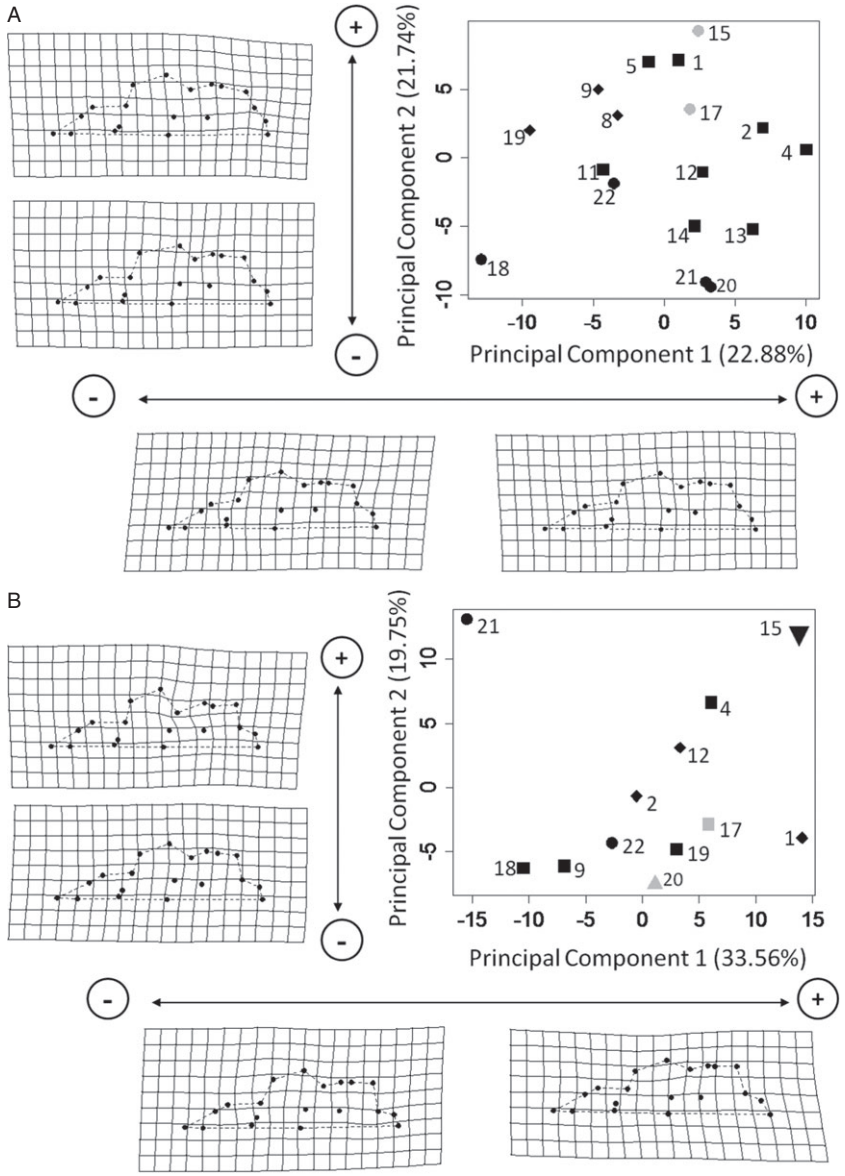

Figure 6. Scatterplots of the first two principal components (PC) on mean shapes of the ventral view of females (A) and males (B). TPS grids are shown for the positive (+) and negative (–) ends of the respective axes, aiming to visualize the shape change along each axis. Percentages in the axes labels indicate the proportion that each PC axis contributed to the overall variation. Black and grey symbols, with locality IDs adjacent, indicate those populations placed in the first and second genetic assemblages, respectively. A, ■ = Winter Rainfall Zone (WRZ); ◆ = Summer Rainfall Zone (SRZ); ● = Year-Round Rainfall Zone (YRZ). B, ■ = *M. u. grantii* (GRA); ● = *M. u. albiensis* (ALB); ○ = *M. u. unisulcatus* (UNI); ▲ = *M. u. bergensis* (BER); ◆ = *M. u. broomi* (BRO).

by the populations. Because museum skins are dried in the process of preserving them, the relationship between body size and environmental variables could not be investigated in the present study.

Congruence with the genetic assemblages was not found for any of the morphological datasets. Instead, cranial size is significantly correlated with mean annual rainfall levels, whereas cranial shape appears to be influenced by mean annual rainfall levels and rainfall seasonality. The female individuals inhabiting the WRZ exhibit larger tympanic bullae compared to those inhabiting the SRZ. It has been suggested that the reason for the presence of an enlarged tympanic bullae is to improve hearing, enabling the animal to better avoid predators in an open habitat (Lay, 1972; Taylor, Kumirai & Contrafatto, 2004). Momtazi *et al*. (2008) investigated tympanic bullae shape using elliptic fourier analyses, in the desert-

adapted gerbilline rodents of the genus *Meriones*, and found that hypertrophism of the auditory meatus was associated with the desert conditions of their environments, and Petter (1961) considered hypertrophism of the bullae to be one of the most important adaptations of desert animals. The bullae of *M. unisulcatus* in general are not as large as those of the sympatric sister genus *Parotomys*, and it is considered that the enlarged bulla is an ancestral trait that has been lost in the *Otomys* and *Myotomys* genera (Taylor *et al*., 2004). Because *M. unisulcatus* inhabits extensive stick-lodges, predator-avoidance is provided by the protection of the nest, and enhanced hearing is not as necessary in this species, as it is in *Parotomys brantsii*, for predator avoidance (Sheets, 1989; Taylor *et al*., 2004). However, the observation that those individuals inhabiting the more arid regions (westerly populations in the WRZ) exhibit larger bullae relative to the rest of the populations may be as a result of a lower density of cover in these areas, thereby necessitating enhanced hearing capabilities for predator avoidance.

The previously described subspecies boundaries were not conclusively recovered in either the molecular or morphological analyses, and so these findings are in accordance with the findings of Van Dyk *et al*. (1991). Because the previous descriptions of subspecies within the species were based on external, and possibly plastic, morphological characteristics (e.g. coat colour, body size; De Graaff, 1981), the recognition of subspecies is not warranted. This is corroborated by the low sequence divergences in the two genetic assemblages and the different geographic distinctions in the skull morphology. This species therefore appears to be prone to rapid cranial morphological adaptation, possibly influenced by rainfall seasonality and mean annual rainfall levels.

Given the outcomes of the present study, it is once again evident that geographic variation across a species range is the result of many factors. Morphologically variable characteristics in *M. unisulcatus* (such as variation in body coloration, body size and cranial shape) as well as behavioural characteristics (such as nesting habits and dietary intakes) appear not to be correlated with evolutionary distinctness in this species but, instead, are environmentally dependant (as in other African rodents such as *Arvicanthis niloticus*; Fadda & Corti, 2001). The outcomes of the present study, as a result of using more sensitive techniques, highlight the fact that the morphological subspecies descriptions are of little value in this species (*sensu* Van Dyk *et al*., 1991) and, from the genetic data, it is reasonable to suggest that regions of sharp elevations, such as those contributed by the Great Escarpment, can significantly influence long-term gene flow in plains-dwelling species in South Africa.

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