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Morphometric and molecular analysis of variation in the southern African hedgehog, *Atelerix frontalis* (Eulipotyphla: Erinaceidae)

By

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Supervisors: Prof C.T. Chimimba
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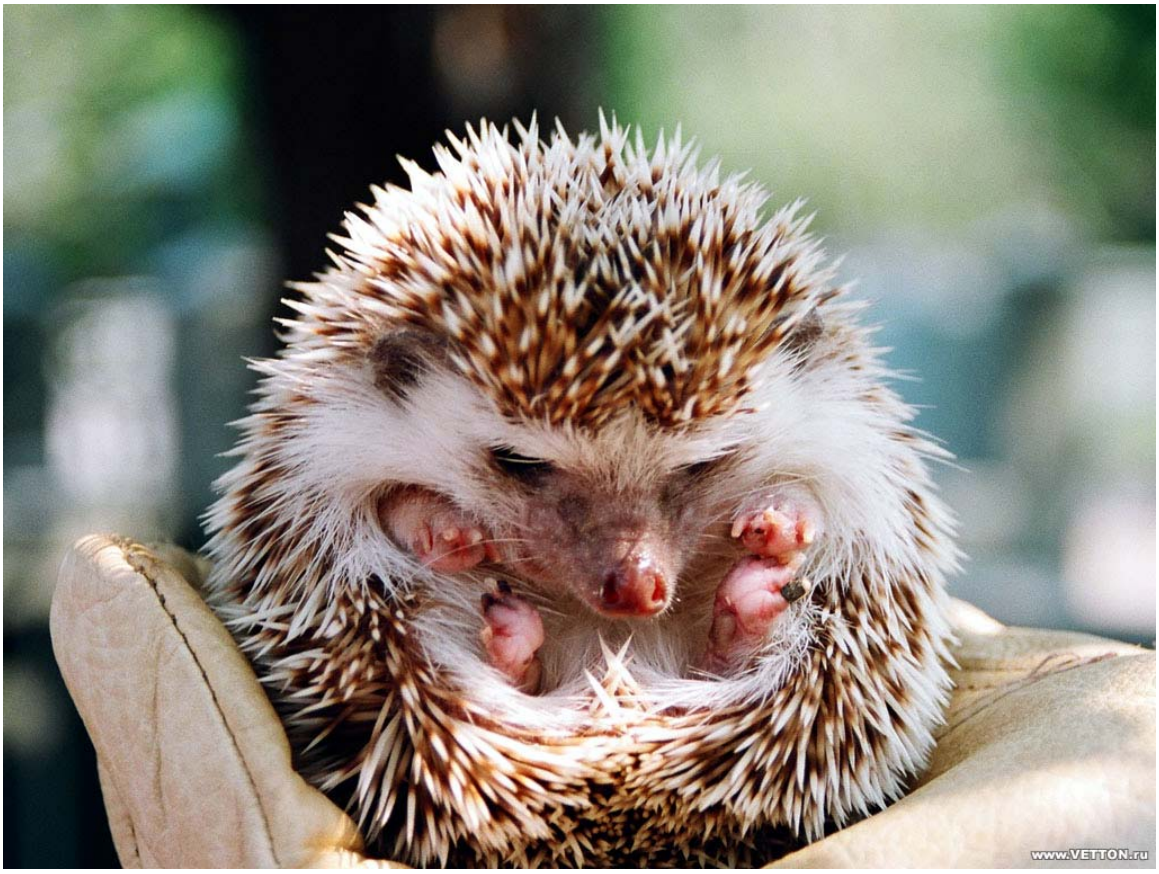
This thesis is dedicated to my family. Your support and encouragement during one of the most difficult times in my life meant a lot to me.



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General Abstract

The near-threatened southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831) is divided into two subspecies based on its disjunct distribution of two allopatric populations. This is despite reservations because its nature and extent of geographic variation remains virtually unknown. The present study, therefore, represents the first analysis of geographic variation within *A. frontalis* and is based on a multidisciplinary approach involving traditional and two-dimensional geometric morphometric analysis of the cranium and mandible, and molecular data in order to test the validity of the subspecies designations. The results of all univariate and multivariate analyses of both traditional and geometric morphometric data were congruent and provide evidence for a north-westerly–south-easterly clinal pattern of variation with cranial configuration being positively correlated with both latitude and longitude. These results are supported by Neighbour-joining, Maximum Likelihood, and Maximum Parsimony analyses of Cyt-*b* and ND2 data that revealed no variation across a 377 bp and 1034 bp region sequenced for each gene, respectively, while a 377 bp control region sequenced revealed low levels of variation between representatives of the two recognized subspecies (0.54 % pairwise sequence divergence). These results together with the lack of pronounced steps in the clinal pattern of variation suggest that the recognition of subspecies within *A. frontalis* may be untenable such that its disjunct distribution may represent a recent divergence event. If this is the case, then the results in this study may have implications in the conservation management strategies for *A. frontalis*, since it could be argued that one disjunct population could act as a source population for the other. However, it is recommended that prior to the implementation of conservation management plans for the species, further studies involving a wide range of alternative systematic techniques need to be undertaken first in order to gain a better understanding of the nature and extent of geographic variation within *A. frontalis*. These suggested studies should focus on comprehensive sampling and analyses involving a range of environmental and/or climatic variables in an attempt to identify factors that may explain the disjunct distribution and the clinal pattern of variation within the southern African hedgehog.

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DISCLAIMER

This thesis consists of a series of chapters that have been prepared as stand-alone manuscripts, for subsequent submission for publication purposes. Consequently, unavoidable and/or repetitions may occur between chapters.

Chapter 1

General introduction

[1] Introduction

The southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831), is listed as near-threatened in the *Red Data Book for South African Mammals* (Friedmann & Daly 2004). It is characterized by dorsal spines that originate from an enlarged sheet of muscle beneath the skin (Mills & Hes 1997; Skinner & Chimimba 2005). The spiny coat extends from the forehead, round behind the ears, and covers the entire dorsal part of the body (Skinner & Chimimba 2005).

The face, limbs and tail are covered with dark brown or grayish-brown hair (Mills & Hes 1997; Skinner & Chimimba 2005). Southern African hedgehogs have characteristic white underparts and a band of white hair across their forehead that extends on either side to below the ears (Mills & Hes 1997; Skinner & Chimimba 2005). However, there is considerable variation in the general pelage colouration due to the width of the white band across their foreheads and their pelage colour (Mills & Hes 1997; Skinner & Chimimba 2005).

Throughout its distributional range, the southern African hedgehog has a preference for semi-arid and sub-temperate regions in a wide variety of habitats with ample ground cover and with an annual rainfall of 300–800 mm, but avoids deserts and mesic habitats as well as wet ground (Louw & Seely 1982; Gillies *et al.* 1991; Mills & Hes 1997; Skinner & Chimimba 2005). Southern African hedgehogs require a habitat with a good supply of insects and other food items as well as adequate amounts of dry cover for refuge and to care for their young (Smithers 1983).

Hedgehogs are considered to have originated in Asia about 25 million years ago and their descendants spread to Europe, Africa, and to North America, where they have since become extinct (Sykes 1995). While ancestral hedgehogs are considered to have appeared about 15 million years ago and are currently extinct (Morris 1994),

extant hedgehogs still retain many primitive features that were probably characteristic of the first mammals (Morris 1994). Although hedgehogs are considered to have no close relatives among mammals, they have some distant links with for example, the moles and shrews that led to their being taxonomically grouped together within the Order Insectivora (Skinner & Smithers 1990; Mills & Hes 1997).

[2] Higher classification

However, the Order Insectivora is considered to be a taxonomic wastebasket (Bronner *et al*. 2003). This is largely because members taxonomically allocated to this Order are not necessarily insectivores and are often dissimilar morphologically (Skinner & Smithers 1990; Mills & Hes 1997; Bronner *et al.* 2003). In southern Africa, the Order Insectivora includes three largely morphologically dissimilar families, namely, the Soricidae, the Chyrochloridae, and the Erinaceidae that include 32 species and 12 genera of shrews, golden moles, and hedgehogs, respectively (Skinner & Smithers 1990; Mills & Hes 1997).

Consequently, these morphological dissimilarities have led to a recent re-classification of the conventionally recognized Order Insectivora (Bronner *et al.* 2003). In this most recent classification, the Order has been split into several Orders (Skinner & Chimimba 2005). For example, while hedgehogs have traditionally been allocated to the Order Insectivora (Skinner & Smithers 1990; Mills & Hes 1997), the current classification allocates them to their own Order, the Eulipotyphla (Bronner *et al.* 2003).

Within the Order Eulipotyphla, two clades are recognized, namely, the Suborder Erinaceomorpha, that includes the hedgehogs, and the Suborder Soricomorpha, that includes the remaining forms within the Order Eulipotyphla, and these include the chrysochlorids and the tenrecs (Bronner *et al.* 2003). However, within the Suborder Erinaceomorpha, the hedgehogs are placed within the traditionally recognized family Erinaceidae (Bronner *et al.* 2003), and the subfamily Erinaceinae.

Historically, the subfamily Erinaceinae has been known to include five nominal genera, namely: *Erinaceus* Linnaeus, 1758; *Atelerix* Pomel, 1848; *Hemiechinus*

Fitzinger, 1866; *Paraechinus* Troussart, 1879; and *Aethechinus* Thomas, 1918. However, the status of these generic designations has ranged from being synonymised into a single genus (*Erinaceus*, Dobson 1882), three genera (*Erinaceus*, *Hemiechinus* and *Paraechinus*), four genera (*Erinaceus*, *Hemiechinus, Paraechinus*, and *Atelerix*), to all five genera being considered valid (Robbins & Setzer 1985). Subsequently, only three genera (*Erinaceus*, *Hemiechinus* and *Paraechinus*) were recognized (Robbins & Setzer 1985). This classification subdivided *Erinaceus* into two subgenera: the subgenus *Erinaceus* with one species and the subgenus *Atelerix* with four species, two of which were previously attributable to *Aethechinus* (Robbins & Setzer 1985).

[3] Generic Classification

Currently, however, two subfamilies are recognized within the family Erinaceidae, namely, Erinaceinae and Hylomyinae. The latter consists of three genera and six species, but does not occur on the African continent. The subfamily Erinaceinae is comprised of four genera and 16 species, and has a wider distribution, occurring in Africa, Europe and Asia (Hutterer 1993). Within sub-Saharan Africa, the subfamily Erinaceinae comprises three genera and four species, of which only one species, *Atelerix frontalis* (A. Smith, 1831), occurs in the southern African sub-region (Mills & Hes 1997).

[4] Species classification

The single southern African species, *A. frontalis* was previously allocated to the genus *Aethechinus* (Allen 1939) by Roberts (1951). Ellerman *et al.* (1953) re-allocated it to *Atelerix* as a subgenus of *Erinaceus*. Robbins & Setzer (1985), however, regarded African hedgehogs as generically distinct from the European hedgehogs in the subgenus *Erinaceus* (Van der Colf 1990).

[5] Subspecific classification

The southern African hedgehog has a disjunct distribution of two allopatric populations occurring in parts of the subregion and extralimitally into Angola and is currently listed as either near-threatened or as approaching vulnerable, with a declining habitat (Friedman & Daly 2004). This disjunct distribution coincides with

subspecific taxonomic designations within the species (Meester *et al.* 1986). The subspecies *A. f. frontalis* (A. Smith, 1831) is restricted to the eastern parts of southern Africa that include eastern Botswana, western Zimbabwe and the Free State, Gauteng, and the central parts of the Cape Provinces of South Africa (Skinner & Chimimba 2005; Fig. 1). The subspecies *A. f. angolae* (Thomas, 1918) is confined to the western parts of the subregion, mostly in Namibia, but with an extralimital occurrence in south-western Angola (Skinner & Chimimba 2005; Fig. 1).

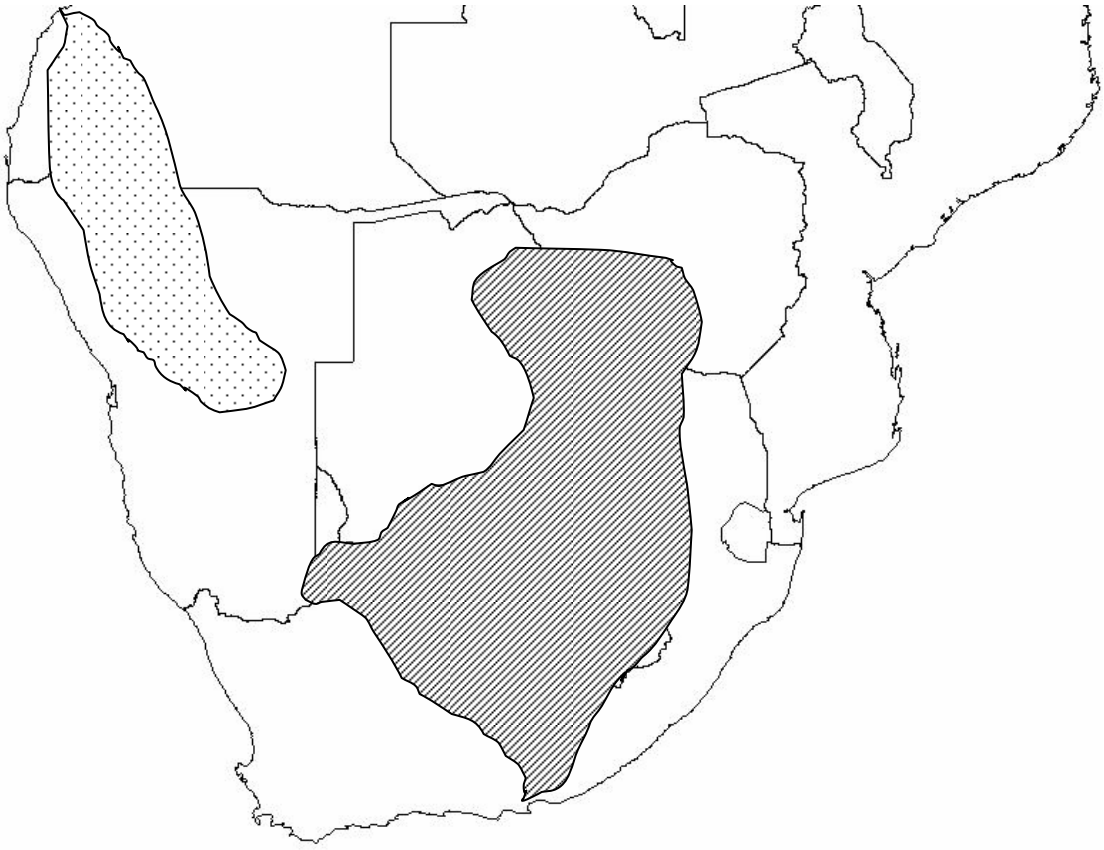

Figure 1. A map of southern Africa showing the distribution of the southern African hedgehog, *Atelerix frontalis* as adopted from Skinner & Chimimba (2005). The western distribution represents that of the subspecies *A. f. angolae* (indicated by dotted shading) while the eastern distribution (indicated by striped shading) represents that of the subspecies *A. f. frontalis*.

Although Rautenbach (1978), based on the complete isolation of the two populations, considered the recognition of the two subspecies within the southern African hedgehog justifiable, there have been reservations on the validity of these subspecific taxonomic designations, particularly that of *A. f. angolae* (Corbet 1974; Gillies 1989; Skinner & Smithers 1990). To date, very little is known about patterns

of intraspecific variation in *A. frontalis* that would either confirm or refute the validity of the current subspecific taxonomic status of the southern African hedgehog. Consequently, there is a critical need to further investigate the nature and extent of variation within the near-threatened *A. frontalis*.

[6] Aim of study

The aim of the present study is, therefore, an attempt to assess the validity of the current subspecific taxonomic status of the southern African hedgehog. This study represents the first attempt to assess intra-specific variation within the species, over a broad geographic area that has previously been considered for the species, based on a multidisciplinary approach that includes traditional and geometric morphometrics, and molecular analyses.

Given the near-threatened listing of the southern African hedgehog in the *Red Data Book for South African Mammals* (Friedman & Daly 2004) and its currently decreasing suitable habitat (Friedman & Daly 2004), study material was generally limited. The general paucity of study material is exacerbated further by the generally secretive nature of hedgehogs (Morris 1994). Consequently, the present multidisciplinary characterization of the southern African hedgehog was largely based on museums specimens for the morphometric as well as molecular analyses but also included opportunistically-obtained fresh material that augmented the molecular part of the study.

[7] Research questions

To this end, the following questions will be addressed in the present investigation:

1. What is the nature and extent of morphometric and molecular variation within *A. frontalis*?
2. Does the nature and extent of morphometric and molecular variation warrant the recognition of subspecies within *A. frontalis*?

[8] Justification

The systematic status of subspecies in the near-threatened *A. frontalis* and the nature and extent of its geographic variation is uncertain. To date, there is no

multidisciplinary systematic study of the southern African hedgehog and the approach adopted in this study may assist nature conservation authorities in formulating conservation management strategies for the species within the southern Africa subregion. Apart from adding to a body of knowledge on small mammal systematics in Africa, the present study may serve as a model for other similar studies in other regions in Africa, particularly with reference to the use of non-destructive techniques to address systematic questions in threatened mammals.

[9] Thesis outline

The first part of this study (Chapter 2) is directed towards selecting meaningful taxonomic characters for use in assessing the nature and extent of variation within the southern African hedgehog based on cranial and mandibular morphology. These measurements were selected to adequately represent cranial and mandibular phenotypes in the southern African hedgehog.

Chapter 3 addresses questions relating to the evaluation of non-geographic variation using a series of univariate and multivariate analyses of the cranium and mandible based on both traditional and geometric morphometric data. This was undertaken with the primary objective of establishing whether: 1) sexes should be treated separately or together; and 2) which specimens have reached adult dimensions and were therefore, suitable for measurement recording and analysis in the subsequent assessment of the nature and extent of variation in the southern African hedgehog.

Chapters 4 and 5 assess the nature and extent of cranial and mandibular morphological variation using traditional (Chapter 4) and geometric (Chapter 5) morphometric data, respectively, while Chapter 6 examines patterns of molecular variation in the southern African hedgehog. Chapters 3–6 also provide overviews of the traditional and geometric morphometric approaches and their associated univariate and multivariate methods, as well as molecular methods used in the present multidisciplinary study, respectively. The final chapter (Chapter 7) provides a general discussion of the major findings of this multidisciplinary study.

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Chapter 2

Character selection for a traditional morphometric analysis of the southern African hedgehog, *Atelerix frontalis* (Eulipotyphla: Erinaceidae)

Abstract

In the present study, a character selection procedure to identify a final character set of 30 out of 70 initial measurements for subsequent morphometric studies of the southern African hedgehog, *Atelerix frontalis*, was followed. Firstly, a preliminary assessment of sexual dimorphism revealed that no sexual dimorphism is present, such that the sexes were pooled in subsequent analyses. A Ward's cluster analysis was used to assess subsets of highly correlated measurements. The final set of measurements was then selected based on their principal components (PCA) loadings, coefficients of variation (CV), percentage measurement error (%ME), ease of measurement, and the potential to capture the overall configuration of the phenotype.

[1] Introduction

The present study forms part of an analysis of morphological geographic variation in the near-threatened southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831), and involves the preliminary selection of quantitative taxonomic characters for use in the study. The selection of quantitative taxonomic characters is critical and yet often neglected in the literature (Strauss & Bookstein 1982; Rohlf 1990). In small mammals, no established procedure is available for selecting appropriate taxonomic characters (Chimimba & Dippenaar 1995). Approaches used to date fall into three categories: 1) the selection of character sets used in the past, with the addition and/or deletion of characters (Power 1971; Chapman *et al.* 1992); 2) the selection of as many measurements as is practically possible (Watson & Dippenaar 1987; Chimimba & Kitchener 1991); and 3) the selection of measurements based on an assessment of functional units of the cranium (Taylor & Meester 1993).

There are various procedures that have been used in the past to screen for reliable taxonomic characters. These range from the use of analysis of variance (ANOVA), Mahalanobis' (1936) D^2 statistic, to correlations among characters as summarized by either principal components analysis (PCA) (Gould *et al.* 1974), factor analysis

(Thomas 1968; Johnston 1973), or cluster analysis (Power 1971; Taylor & Meester 1993) with the selection of characters from within highly correlated subsets of characters (Chimimba & Dippenaar 1995). Although some of these procedures may perform well, others either ignore redundancy or can be unstable because of small sample sizes (Thorpe 1976). The morphometric character selection in the present study is based on a procedure previously applied to a murid rodents from southern Africa (Chimimba & Dippenaar 1995) and weevils from the sub-Antarctic Marion Island (Janse van Rensburg *et al.* 2003). In these studies, the final set of measurements was selected based on their principal components (PCA) loadings, coefficients of variation (CV), percentage measurement error (%ME), ease of measurement, and the potential of a measurement to capture the overall configuration of the phenotype.

[2] Materials and methods

2.1 *Samples*

In order to address the potentially confounding effect of geographic variation, the character selection procedure in the present study was based on a homogenous sample of the southern African hedgehog from Gauteng Province, South Africa. Similarly, in order to limit the potentially confounding effect of age variation, only one adult relative age class (age class 3) based on the extent of eruption of the last molar was used. All specimens examined in this study were obtained from the mammal collection of the Transvaal Museum(TM) of the Northern Flagship Institute (NFI), Pretoria, South Africa and are listed in Appendix 1.

2.2 *Morphometric analysis*

An initial set of 70 measurements (adopted from Chimimba & Dippenaar 1995) defined and illustrated in Fig. 1 was selected to represent the cranial and mandibular phenotype of the southern African hedgehog. These measurements were recorded to the nearest 0.01 mm using a pair of Mitutoyo® digital callipers (Mitutoyo American Corporation, Aurora, Illinois, U.S.A.). However, due to consistent damage in most specimens, the greatest zygomatic width (ZYW) was excluded from further analysis.

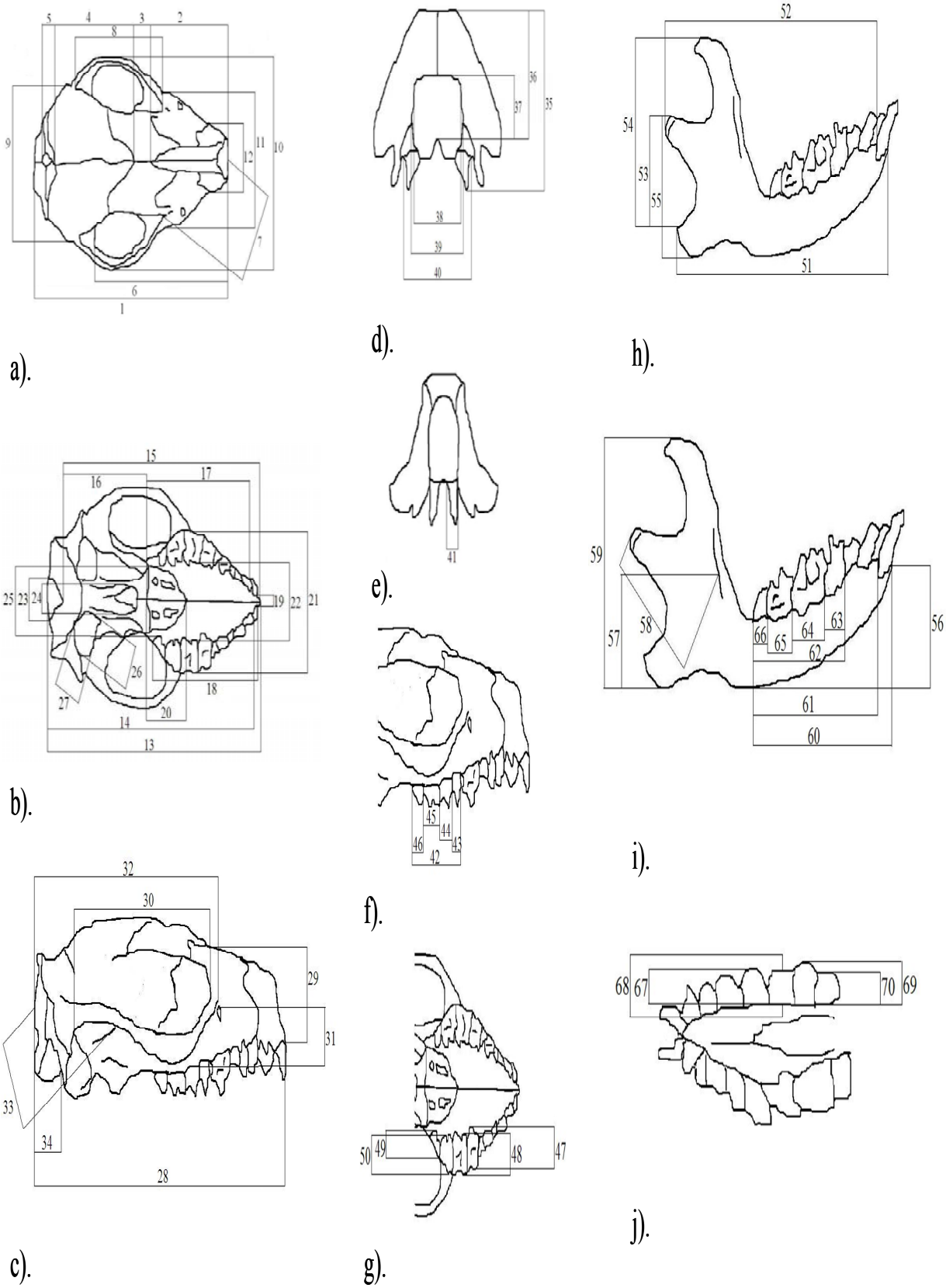

Figure 1. Definitions and an illustration of the skull measurements (a – j) used in the present study: 1. GLS = Greatest length of skull, from anterior edge of nasals to posterior edge of occipital condyle,

along longitudinal axis of skull. 2. GLN = Greatest length of nasals, from longest posterior projection of nasal wings to anteriormost edge of nasal bones. 3. FRO = Greatest length of frontals. 4. PAR = Greatest length of parietals. 5. INT = Interparietal length, from intersection of sagittal suture and posterior end of parietal, perpendicular to posterior end of interparietal. 6. NPP = Distance from anterior edge of nasals to anterior edge of posterior part of zygomatic arch. 7. NPO = Distance from anterior edge of nasals to posterior edge of postorbital bar. 8. ZAL = Zygomatic arch length, from posteriormost part of anterior part of zygomatic arch to anteriormost part of posterior part of zygomatic arch. 9. BBC = Breadth of braincase, width at dorsal root of squamosals. 10. ZYW = Greatest zygomatic width, between outer margins of zygomatic arches, perpendicular to longitudinal axis of skull. 11. IOB = Least breadth of interorbital constriction, least distance dorsally between orbits. 12. NAS = Nasal width, at anteriormost point where nasals join premaxillae. 13. CBL = Condylobasal length of skull, from posteriormost projection of occipital condyles to anterior edge of premaxillae. 14. PIC = Incisor to condyle length, from posterior surface of I^1 at alveolus to posteriormost projection of occipital condyle. 15. BSL = Basal length of skull, from anteriormost point of lower border of foramen magnum to anterior edge of premaxilla. 16. PPL = Postpalatal length, from anteriormost edge of hard palate to anteriormost point on lower border of foramen magnum. 17. PAL = Palatilar length, from posterior edge of I^1 alveolus to posterior edge of hard palate. 18. TRL = Toothrow length, from anterior alveolus to posterior surface of M^1 alveolus. 19. WGI = Width of gap between the incisors. 20. LPF = Greatest length of longest palatal foramen. 21. MAW = Greatest maxillary width between labial crown edges of M^1. 22. PWM = Hard palate width at first upper molar measured on lingual side of teeth at alveolus. 23. PAC = Hard palate width at point of constriction immediately posterior to third upper molar. 24. VCW = Vidian canal width of foramen lateral to pterygoid processes. 25. FJW = Least distance between foramina jugulare on posterior edge of bullae. 26. BUL = Greatest bulla length at 45º angle to the skull axis. 27. BUW = Greatest bulla width at 45º angle to skull axis. 28. ITC = Incisor to condyle length, from anterior surface of first upper incisor at alveolus to posteriormost projection of the occipital condyle. 29. HOR = Height of rostrum, perpendicularly from a point directly behind upper incisors. 30. IOE = Distance from anterior base of zygomatic plate to anterior edge of ear opening. 31. IZD = Infaorbital-zygomatic plate distance, from dorsal edge of infraorbital foramen to anterior base of zygomatic plate. 32. MPO = Foramen magnum-postorbital bar length, from lateral edge of foramen magnum to anterior edge of postorbital bar. 33. MPZ = Foramen magnum-zygomatic arch length, from lateral of foramen magnum to anterior edge of posterior part of zygomatic arch. 34. FME = Foramen magnum-external auditory meatus length, from lateral edge of foramen magnum to posterodorsal edge of external auditory meatus. 35. GHS = Greatest height of skull perpendicular to horizontal plane through bullae. 36. BCH = Braincase height, from dorsal surface of sagittal crest to midventral surface of basioccipital between anterior bullae. 37. FMH = Foramen magnum height, widest part of foramen in vertical plane. 38. FMW = Foramen magnum width, widest part of foramen magnum in a horizontal plane. 39. CNW = Greatest occipital condyle width perpendicular to skull axis. 40. WAB = Width at bullae on ear openings perpendicular to skull axis. 41. FIB= First incisor breadth, breadth of principal upper incisor at level of median edge of alveolus. 42. UTR = Crown length of maxillary toothrow, from anterior edge of first upper molar at alveolus to posterior edge of third molar at alveolus. 43. LPM =

Length of the upper premolar 44. LFM = Length of upper first molar along cingulum. 45. LSM = Length of upper second molar along cingulum. 46. LTM = Length of upper third molar along cingulum. 47. WPM = Greatest cross-sectional crown width of upper premolar. 48. WFM = Greatest cross-sectional crown width of first upper molar. 49. WSM = Greatest cross-sectional crown width of second upper molar. 50. WFM = Greatest cross-sectional crown width of third upper molar. 51. GML = Greatest mandible length, in a straight line from anterior edge of first lower incisor alveolus to posterior surface of angular process. 52. MDL = Greatest length of mandible (excluding teeth), from posterior surface of condylar process to anteroventral edge of incisor alveolus. 53. AFA = Angular process-mandibular condyle length. 54. MRH = Mandible-ramas height, from dorsal edge of coronoid process to ventral edge of angular process. 55. MCA = Mandibular condyle-angular process distance, in straight line from dorsal edge of mandibular condyle to ventral edge of angular process. 56. LHM = Least mandible height, perpendicularly from between posterior first lower molar alveolus and anterior second lower molar alveolus. 57. MFA = Mandibular foramen-angular process length, from anterior edge of mandibular foramen to posterior edge of angular process. 58. MAF = Mandibular foramen-articular facet length, from ventral edge of mandibular foramen to midposteriodorsal edge of articulating facet. 59. CMH = Coronoid mandible height, from dorsal edge of coronoid process to ventral edge of mandible in line with mandibular foramen. 60. MTL = Mandibular toothrow, from anterior edge of first lower incisor alveolus to posterior edge of third lower molar alveolus. 61. IML = Posterior incisor-third lower molar length, in a straight line from posterior edge of first lower incisor alveolus to posterior edge of third lower molar alveolus. 62. MTR = Mandibular toothrow length, from anterior edge of lower first molar alveolus to posterior edge of third lower molar alveolus. 63. LMP = Length of lower premolar along cingulum. 64. LLM = Length of first lower molar along cingulum. 65. LMS = Length of second lower molar along cingulum. 66. LMT = Length of third lower molar along cingulum. 67. WMP = Greatest cross-sectional crown width of lower premolar. 68. WLM = Greatest cross-sectional crown width of first lower molar. 69. WMS = Greatest cross-sectional crown width of second lower molar. 70. WMT = Greatest cross-sectional crown width of third lower molar (modified from Chimimba & Dippenaar 1995).

Sexual dimorphism was first assessed independently using the univariate one-way analysis of variance (ANOVA; Zar 1996). To assess the nature and extent of sexual dimorphism multivariately, an unweighted pair-group arithmetic average (UPGMA) cluster analysis and principal components analysis (PCA) based on standardised variables (Sneath & Sokal 1973) were used. UPGMA cluster analysis was based on both Euclidean distances as well as correlation coefficients among groups, while the PCA was computed from correlation coefficients among variables.

In order to assess measurement error, measurements were recorded by one observer (LR) on three separate occasions and were followed by a one-way ANOVA in order to compute percent measurement error (%ME) for each measurement (Pankakoski *et al.* 1987; Bailey & Byrnes 1990). In order to assess the relative importance of each measurement, the measurements were then subjected to an R-mode PCA (Sneath & Sokal 1973). Character associations were assessed by PCA loadings of measurements after transposing the measurement loadings from an R-mode PCA in order to conduct a Q-mode PCA to assess relationships among the measurements (Sneath & Sokal 1973). The Q-mode PCA loadings were then subjected to a Ward's (1963) cluster analysis, which produces more homogenous clusters. The selection of characters from within sub-clusters generated by the Ward's (1963) cluster analysis was based on a combination of the following criteria:

1. Relative loadings of measurements as derived by an R-mode PCA (James & McCulloch 1990);
2. The magnitude of coefficients of variation (CV) as a measurement of relative variability between measurements;
3. The degree of measurement error (ME) expressed as a percentage (%ME) of the total variability due to *within*-individual variation (Pankakoski *et al.* 1987; Bailey & Byrnes 1990);
4. Relative ease of the measurement; and
5. The potential for a measurement to capture the overall configuration of the phenotype.

[3] Results

3.1 *Sexual dimorphism*

An ANOVA revealed no statistically significant differences between measurements (Table 1) with reference to sexual dimorphism.

TABLE 1. The results of a 1-way ANOVA of relative age class 3 of the southern African hedgehog, *Atelerix frontalis* indicating the significance level of the initial 70 measurements. Measurements are defined and illustrated in Fig. 1.

Measurement	*F*-value	Measurement	*F*-value	Measurement	*F*-value
GLS	0.43^{ns}	FJW	0.18^{ns}	WFM	0.01^{ns}
GLN	0.43^{ns}	BUL	0.01^{ns}	WSM	1.09^{ns}
FRO	1.11^{ns}	BUW	1.64^{ns}	WTM	8.95^{ns}
PAR	0.51^{ns}	ITC	0.42^{ns}	GML	0.03^{ns}
INT	1.55^{ns}	HOR	0.44^{ns}	MDL	0.36^{ns}
NPP	0.80^{ns}	IOE	0.0001^{ns}	AFA	0.60^{ns}
NPO	0.001^{ns}	IZD	0.11^{ns}	MRH	0.26^{ns}
ZAL	2.64^{ns}	MPO	0.01^{ns}	MCA	1.09^{ns}
BBC	1.70^{ns}	MPZ	0.19^{ns}	LMH	0.37^{ns}
IOB	0.84^{ns}	FME	0.19^{ns}	MFA	0.17^{ns}
NAS	0.87^{ns}	GHS	2.30^{ns}	MAF	0.07^{ns}
CBL	0.37^{ns}	BCH	0.43^{ns}	CMH	0.01^{ns}
PIC	0.59^{ns}	FMH	3.20^{ns}	MTL	0.09^{ns}
BSL	0.58^{ns}	FMW	0.78^{ns}	IML	0.07^{ns}
PPL	0.12^{ns}	CNW	1.75^{ns}	MTR	0.01^{ns}
PAL	0.57^{ns}	WAB	2.32^{ns}	LMP	0.18^{ns}
TRL	0.000^{ns}	FIB	0.18^{ns}	LLM	0.30^{ns}
WGI	0.35^{ns}	UTR	0.56^{ns}	LSM	0.33^{ns}
LPF	0.53^{ns}	LPM	3.13^{ns}	LMT	1.23^{ns}
MAW	0.08^{ns}	LFM	0.01^{ns}	WMP	0.90^{ns}
PWM	1.11^{ns}	LSM	0.53^{ns}	WLM	0.36^{ns}
PAC	0.79^{ns}	LTM	1.33^{ns}	WMS	0.66^{ns}
VCW	0.09^{ns}	WPM	0.03^{ns}	WMT	1.13^{ns}

^{ns}= non-significant

The lack of sexual dimorphism was also apparent in the UPGMA cluster analysis phenogram (Fig. 2) and the PCA scatterplot (Fig. 3). Consequently, the sexes were pooled in the assessment of character associations and in all subsequent analyses of geographic variation within the southern African hedgehog.

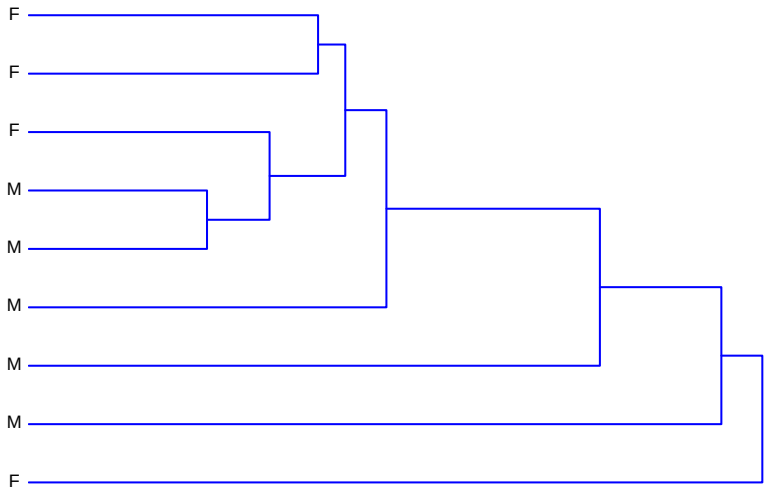

Figure 2. The phenogram of an unweighted-pair group arithmetic average (UPGMA) cluster analysis of relative age class 3 for the southern African hedgehog, with M indicating male and F indicating female. The UPGMA cluster analysis indicates the lack of sexual dimorphism.

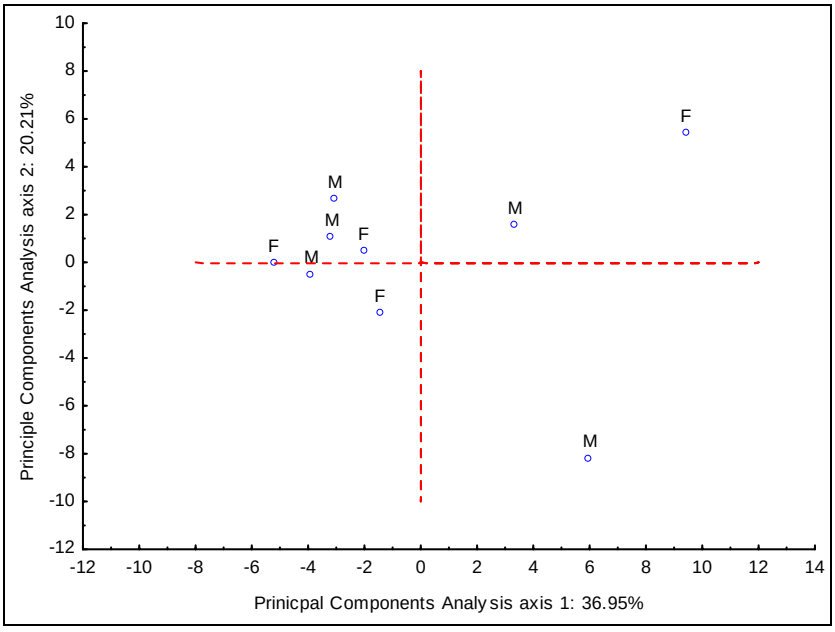

Figure 3. Relative age class 3 of the southern African hedgehog indicates a lack of sexual dimorphism using a principal components analysis (PCA). M is abbreviated for male and F for female.

3.2 *Analysis of character association*

The phenogram derived from Ward's (1963) cluster analysis of the 70 measurements is illustrated in Fig. 4. There are 6 major clusters of characters designated I–VI. Major

cluster I consists of mostly length measurements of the cranium and mandible. Among this grouping of measurements are GLS and GML that reflect the overall size of the cranium and mandible. Major cluster II consists of length and width measurements of the cranium and measurements such as BBC, TRL, MPO, and MTL. Major cluster III mainly consists of teeth measurements and includes measurements such as MAW, PMW, MCA and IML. Major cluster IV represents depth and width measurements of the cranium and includes measurements such as IOB, PAC, IZD and GHS. Major cluster V represents depth and width measurements of the cranium and the mandible and includes measurements such as HOR, FMH, FMW and MRH. Major cluster VI also represents teeth measurements and includes measurements such as LFM, LSM, WFM, WSM, LLM, LMS, WLM and WMS.

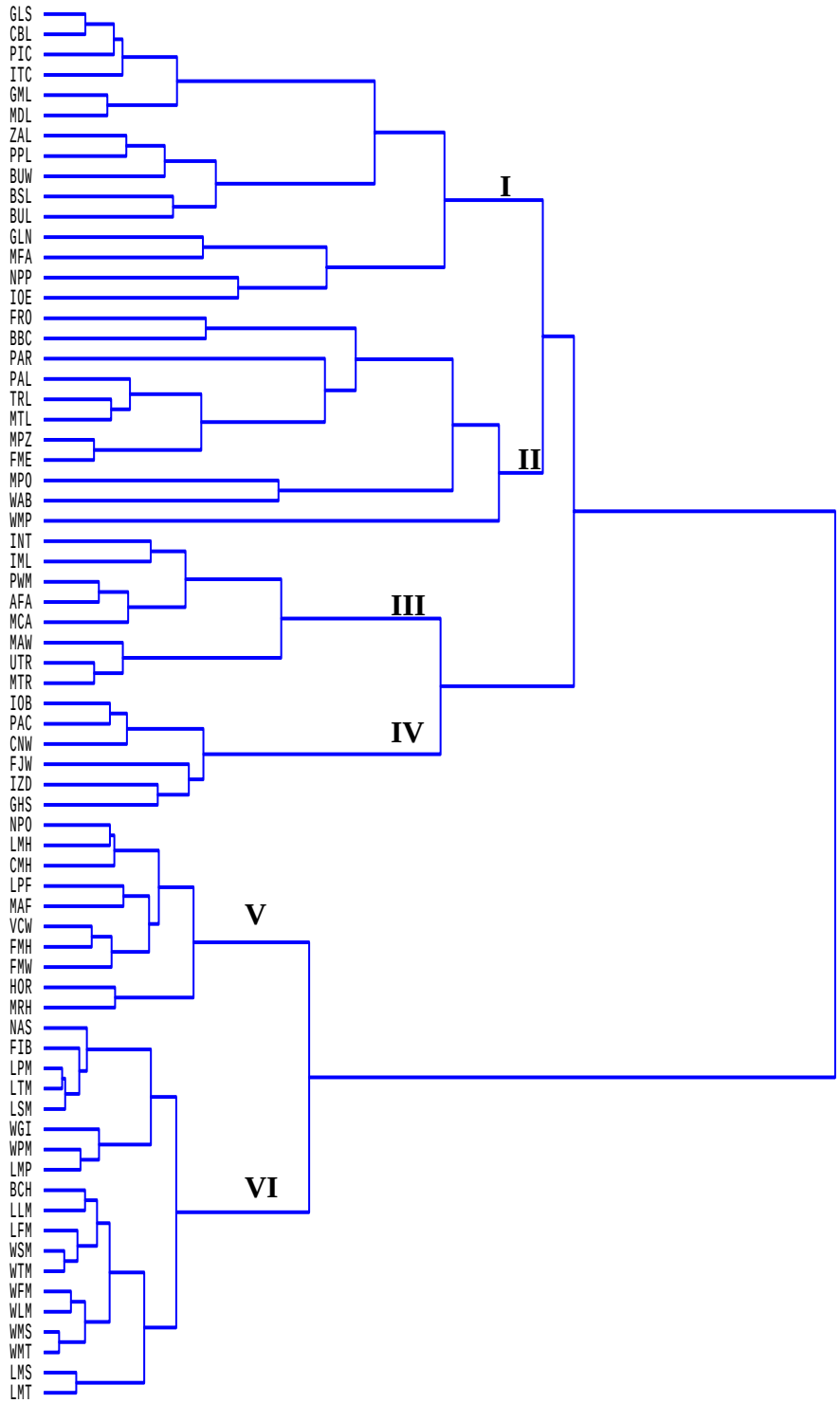

Figure 4. A Ward's (1963) cluster analysis of the 70 initial skull measurements. The cluster analysis illustrates the 6 major clusters (designated as I-VI) that were used to determine the final set of 30 measurements.

3.3 *Selection of measurements within major clusters*

The R-mode PCA loadings (Table 2) were used as one of the criteria for selecting characters within Ward's (1963) cluster analysis-derived subsets of measurements. Only PCA axes I and II were used as they contributed highly to the total variance (PCA I: 30.20% of the total variance; PCA II: 20.77% variance). Table 2 arranged according to the 6 major clusters derived from the Ward's (1963) cluster analysis summarizes the criteria used in the selection procedure in this study.

Apart from relative loadings in PCA I and II, additional criteria used as summarized in Table 2 included the CV, %ME, ease of measurement, and the potential for a measurement to capture the overall configuration of the phenotype. The following section provides the rationale behind the selection of characters within the major clusters. Since there were sub-clusters within the major clusters outlined above, it was decided to also select some measurements within these sub-clusters as long as they satisfied the set criteria in order to represent the overall phenotype of the cranium and mandible. Although some measurements such as NAS unequivocally clustered with teeth measurements for example, such measurements were also selected in the final data set so long as they contributed to the capturing of the overall configuration of the cranium and mandible. Apart from the criteria outlined above, measurements were also selected to reflect length, width, depth, as well as oblique measurements in order to maximize the capturing of the overall configuration of both the cranium and the mandible.

TABLE 2. A table representing the character selection criteria used in the selection of measurements for a morphometric study of the southern African hedgehog, *Atelerix frontalis*. Characters marked with an asterisk denote those characters that were selected. Ease of measurement is denoted as 1 = easy and 2 = difficult, the capturing of configuration is denoted as 1 = captures configuration, 2 = does not capture configuration.

Measurement	PCA I	PCA II	CV	%ME	Ease of measurement	Capture configuration
Major cluster I: Length measurements of cranium and mandible						
GLS*	-0.04	-0.87	0.14	0.06	1	1
GLN*	-0.51	-0.72	0.7	0.16	1	1
NPP	-0.33	-0.78	0.49	0.37	2	2
ZAL*	0.79	-0.4	0.55	0.12	1	1
CBL	-0.15	-0.87	0.21	0.13	1	2
PIC*	-0.05	-0.86	0.24	0.16	1	2
BSL	0.02	-0.76	0.21	0.11	1	2
PPL	0.15	-0.43	0.41	0.08	1	2
BUL	0.09	-0.45	2.03	1.37	2	2
BUW	0.02	-0.44	2.4	0.47	2	2
ITC	-0.15	-0.87	0.32	0.29	1	2
IOE	0.69	-0.38	0.1	0.01	2	2
GML*	0.91	-0.22	0.81	1.08	1	1
MDL	0.91	-0.25	0.93	1.41	1	2
MFA	0.76	-0.05	3.37	1.71	2	2
Major cluster II: Length and width measurements of cranium						
FRO	0.11	0.07	3.16	0.99	1	2
PAR	0.54	-0.16	0.47	0.09	1	2
BBC*	0.74	-0.31	0.39	0.09	1	1
PAL	-0.45	-0.73	0.23	0.04	1	2
TRL*	-0.78	-0.17	0.44	0.15	1	1
MPO*	0.19	-0.44	0.91	1.43	1	1
Major cluster II: Length and width measurements of cranium						
MPZ	0.07	-0.44	1.76	1.16	2	2
FME	0.05	-0.44	0.16	0.01	2	2
WAB	0.06	-0.42	1.95	1.08	2	2
MTL*	0.83	-0.32	0.42	0.08	1	1
WMP	0.01	0.03	0.76	0	1	2
Major cluster III: Teeth measurements and length of cranium						
INT	-0.6	-0.66	1.73	0.09	1	2
MAW*	0.82	0.1	0.04	0	1	1
PWM*	0.73	-0.37	0.51	0.03	1	1
UTR	-0.51	0.47	0.06	0	1	2
AFA	0.87	-0.19	0.86	0.09	1	2
MCA*	0.86	-0.1	0.69	0.11	1	1
IML*	0.86	-0.31	0.78	0.26	1	1
MTR	0.71	0.1	0.25	0.01	1	2
Major cluster IV: Depth and width measurements of the cranium:						
IOB*	0.78	-0.45	0.46	0.05	1	1
PAC*	-0.5	0.44	1.22	0.2	1	1
FJW	0.08	-0.45	0.33	0.02	2	2
IZD*	0.87	0.12	0.68	0.09	1	1
GHS*	-0.09	-0.2	0.64	0.13	1	1
CNW	0.07	-0.44	1.13	0.16	2	2

TABLE 2 continued

Measurement	PCA I	PCA II	CV	%ME	Ease of measurement	Capture configuration
Major cluster V: Depth and width measurements of cranium and mandible						
NPO	-0.51	-0.71	0.72	0.21	2	2
LPF	0.69	-0.13	0.52	0.03	1	2
VCW	0.89	-0.26	3.65	0.42	1	2
HOR*	-0.58	-0.68	0.9	0.08	1	1
FMH*	-0.24	-0.73	0.39	0.01	1	1
FMW*	0.06	-0.44	0.7	0.03	1	1
MRH*	0.89	-0.24	2.13	1.83	1	1
LMH	0.8	-0.14	6.12	1.77	1	2
MAF	0.88	-0.04	5.21	3.68	2	2
CMH	0.91	-0.18	1.52	1.04	1	2
Major cluster VI: Teeth measurements						
NAS*	-0.58	-0.68	0.46	0.02	1	1
WGI	-0.59	-0.67	3.83	0.11	1	2
BCH	0.07	-0.44	0.33	0.02	1	2
FIB	-0.61	-0.66	1.35	0.01	1	2
LPM	0.3	-0.19	1.19	0.02	1	2
LFM*	0.09	0.09	1.21	0.03	1	1
LSM*	-0.19	0.4	3.86	0.24	1	1
LTM	-0.36	0.52	4.73	0.24	1	2
WPM	0.4	0.04	0.53	0	1	2
WFM*	0.09	0.09	1.26	0.04	1	1
WSM*	-0.19	0.4	0.75	0.01	1	1
WTM	-0.36	0.52	1.13	0.01	1	2
LMP	-0.26	-0.45	2.54	0.06	1	2
LLM*	0.71	-0.2	0.55	0.01	1	1
Major cluster VI: Teeth measurements						
LMS*	-0.6	-0.66	0.9	0.01	1	1
LMT	-0.46	-0.09	1.68	0.01	1	2
WLM*	0.63	0	2.24	0.06	1	1
WMS*	-0.6	-0.66	2.44	0.07	1	1
WMT	-0.45	-0.09	10.65	0.5	1	2
% trace	30.2	20.77				

Major cluster I: Length measurements of the cranium and mandible

Measurement 1: Greatest length of skull (GLS) – GLS was selected because of its low CV and %ME, ease of the measurement, and its potential to capture the overall configuration of the cranium.

Measurement 2: Greatest length of nasals (GLN) – GLN was selected because of its low %ME, relative ease to measure, and its potential to capture the overall configuration of the anterior-dorsal part of the cranium.

Measurement 3: Zygomatic arch length (ZAL) – ZAL was selected because of its relatively high loading on PCA axis I, a low %ME, its relative ease to measure, as well its potential to capture the overall configuration of the middle part of the cranium.

Measurement 4: Incisor to condyle length (PIC) – PIC was selected because of a low CV and %ME, its relative ease of measurement, and its potential to capture the overall configuration of the basio-cranial part of the cranium.

Measurement 5: Greatest mandible length (GML) – GML was selected because of its high loading on PCA axis I, relative ease of measurement, and its potential to capture the overall configuration of the mandible.

Major cluster II: Length and width measurements of the cranium

Measurement 6: Breadth of braincase (BBC) – BBC was selected because of its relatively high loading on PCA axis I, relatively low CV and %ME, relative ease of measurement, and its potential to capture the overall configuration of the posterior-lateral part of the cranium.

Measurement 7: Toothrow length (TRL) – TRL was selected because of its low CV and %ME, relative ease of the measurement, as well as its potential to capture the overall tooth configuration.

Measurement 8: Foramen magnum-postorbital bar length (MPO) – MPO was selected due to its relative ease of measurement as well as its potential to capture the overall configuration of the posterior part of the cranium.

Measurement 9: Mandibular toothrow (MTL) – MTL was selected because of its low %ME, relative ease of the measurement, and its potential to capture the overall configuration of the mandibular toothrow.

Major cluster III: Teeth measurements

Measurement 10: Greatest maxillary width between labial crown edges of M^1 (MAW) – MAW was selected because of its relatively high loading on the PCA axis I, a low

CV and %ME, its relative ease of measurement, and its potential to capture the overall maxillary teeth.

Measurement 11: Hard palate width M^1 measured on lingual side of teeth at alveolus (PMW) – PWM was selected due to its relatively high loading on PCA axis I, a low %ME, its relative ease of measurement, as well as its potential to capture the overall configuration of the ventral part of the cranium.

Measurement 12: Mandibular condyle-angular process distance, in straight line from dorsal edge of mandibular condyle to ventral edge of angular process (MCA) – MCA was selected because of its relatively high loading on PCA axis I, a low %ME, relative ease of measurement, and its potential to capture the overall configuration of the mandible.

Measurement 13: Posterior incisor-M_3 length (IML) – IML was selected because of its relatively high loading on PCA axis I, a relatively low %ME, its relative ease of measurement, as well as its potential to capture the overall configuration of mandibular teeth.

Major cluster IV: Depth and width measurements of the cranium

Measurement 14: Least breadth of interorbital constriction (IOB) – IOB was selected because of its relatively high loading on PCA axis I, a relatively low CV and % ME, relative ease of measurement, and its potential to capture the overall configuration of the dorsal part of the cranium.

Measurement 15: Greatest maxillary width between labial crown edges of M^1 (PAC) – PAC was selected because of its low %ME, relative ease of measurement, and its potential to capture the overall maxillary teeth configuration.

Measurement 16: Infaorbital-zygomatic plate distance (IZD) – IZD was selected because of its relatively high loading on PCA axis I, a low CV %ME, relative ease of measurement, and it has the potential to capture the overall configuration of the dorsal part of the cranium.

Measurement 17: Greatest height of skull perpendicular to horizontal plane through bullae (GHS) – GHS was selected because of its low %ME, relative ease of measurement, as well as its potential to capture the overall configuration of the lateral part of cranium.

Major cluster V: Depth and width measurements of the cranium and mandible

Measurement 18: Height of rostrum (HOR) – HOR was selected because of its low %ME, relative ease of measurement, and has the potential to capture the overall configuration of the antero-lateral part of the cranium.

Measurement 19: Foramen magnum height (FMH) – FMH was selected due to its low CV and %ME, relative ease of measurement, and its potential to capture the overall configuration of the posterior part of the cranium.

Measurement 20: Foramen magnum width (FMW) – FMW was selected because of its low %ME, relative ease of the measurement, and its potential to capture the overall configuration of the posterior part of the cranium.

Measurement 21: Mandible-ramus height (MRH) – MRH was selected because of its relative ease of measurement, and its potential to capture the overall configuration of the posterior part of the mandible.

Major cluster VI: Teeth measurements

Measurement 22: Nasal width (NAS) – NAS was selected because of its low CV and %ME, relative ease of measurement and its potential to capture the overall configuration of the antero-dorsal part of the cranium.

Measurement 23: Length of M^1 along cingulum (LFM) – LFM was selected due to its low %ME, relative ease of measurement and its potential to capture the overall configuration of maxillary teeth.

Measurement 24: Length of M^2 along cingulum (LSM) – LSM was selected because of its low %ME, relative ease of measurement, and has the potential to capture the overall configuration of maxillary teeth.

Measurement 25: Greatest cross-sectional crown width of M^1 (WFM) – WFM was selected because low %ME, relative ease of measurement, as well as its potential to capture the overall configuration of maxillary teeth.

Measurement 26: Greatest cross-sectional crown width of M^2 (WSM) – WSM was selected because of its low %ME, relative ease of measurement, and its potential to capture the overall configuration of maxillary teeth.

Measurement 27: Length of M_1 along cingulum (LLM) – LLM was selected because of its low CV and %ME, relative ease of measurement, and its potential to capture the overall maxillary teeth configuration.

Measurement 28: Length of M_2 along cingulum (LMS) – LMS was selected due to its low %ME, relative ease of measurement, and its potential to capture the overall mandibular teeth configuration.

Measurement 29: Greatest cross-sectional crown width of M_1 (WLM) – WLM was selected due to its low %ME, relative ease of measurement, and its potential to capture the overall configuration of mandibular teeth.

Measurement 30: Greatest cross-sectional crown width of M_2 (WMS) – WSM was selected because of its low %ME, relative ease of measurement, and its potential to capture the overall mandibular teeth configuration.

[4] Discussion

A preliminary assessment of sexual dimorphism in the present study revealed a lack of sexual dimorphism among measurements within the southern African hedgehog. None of the measurements showed statistically significant sexual dimorphism in the univariate analyses and were supported by the results of the multivariate UPGMA cluster analysis and PCA. Consequently, the sexes were pooled in the analyses of character associations as well as in all subsequent analyses of geographic variation within the revision of the southern African hedgehog.

The present investigation was based on a measurement selection procedure in *Aethomys* (Rodentia: Muridae) from southern Africa (Chimimba & Dippenaar 1995) and weevils from the sub-Antarctic Marion Island (Janse van Rensburg *et al.* 2003). The procedure applied attempted to identify a reduced number of measurements that could summarize morphometric variation in the overall cranial and mandibular configuration in the southern African hedgehog. The selection of the final set of measurements was based on variable loadings from an R-mode PCA, coefficients of variation, percent measurement error, relative ease of measurement, and the potential to capture the overall configuration of the phenotype.

A Ward's (1963) cluster analysis (based on the principal components scores of a Q-mode PCA) generated six major clusters of highly correlated measurements from within which a final character set of 30 measurements from an initial 70 measurements was selected based on the criteria outlined above. Overall, percentage measurement error in the southern African hedgehog was negligible. Low percent measurement error was also apparent in both the study on *Aethomys* (Chimimba & Dippenaar 1995) as well as that of the weevils from the sub-Antarctic Marion Island (Janse van Rensburg *et al.* 2003). However, percent measurement error values of over 50% have been recorded in studies on birds and mussels (Bailey and Byrnes 1990).

The 30 measurements chosen for the subsequent revision of the southern African hedgehog include: greatest length of skull (GLS), greatest length of nasals (GLN), zygomatic arch length (ZAL), incisor to condyle length (PIC), greatest mandible length (GML), breadth of braincase (BBC), toothrow length (TRL), foramen magnum-postorbital bar length (MPO), mandibular toothrow (MTL), greatest maxillary width between labial crown edges of M^1 (MAW), hard palate width at M^1 measured on lingual side of teeth at alveolus (PMW), mandibular condyle-angular process distance, in straight line from dorsal edge of mandibular condyle to ventral edge of angular process (MCA), posterior incisor-M_3 length (IML), least breadth of interorbital constriction (IOB), greatest maxillary width between labial crown edges of M^1 (PAC), infaorbital-zygomatic plate distance (IZD), greatest height of skull perpendicular to horizontal plane through bullae (GHS), height of rostrum (HOR), foramen magnum height (FMH), foramen magnum width (FMW), mandible-ramus height (MRH), nasal width (NAS), length of M^1 along cingulum (LFM), length of M^2

along cingulum (LSM), greatest cross-sectional crown width of M^1 (WFM), greatest cross-sectional crown width of M^2 (WSM), length of M_1 along cingulum (LLM), length of M_2 along cingulum (LMS), greatest cross-sectional crown width of M_1 (WLM), greatest cross-sectional crown width of M_2 (WMS).

These measurements were selected in an attempt to fulfill two important requirements namely, "comprehensiveness" through the consideration of adequate coverage of the phenotype, and "economy" through the removal of redundant measurements. It has been reported that the use of unevaluated measurements may have an effect on analyses (Chimimba & Dippenaar 1995). These range from distortions in inter-operational taxonomic units (OTU; Sneath & Sokal 1973) relationships to an increase in analysis time that results in analytical problems while processing large data matrices. It has been shown that after the assessment of redundancy (or linear dependency) and co-linearity, large quantitative measurement sets can be reduced to a few and still contain equivalent information. More importantly, the procedure followed can have a wide application in a range of taxa.

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Appendix I

A gazetteer and geographic coordinates of sampled localities and specimens of the southern African hedgehog, *Atelerix frontalis* examined in the present study. TM denotes the Transvaal Museum of the Northern Flagship Institute (NFI), Pretoria, South Africa.

Locality	Geographic co-ordinates	Museum number
Pretoria, Irene	25º 45’ S; 28º 00’E	TM 25699; 25700
Rooiberg	24º 50’S; 27º 44’E	TM 749; 750
Heidelberg	26º 30’S; 28º 00’E	TM 25197
Waterpoort, Rochdale	22º 45’S; 28º 30’E	TM 19970
Pretoria	25º 42’S; 28º 13’E	TM 2857; 4113; 5686; 7375; 16603; 16611; 25942; 27406; 40314
Pretoria, Silverton	25º 43’S; 28º 20’E	TM 27408
Pretoria, Derdepoort	25º 40’S; 28º 20’E	TM 27684
Waterberg	25º 44’S; 28º 01’E	TM 1570
Settlers	24º 57‘S; 28º 32’E	TM 28496
Pretoria, De Wildt	25º 37’S; 27º 57’E	TM 5554; 5687
Zebediela	24º 18’S; 29º 15’E	TM 12203
Pretoria, Waterkloof	25º 47’S; 28º 16’E	TM 15504
Delarayville	26º 41’S; 25º 28’E	TM 23439
Pretoria, Hatfield	25º 44’S; 28º 13’E	TM 1830
Pietersburg	23º 54’S; 29º 27’E	TM 12470
Krugersdorp	26º 06’S; 27º 46’E	TM 27409

Chapter 3

Non-geographic variation in the southern African hedgehog, *Atelerix frontalis*

Abstract

Prior to a systematic revision of the near-threatened southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831), the nature and extent of non-geographic variation due to age variation and sexual dimorphism were first examined using both traditional and geometric morphometric analyses of the cranium and mandible. These analyses, based on the largest available geographically proximal sample from a uniform habitat in South Africa were undertaken with the primary objective of establishing criteria for the selection of adult specimens to consider for subsequent data recording and analysis, and whether to analyse sexes separately or together during the systematic revision of *A. frontalis* from southern Africa. The results of both traditional and geometric morphometric analyses were similar and showed a lack of sexual dimorphism in the specimens examined. However, these analyses showed marked morphometric variation between four relative age classes based on the degree of maxillary molar eruption and wear. All analyses suggested that individuals of age classes I and II represent either juvenile or subadult individuals, while those of age classes III and IV represent adult individuals. These results justified the pooling of sexes as well as individuals of age classes III and IV for subsequent data recording and analysis. The present study represents the first known analysis of non-geographic variation in the southern African hedgehog.

[1] Introduction

The present study examines the nature and extent of non-geographic variation at the level of sexual dimorphism and age variation in the near-threatened (Friedman & Daly 2004) southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831) based on the largest available geographically proximal sample from a uniform habitat in South Africa. The study represents a preliminary analysis to a systematic revision of the southern African hedgehog.

The assessment of non-geographic variation is fundamental to morphometric studies of geographic variation (Thorpe 1976; Straney 1978; Leamy 1983; Webster &

Jones 1985; Dippenaar & Rautenbach 1986; Van der Straeten & Dieterlen 1992). While some authors (e.g., Leamy & Bader 1968) consider non-geographic variation to be composed of genetic and non-genetic components, most authors view it as a function of differences in sex, age, season, cohort, and individuals within a population (e.g., Thorpe 1976; Straney 1978; Leamy 1983; Webster & Jones 1985; Dippenaar & Rautenbach 1986; Van der Straeten & Dieterlen 1992). However, due to the unavailability of the appropriate data and for practical reasons, particularly for mammals, most analyses of non-geographic variation are restricted to the analyses of sexual dimorphism and age variation (Dippenaar & Rautenbach 1986; Chimimba & Dippenaar 1994).

Nevertheless, absolute mammalian age is difficult to measure directly (Fairall 1980). Consequently, various methods for its estimation have been proposed. Among these methods, the degree of molar eruption and wear is considered a reliable indicator of age, and has been applied to various mammals ranging from mole-rats (Taylor *et al.* 1985, Janse van Rensburg *et al.* 2004; Hart *et al.* in press) to murid rodents (Dippenaar & Rautenbach 1986, Chimimba and Dippenaar 1994).

However, the use of molar eruption and wear for ageing in mammals has been criticised due to the potential influence of factors such as genetic differences in enamel hardness, nutrition, diet, and health (Hall *et al.* 1957; Keiss 1969; Gilbert & Stolt 1970; Chaplin & White 1969; Morris 1972). Nevertheless, the use of the degree of molar eruption and wear to estimate relative rather than absolute mammalian age is considered to be appropriate for a wide range of mammals, particularly if the sample examined is from a homogenous population which reduces the potentially confounding factors mentioned, and those associated with geographic variation (Chaplin & White 1969; Gilbert *et al.* 1970; Taylor *et al.* 1985; Dippenaar & Rautenbach 1986; Chimimba & Dippenaar 1994; Janse van Rensburg *et al.* 2004; Hart *et al.* in press).

Consequently, prior to the systematic revision of the southern African hedgehog, the present study uses the degree of molar eruption and wear to assess sexual dimorphism and relative age variation using both traditional and geometric morphometric analyses of the cranium and mandible. These analyses are undertaken

with the primary objective of establishing criteria for the selection of specimens to consider for data recording and analysis and whether to analyse the sexes separately or together during the systematic revision of the southern African hedgehog. The present study represents the first known analysis of non-geographic variation in the southern African hedgehog.

[2] Materials and methods

2.1 *Specimens examined*

The analysis of age variation in the southern African hedgehog was based on 27 specimens, while the analysis of sexual dimorphism was based on 19 specimens. These specimens represented samples in the mammal collection of the Transvaal Museum (TM) of the Northern Flagship Institute (NFI), Pretoria, South Africa. This selection of 27 specimens represents the largest available geographically proximal sample from a uniform habitat in South (Table 1).

TABLE 1. A gazetteer and geographic coordinates of sampled localities and specimens of the southern African hedgehog, *Atelerix frontalis* examined in the present study. TM denotes the Transvaal Museum of the Northern Flagship Institute (NFI), Pretoria, South Africa.

Locality	Geographic co-ordinates	Museum number
Rooiberg	24º 50'S; 27º 44'E	TM 749
Pretoria	25º 42'S; 28º 13'E	TM 7375; 16611; 25942; 27406; 40314
Pretoria, Silverton	25º 43'S; 28º 20'E	TM 27408
Pretoria, Derdepoort	25º 40'S; 28º 20'E	TM 27684
Waterberg	25º 44'S; 28º 01'E	TM 1570
Settlers	24º 57'S; 28º 32'E	TM 28496
Pretoria, De Wildt	25º 37'S; 27º 57'E	TM 5554
Zebediela	24º 18'S; 29º 15'E	TM 12203
Pretoria, Waterkloof	25º 47'S; 28º 16'E	TM 15504
Delarayville	26º 41'S; 25º 28'E	TM 23439

2.2 *Ageing of specimens*

Relative ageing of specimens was based on the degree of molar eruption and wear as illustrated in Fig. 1 and defined as follows: 1) age class I: M^3 not erupted; 2) age class II: M^3 erupted but not fully; 3) age class III: M^3 fully erupted but with no evidence of tooth wear; and 4) age class IV: M^3 fully erupted and with evidence of tooth wear.

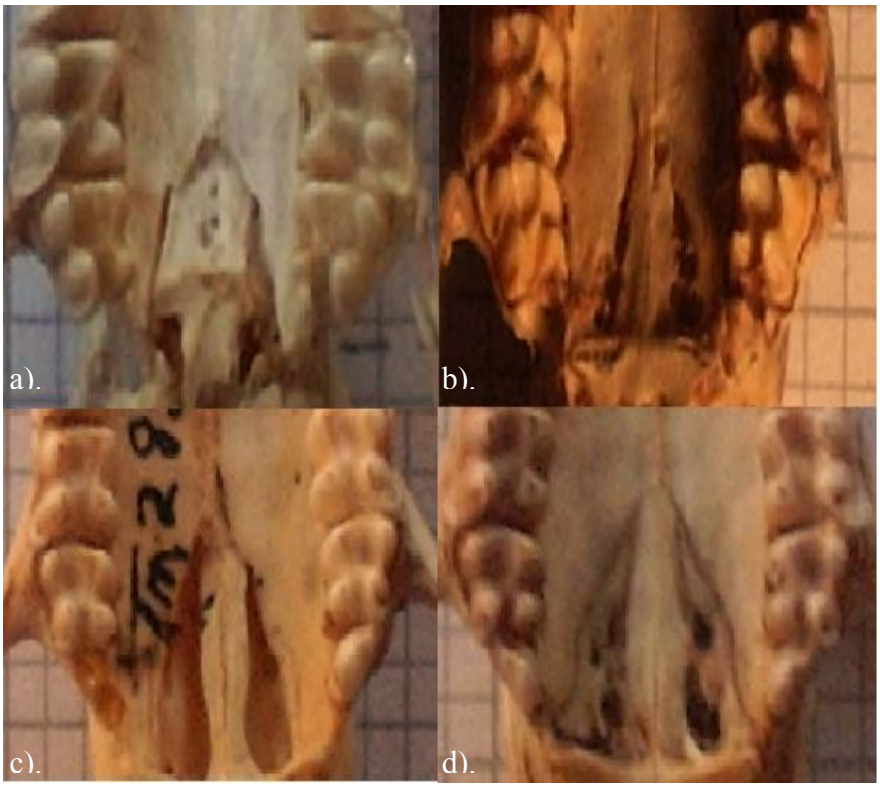

Figure 1. An illustration of the relative age classes assigned to specimens of the southern African hedgehog, *Atelerix frontalis* for the analysis of age variation: a) Age class I; b) Age class II; c) Age class III; and d) Age class IV. Age classes are defined in the section on "Ageing of specimens" above.

2.3 *Morphometrics*

Analytical subdivision of the data into sex and age class groupings precluded the simultaneous univariate morphometric assessment of sexual dimorphism and age variation within the sample because of *within*-group sample size limitations. Consequently, sexual dimorphism was first assessed independently by one-way analysis of variance (ANOVA; Zar 1996) using adequately represented samples of age classes III and IV. These analyses were followed by the independent assessment of the nature and extent of age variation within the sample using one-way ANOVA of adequately represented samples of age classes II, III and IV. All ANOVAs were undertaken after tests for normality and homogeneity of variances showed that the data satisfied the assumptions of ANOVA (Zar 1996).

The univariate analyses of sexual dimorphism and age variation were followed by multivariate analyses of both traditional and geometric morphometric data. Among

other uses, morphometrics is useful as a systematic tool to quantify morphological differences both *within* and *among* operational taxonomic units (OTUs; Sneath & Sokal 1973), where joint relationships in character complexes are assessed simultaneously by the reduction of large character sets to a few dimensions (James & McCulloch 1990). This can be achieved by linear/orthogonal measurement-based traditional morphometrics and/or unit-free landmark/outline-based geometric morphometrics (Marcus 1990; Rohlf & Marcus 1993).

2.4 *Traditional morphometrics*

All traditional morphometric analyses were based on 21 cranial and 9 mandibular measurements from the character selection procedure in Chapter 2 and are defined and illustrated in Fig. 2. These measurements were selected to adequately represent cranial and mandibular phenotypes in the southern African hedgehog. All measurements were recorded to the nearest 0.05 mm by one observer (LR) using a pair of Mitutoyo® digital callipers (Mitutoyo American Corporation, Aurora, Illinois, U.S.A).

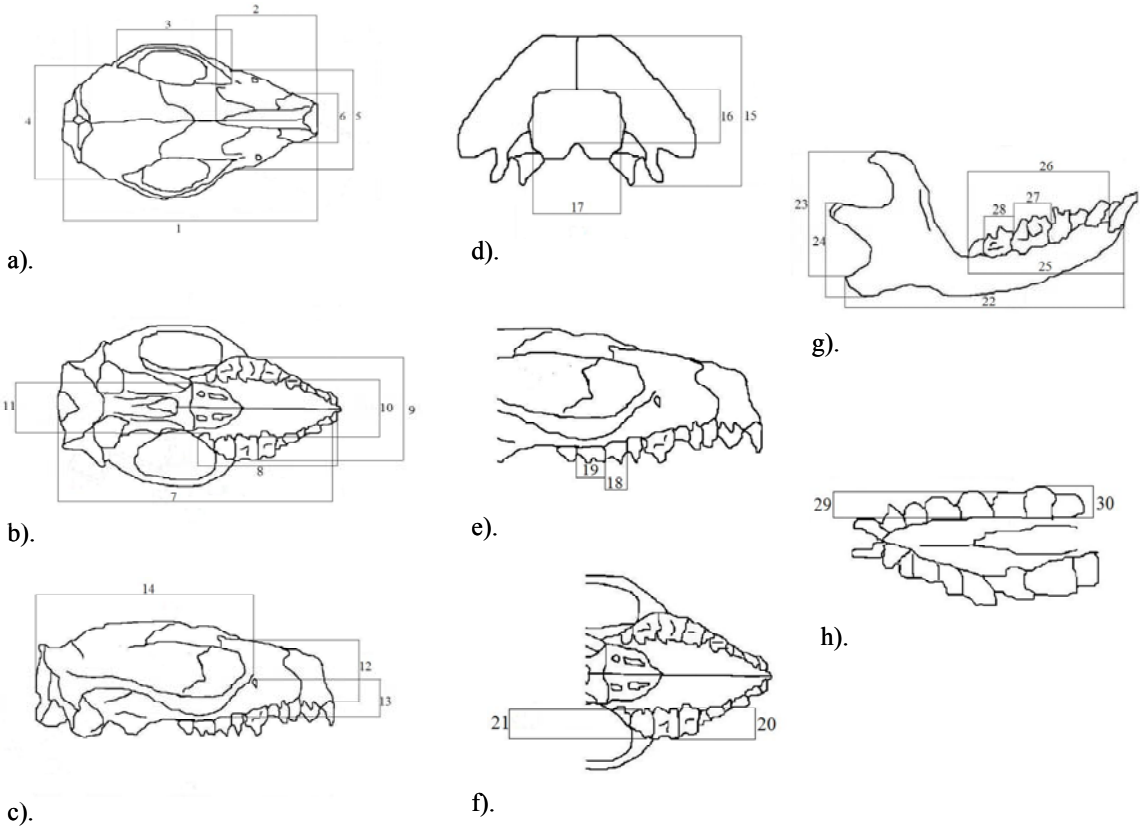

Figure 2. Cranial and manibular measurements and their measuring points recorded in various views (a-h) of the southern African hedgehog, *Atelerix frontalis* in the present study: 1. GLS – greatest length of skull, from anterior edge of nasals to posterior edge of occipital condyle, along longitudinal axis of skull; 2. GLN – greatest length of nasals, from posterior projection of nasal wings to anterior-most edge of nasal bones; 3. ZAL – zygomatic arch length, from posterior-most part of anterior part of zygomatic arch to anterior-most part of posterior part of zygomatic arch; 4. BBC – breadth of braincase width at dorsal root of squamosals; 5. IOB – least breadth of interorbital constriction, least distance dorsally between orbits; 6. NAS – nasal width, at anterior-most point where nasals join premaxillae; 7. PIC – incisor to condyle length, from posterior surface of I^1 at alveolus to posterior-most projection of occipital condyle; 8. TRL – toothrow length, from anterior alveolus to posterior surface of M^1 alveolus; 9. MAW – greatest maxillary width between labial crown edges of M^1; 10. PWM – hard palate width at M^1 measured on lingual side of teeth at alveolus; 11. PAC – hard palate width at point of constriction immediately posterior to M^3; 12. HOR – height of rostrum, perpendicularly from a point directly behind upper incisors; 13. IZD – infraorbital-zygomatic plate distance, from dorsal edge of infraorbital foramen to anterior base of zygomatic plate; 14. MPO – foramen magnum-postorbital bar length, from lateral edge of foramen magnum to anterior edge of postorbital bar; 15. GHS – greatest height of skull perpendicular to horizontal plane through bullae; 16. FMH – foramen magnum height, widest part of foramen in vertical plane; 17. FMW – foramen magnum width, widest part of foramen magnum in horizontal plane; 18. LFM – length of M^1 along cingulum; 19. LSM – length of M^2 along cingulum; 20. WFM – greatest cross-sectional crown width of M^1; 21. WSM – greatest cross-sectional crown width of M^2; 22. GML – greatest mandible length, in a straight line from anterior edge of I_1 alveolus to

posterior surface of angular process; 23. MRH – mandible-ramus height, from dorsal edge of coronoid process to ventral edge of angular process; 24. MCA – mandibular condyle-angular process distance, in straight line from dorsal edge of mandibular condyle to ventral edge of angular process; 25. MTL – mandibular toothrow length, from anterior edge of I_1 alveolus to posterior edge of M_3 alveolus; 26. IML – posterior incisor- M_3 length, in a straight line from posterior edge of I_1 alveolus to posterior edge of M_3 alveolus; 27. LLM – length of M_1 along cingulum; 28. LMS – length of M_2 along cingulum; 29. WLM – greatest cross-sectional crown width of M_1; 30. WMS – greatest cross-sectional crown width of M_2.

2.5 *Geometric morphometrics*

Geometric morphometrics (Marcus & Corti 1996), which is considered to be more superior in assessing organismal shape differences in morphology than traditional morphometrics (Marcus & Corti 1996), was also used to assess age and sexual dimorphism-related cranial shape differences in the southern African hedgehog. A Pentax® Opti 33I digital camera attached to a tripod stand was used to capture images of the dorsal, ventral, and lateral views of the cranium, as well as lateral views of the mandible of each specimen (Fig. 3). To standardize the image capturing procedure, each specimen was placed on a fixed piece of marked graph paper. All images were captured by one observer (LR).

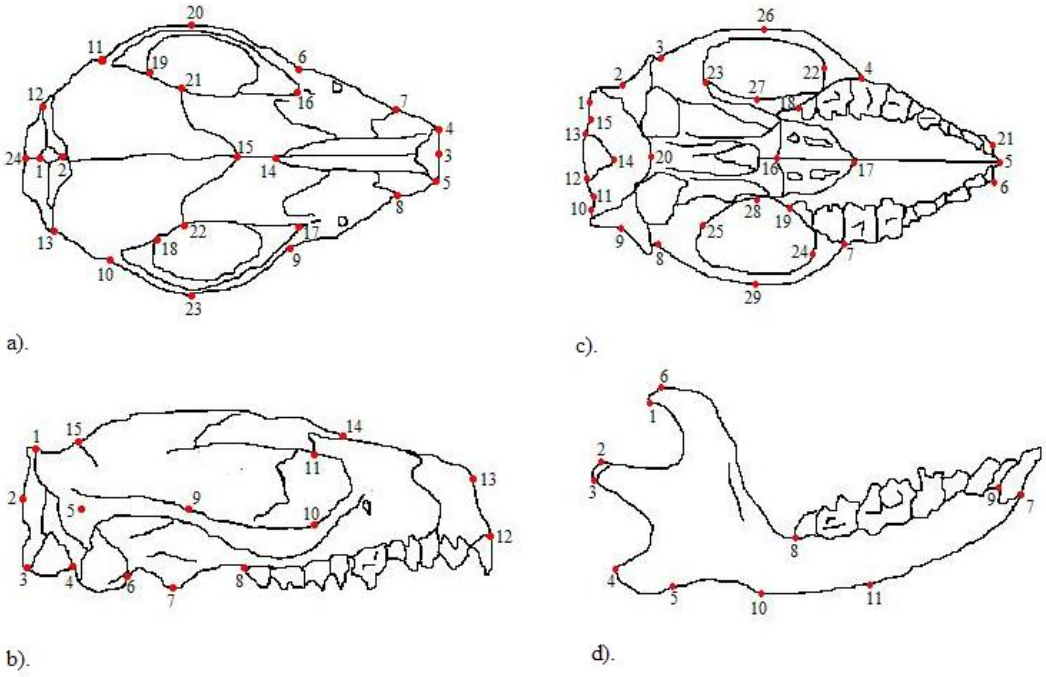

Figure 3. Landmarks of the dorsal (a), lateral (b), and ventral (c) views of the cranium, and the lateral view of the mandible (d) used in the geometric morphometric analyses of age variation and sexual dimorphism in the southern African hedgehog, *Atelerix frontalis* in the present study.

2.6 *Digitizing error*

A Thin Plate Spline (TPS) sub-routine, TPSDig (Rohlf 2004a) was used to digitize landmarks, each with an (*x*,*y*) coordinate, on each of the four views for each specimen. Landmarks captured included 24, 15, and 29 landmarks of the dorsal, lateral and ventral views of the cranium, respectively, and 12 landmarks of the lateral view of the mandible (Fig. 3). In order to assess the degree of landmark digitizing error (DE), the degree of error was expressed as a percentage (%DE) of the total variability due to within-individual variation (Pankakoski *et al.* 1987; Bailey & Byrnes 1990). The %DE analysis was based on three independent data sets of repeated digitized landmarks on the sample derived by LR on three separate occasions. Because the analyses revealed very low %DE values, averages of landmarks were computed and used in all subsequent geometric morphometric analyses. A TPS sub-routine, TPSSpline (Rohlf 2004b), was used to compute splines in order to compare each specimen to a consensus configuration in order to detect any subtle differences in cranial and mandibular shape morphology (Marcus & Corti 1996) with reference to age variation and sexual dimorphism.

2.7 *Morphometric analysis*

All generated traditional and geometric morphometric data were subjected to a series of analyses to identify phenetic groupings in which no *a priori* sub-divisions of samples were presumed using an Unweighted-pair group arithmetic average (UPGMA) cluster analysis and principal components analysis (PCA; Jolliffe 1986) of standardized data (Sneath & Sokal 1973). Cluster analysis is a multivariate method used to group entities *a priori* based on distances with sets arranged hierarchically and represented in a phenogram (or dendogram) in which similar entities are clustered together.

Among the various clustering methods, UPGMA cluster analysis is recommended in systematics (Sneath & Sokal 1973) because being a cross-averaging algorithm, it conserves space by minimizing input and output distances leading to a distribution of Operational Taxonomic Units (OTUs; Sneath & Sokal) into a reasonable number of groups (James & McCulloch 1990). The UPGMA cluster analysis of the traditional morphometric data was based on both Euclidean distances and correlation coefficients among groups (Sneath & Sokal 1973). The former coefficient focuses on size, while the latter reduces the influence of absolute size to allow a focus on shape (James and McCulloch 1990). The UPGMA cluster analysis of the geometric morphometric data was based on procrustes distances generated from the TPS sub-routine, TPSSmall (Rohlf 2004c).

PCA is also an *a priori* data reduction method in which variables or components of linear combinations of original data responsible for much of the variation in the data set are shown (Jolliffe 1986). PCA projects points from the original data on two dimensions with axes corresponding to the two most essential components. Minimal information is lost during its computation and it is recommended for analyzing morphometric data. The PCA of the traditional morphometric data was based on product-moment correlation coefficients among variables (Sneath & Sokal 1973). The PCA of the geometric morphometric data was based on a weighted matrix generated from the TPS sub-routine TPSRelw (Rohlf 2004d) that was used to perform a relative warps analysis, which is equivalent to a PCA.

Other analyses in the study included the generation of standard univariate descriptive statistics for each age/sex phenetic groups. All analyses in this study were accomplished using algorithms in Statistica version 6.0 (StatSoft Inc. 2004) and/or sub-routines in the TPS (Rohlf 2004a-d) series of programmes.

[3] Results

Preliminary analyses include the assessment of sexual dimorphism within each age class. However, due to sample size limitations this was only possible in age classes III and IV that had adequate sample sizes for both male and female individuals. The lack of sexual dimorphism necessitated the pooling of individuals of age class III and IV in order to assess sexual dimorphism in the southern African hedgehog.

3.1 Sexual dimorphism

3.1.1 *Traditional morphometric data*

Due to damage in some specimens that resulted in missing data, the ANOVA of the traditional morphometric data used to asses sexual dimorphism in the southern African hedgehog was based on 22 of the 30 initial measurements. Similarly, because of sample size limitations, the ANOVA was only based on individuals of age classes III and IV that had adequate sample sizes of both males and females. *F*-values from a one-way ANOVA of the sample showed no measurement to be sexually dimorphic in individuals of age classes III and IV (Table 2).

TABLE 2. *F*-values from a one-way analysis of variance (ANOVA) used to assess sexual dimorphism in the southern African hedgehog, *Atelerix frontalis* using measurements as defined and illustrated in Figure 2.

Measurement	*F*-value
Greatest length of skull	0.73^{ns}
Greatest length of nasals	0.52 ^{ns}
Zygomatic arch length	3.31 ^{ns}
Breadth of braincase width	3.22 ^{ns}
Least breadth of interorbital constriction	1.01 ^{ns}
Nasal width	0.16 ^{ns}
Incisor to condyle length	0.83 ^{ns}
Greatest maxillary width between labial crown edges of M^1	0.64 ^{ns}
Hard palate width at M^1	3.74 ^{ns}
Height of rostrum	0.67 ^{ns}
Infraorbital-zygomatic plate distance	0.30 ^{ns}
Length of M^1	0.02 ^{ns}
Greatest cross-sectional crown width of M^1	1.38 ^{ns}
Greatest mandible length	0.13 ^{ns}
Mandible-ramus height	0.36 ^{ns}
Mandibular condyle-angular process distance	0.38 ^{ns}
Mandibular toothrow length	0.07 ^{ns}
Posterior incisor-M_3 length	0.08 ^{ns}
Length of M_1	1.36 ^{ns}
Length of M_2	0.00 ^{ns}
Greatest cross-sectional crown width of M_1	0.17 ^{ns}
Greatest cross-sectional crown width of M_2	0.26 ^{ns}

^{ns} = not statistically significant.

Similarly, a PCA scatterplot of the first and second principal components axes (Fig. 4) that explained 36.12 % and18.46 % of the total variance, respectively (Table 3), showed a lack of sexual dimorphism in multivariate space. The general lack of sexual dimorphism was also evident in all subsequent PCA axes generated (i.e., PCA axes III-XXII).

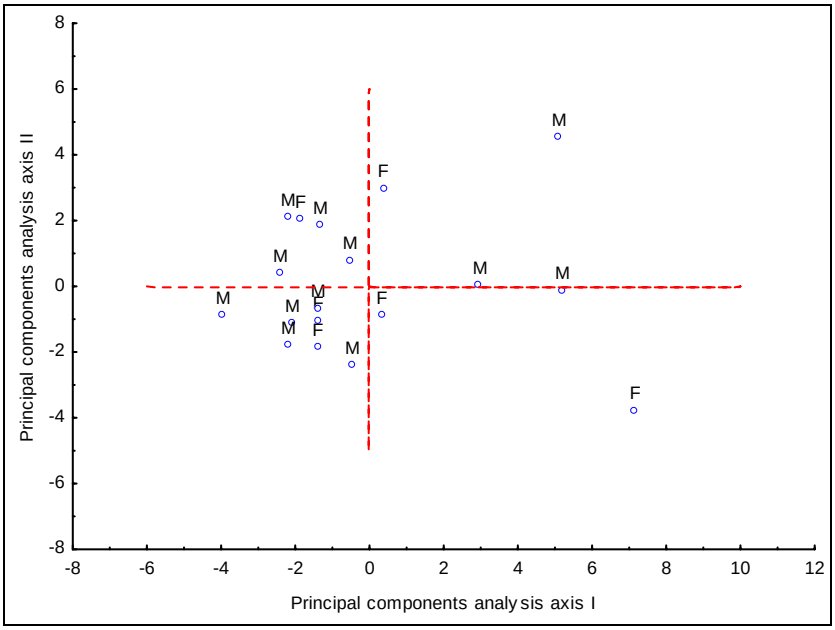

Figure 4. Axes I and II from a principal components analysis (PCA) used to assess sexual dimorphism (M = males; F = females) in the southern African hedgehog, *Atelerix frontalis*.

TABLE 3. Loadings of variables on the first and second principal components from a principal components analysis (PCA) used to assess sexual dimorphism in the southern African hedgehog *Atelerix frontalis*. Measurements are defined and illustrated in Figure 2.

Measurement	PCA I	PCA II
Greatest length of skull	-0.92	0.10
Greatest length of nasals	-0.65	0.17
Zygomatic arch length	-0.76	0.30
Breadth of braincase width	-0.63	0.14
Least breadth of interorbital constriction	-0.70	0.30
Nasal width	-0.55	-0.24
Incisor to condyle length	-0.88	0.12
Greatest maxillary width between labial crown edges of M^1	-0.47	-0.35
Hard palate width at M^1	-0.59	-0.04
Height of rostrum	-0.08	0.74
Infraorbital-zygomatic plate distance	-0.38	0.52
Length of M^1	0.27	-0.29
Greatest cross-sectional crown width of M^1	-0.12	-0.43
Greatest mandible length	-0.95	0.09
Mandible-ramus height	-0.65	-0.40
Mandibular condyle-angular process distance	-0.70	-0.40
Mandibular toothrow length	-0.66	-0.40
Posterior incisor-M_3 length	-0.63	-0.45
Length of M_1	-0.05	-0.80
Length of M_2	-0.43	-0.75
Greatest cross-sectional crown width of M_1	-0.54	-0.39
Greatest cross-sectional crown width of M_2	-0.44	-0.69
% trace	36.12%	18.46%

The results of the UPGMA cluster analysis based on both Euclidean distances and correlation coefficients were similar. As exemplified by a Euclidean distance phenogram (Fig. 5), similar to the ANOVA and PCA of sexual dimorphism based on traditional morphometric data above, the UPGMA cluster analysis also showed a general lack of sexual dimorphism in the southern African hedgehog.

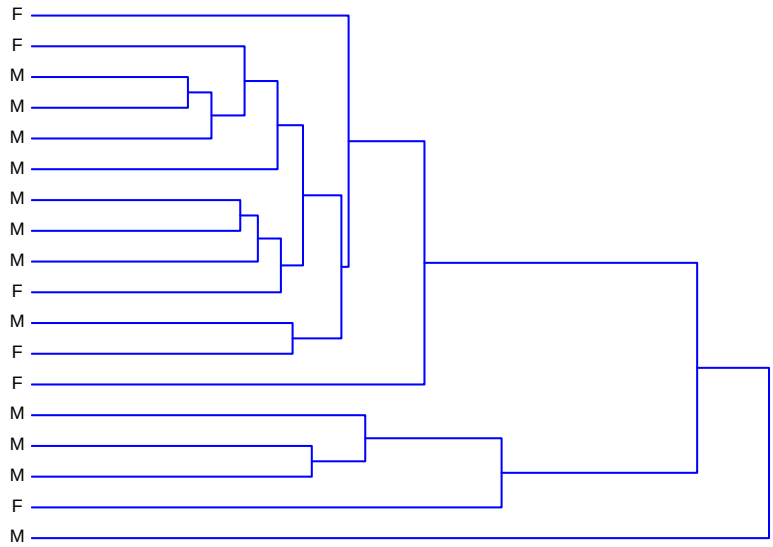

Figure 5. A Euclidean distance phenogram from an Unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis to assess sexual dimorphism (M = males; F = females) in the southern African hedgehog, *Atelerix frontalis*.

3.1.2 *Geometric morphometric data*

The results of the geometric morphometric analyses of the dorsal, lateral, and ventral views of the cranium, and the lateral view of the mandible to assess sexual dimorphism in *A. frontalis* were similar, and these results are best exemplified by those of the PCA (Fig. 6) and UPGMA cluster analysis (Fig. 7) of the dorsal view of the cranium. The PCA scatterplot (Fig. 6) of the first relative warp (RW) explained 25.58 % of the total variance and the second RW accounts for 19.02 % of the total variance in the geometric morphometric data of individuals of age classes III and IV. Similar to the ANOVA, PCA and UPGMA cluster analysis of the traditional morphometric data, the PCA of the geometric morphometric data showed no separation between the sexes in multivariate space.

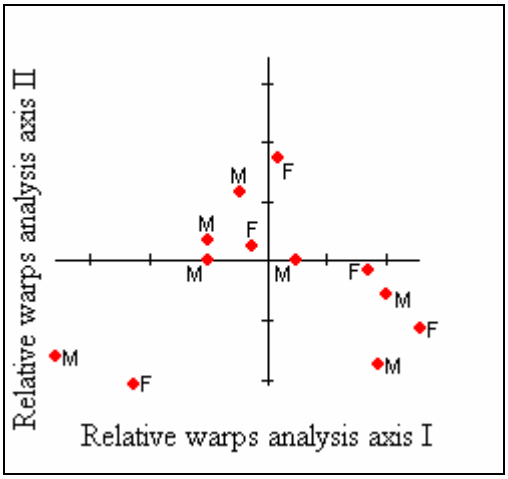

Figure 6. A scatterplot of relative warps (RW) I and II from a principal components analysis (PCA) of geometric morphometric data of the dorsal view of the cranium used to assess sexual dimorphism (M = males; F = females) in the southern African hedgehog, *Atelerix frontalis*.

Similarly, the procrustes distance phenogram from the UPGMA cluster analysis (Fig. 7) showed no discrete groupings of the sexes. The lack of sexual dimorphism is also shown by minimal changes in the position of landmarks for males and females with reference to a consensus configuration of the dorsal view of the cranium derived from TPSSpline (Fig. 8). The splines for males and females were very similar and as exemplified by that of males are illustrated against the consensus (Fig. 8). These results confirm the lack of sexual dimorphism in the southern African hedgehog.

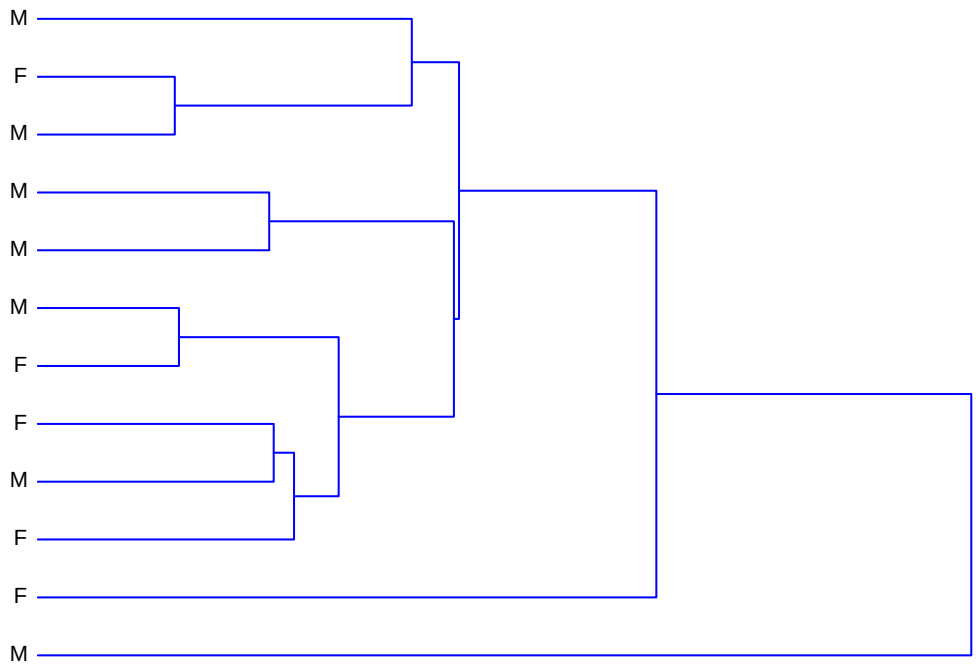

Figure 7. A procrustes distance phenogram from an Unweighted-pair group arithmetic average (UPGMA) cluster analysis of geometric morphometric data of the dorsal view of the cranium used to assess sexual dimorphism (M = males; F = females) in the southern African hedgehog, *Atelerix frontalis*.

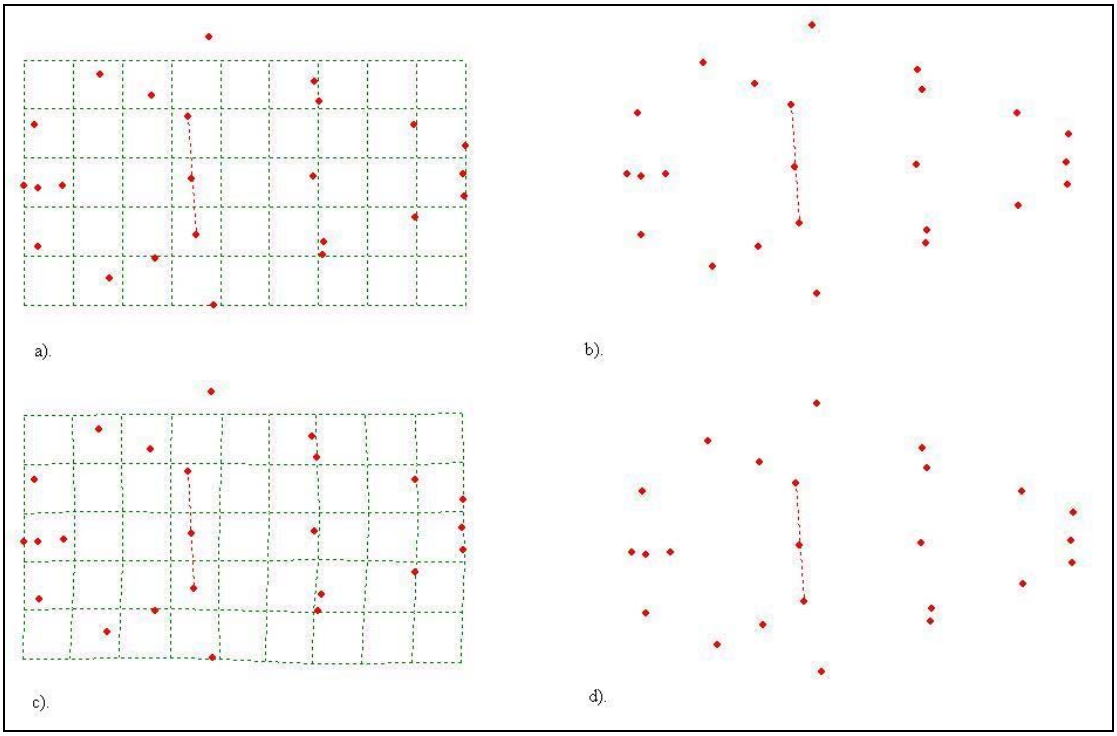

Figure 8. Changes in the position of landmarks with reference to a consensus configuration (splines) of the dorsal view of the cranium of the southern African hedgehog, *Atelerix frontalis*, derived from TPSSpline (Rohlf 2004b) are indicated for the consensus configuration (a & b) and exemplified by that of the males (c & d) which was essentially similar to that of females.

While ideally, a canonical variates analysis (CVA, Sneath & Sokal 1973) that maximizes variation *among* and minimizes variation *within* specified groups should also have been undertaken using both traditional and geometric morphometric data, the analysis was not possible due to *within*-cell sample size limitation. However, collations of all traditional and geometric morphometric results in the present study unequivocally suggest the lack of sexual dimorphism within the southern African hedgehog. These results justified the pooling of sexes in all subsequent analyses to assess the nature and extent of age variation and in the systematic revision of the southern African hedgehog.

3.2 Age variation

3.2.1 *Traditional morphometric data*

After pooling of the sexes, one-way ANOVA of traditional morphometric data was used to independently assess the nature and extent of age variation in the southern

African hedgehog. Due to the damage of some specimens that resulted in missing data, the ANOVA of the traditional morphometric data used to assess the nature and extent of age variation in the southern African hedgehog was based on 22 of the 30 initial measurements. Similarly, because of small *within*-age class sample sizes, the ANOVA was based on individuals of age classes II, III and IV that had adequate sample sizes.

F-values from the one-way ANOVA showed 19 of the 22 measurements analyzed had statistically significant *F*-values for age (Table 4). Because of limited sample size particularly for individuals of age class II, *post-hoc* analyses such as the Student-Newman-Keuls (SNK; Zar 1996) tests could not be undertaken. However the overall pattern of age variation shown by the ANOVA is further supported by standard descriptive statistics of the sample analyzed (Table 5) that show a direct relationship between measurement magnitude and increasing age.

TABLE 4. *F*-values from a one-way analysis of variance (ANOVA) used to assess the nature and extent of age variation in three age classes (II, III and IV) of pooled males and females of the southern African hedgehog, *Atelerix frontalis*. Measurements are defined and illustrated in Figure 2.

Measurement	*F*-value
Greatest length of skull	43.07***
Greatest length of nasal	8.67***
Zygomatic arch length	50.64***
Breadth of braincase width	16.00***
Least breadth of interorbital constriction	7.05**
Nasal width	20.16***
Incisor to condyle length	41.26***
Greatest maxillary width	10.98***
Hard palate width at M^1	12.50***
Height of rostrum	14.54***
Infraorbital zygomatic plate distance	15.30***
Length of M^1	3.99**
Greatest cross-sectional width of M^1	3.00^{ns}
Greatest mandible length	42.98***
Mandible-ramus height	43.98***
Mandibular condyle-angular process distance	25.48***
Mandibular toothrow length	13.72***
Posterior incisor-M_3 length	19.51***
Length of M_1	2.99^{ns}
Length of M_2	0.77^{ns}
Greatest cross-sectional width of M_1	2.34^{ns}
Greatest cross-sectional width of M_2	4.35*

Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ^{ns} = no statistically significant differences.

TABLE 5. Standard descriptive statistics of 30 measurements of male and female southern African hedgehogs, *Atelerix frontalis*, of age classes II, III and IV) as defined and illustrated in Fig. 1.

Measurement	Age class II (Males)					Age class II (Females)					Age class III (Males)				
	$\bar{X}$	SD	*n*	CV	Range	$\bar{X}$	SD	*n*	CV	Range	$\bar{X}$	SD	*n*	CV	Range
Greatest length of skull	35.41	–	1	–	–	31.15	5.87	2	18.84	27.00–35.30	45.92	2.13	7	4.80	42.50–48.27
Greatest length of nasals	14.79	–	1	–	–	10.43	3.08	2	29.56	8.25–12.61	15.96	1.40	7	9.06	14.35–18.42
Zygomatic arch length	14.83	2.38	2	18.08	13.14–16.51	11.56	2.04	2	17.62	10.12–13.00	18.04	0.83	7	4.75	16.53–19.12
Breadth of braincase width	19.25	0.41	2	2.40	18.96–19.54	16.40	2.15	2	13.11	14.88–17.92	21.11	0.95	7	4.68	20.07–21.87
Least breadth of interorbital constriction	11.92	0.55	2	5.21	11.53–12.31	10.54	1.56	2	14.83	9.43–11.64	12.36	0.58	7	4.85	11.41–13.06
Nasal width	8.27		1	–	–	7.16	0.25	2	3.56	6.98–7.34	9.32	0.39	7	4.31	8.88–9.82
Incisor to condyle length	30.75	–	1	–	–	29.73	5.70	2	19.17	25.70–33.76	44.26	2.26	7	5.30	40.56–46.47
Toothrow length	18.25	–	1	–	–	19.14	–	1	–	–	23.03	1.44	7	6.46	20.82–24.30
Greatest maxillary width between labial crown edges of M1	13.19	0.76	2	6.46	12.65–13.72	14.18	1.33	2	9.37	13.24–15.12	16.89	1.17	7	7.16	15.37–19.03
Hard palate width at M^1	8.32	0.82	2	11.09	7.74–8.90	6.53	1.60	2	24.47	5.40–7.66	9.19	0.54	7	6.11	8.55–9.90
Hard palate width at M^3	7.47	0.09	2	1.39	7.40–7.53	–	–	0	–	–	9.40	0.74	7	8.15	8.16–10.11
Height of rostrum	6.37	–	1	0.00	6.37	4.87	0.86	2	17.59	4.26–5.47	7.37	0.41	7	5.81	6.70–8.06
Infraorbital–zygomatic plate distance	6.99	2.33	2	37.56	5.34–8.64	5.38	1.38	2	25.65	4.40–6.35	10.10	0.53	7	5.47	9.27–10.99
Foramen magnum–postorbital bar length	22.99	–	1	–	–	22.32	2.02	2	9.03	20.89–23.74	33.59	3.48	7	10.72	28.16–36.95
Greatest height of skull	13.20	–	1	–	–	11.78	2.43	2	20.60	10.06–13.49	15.56	0.81	7	5.37	14.36–16.98
Foramen magnum height	–	–	–	–	–	5.51	0.49	2	8.86	5.16–5.85	7.34	0.28	7	4.06	7.14–7.38
Foramen magnum width	6.13	–	1	–	–	6.45	1.13	2	17.54	5.65–7.25	6.87	0.33	7	5.00	6.44–7.30
Length of M^1	3.78	0.33	2	9.68	3.55–4.01	3.59	0.45	2	12.43	3.27–3.90	4.23	0.26	7	6.39	3.79–4.64
Length of s M^2	3.12	0.13	2	4.85	3.02–3.21	3.78	–	1	–	–	3.59	0.25	7	7.26	3.18–3.88
Greatest cross–sectional crown width of M^1	4.25	0.33	2	8.61	4.02–4.48	3.76	0.27	2	7.15	3.57–3.95	4.49	0.19	7	4.31	4.24–4.75
Greatest cross–sectional crown width of M^2	3.10	0.07	2	2.57	3.05–3.15	3.23	–	1	–	–	3.58	0.19	7	5.38	3.23–3.79
Greatest mandible length	25.73	0.36	2	1.58	25.47–25.98	23.50	3.66	2	15.59	20.91–26.09	33.79	1.86	7	5.70	31.20–35.75
Mandible–ramus height	11.43	0.06	2	0.63	11.38–11.47	9.30	2.43	2	26.16	7.58–11.02	15.99	0.60	7	3.89	15.06–16.88
Mandibular condyle–angualr process distance	8.44	0.54	2	7.26	8.05–8.82	7.19	1.54	2	21.44	6.10–8.28	11.37	0.64	7	5.81	10.61–12.27
Mandibular toothrow length	15.72	0.12	2	0.86	15.63–15.80	15.74	3.37	2	21.38	13.36–18.12	18.11	0.90	7	5.14	16.69–19.43
Posterior incisor–third lower molar length	15.67	0.23	2	1.68	15.50–15.83	14.72	2.08	2	14.12	13.25–16.19	18.11	0.86	7	4.92	16.97–18.93
Length of M_1	3.70	0.64	2	19.59	3.24–4.15	3.18	1.51	2	47.59	2.11–4.25	4.38	0.38	7	8.92	3.71–4.89
Length of M_2	3.38	–	1	–	–	3.50	0.28	2	8.08	3.30–3.70	3.46	0.70	7	20.88	1.96–3.98
Greatest cross–sectional crown width of M_1	2.85	0.29	2	11.46	2.64–3.05	2.61	0.04	2	1.36	2.58–2.63	3.13	0.32	7	10.68	2.64–3.63
Greatest cross–sectional crown width of M_2	2.86	–	1	–	–	2.51	0.48	2	19.16	2.17–2.85	2.88	0.46	7	16.72	1.89–3.32

$\bar{X}$ = arithmetic mean; SD = standard deviation; *n* = sample size; CV = coefficient of variation. Measurements are defined and illustrated in Figure 2

TABLE 5 continued

Measurement	Age class III (Females)					Age class IV (Males)				
	$\bar{X}$	SD	*n*	CV	Range	$\bar{X}$	SD	*n*	CV	Range
Greatest length of skull	45.46	2.76	5	6.37	40.73–47.93	48.18	0.77	8	1.66	47.44–49.61
Greatest length of nasals	15.54	2.08	5	14.06	12.04–17.10	16.17	1.32	8	8.40	13.82–18.13
Zygomatic arch length	17.40	1.00	5	6.04	16.01–18.79	19.24	0.53	8	2.86	18.48–19.92
Breadth of braincase width	20.07	1.75	5	9.13	17.35–21.35	22.54	0.70	8	3.22	21.44–23.37
Least breadth of interorbital constriction	12.14	0.55	5	4.78	11.49–12.88	12.78	0.36	8	2.92	12.30–13.28
Nasal width	9.22	0.41	5	4.64	8.73–9.70	9.50	0.57	8	6.16	9.02–10.43
Incisor to condyle length	43.53	3.46	5	8.34	37.58–46.50	46.18	0.84	8	1.89	44.64–47.08
Toothrow length	23.47	1.23	5	5.52	21.62–25.04	23.88	0.66	8	2.87	23.03–24.82
Greatest maxillary width between labial crown edges of M1	17.38	1.07	5	6.48	16.18–18.76	17.81	1.04	8	6.04	16.40–19.33
Hard palate width at M^1	9.73	0.68	5	7.29	8.88–10.69	9.88	0.60	8	6.23	9.15–10.92
Hard palate width at M^3	8.85	0.97	5	11.55	7.63–9.85	10.20	0.68	8	6.83	8.87–11.07
Height of rostrum	7.48	0.70	5	9.76	6.80–8.46	7.79	0.88	8	11.59	6.97–9.73
Infraorbital–zygomatic plate distance	10.24	1.21	5	12.41	8.97–12.09	9.47	2.02	8	21.95	6.17–11.48
Foramen magnum–postorbital bar length	34.18	3.22	5	9.89	28.87–37.07	33.44	4.49	8	13.90	23.55–36.58
Greatest height of skull	14.90	1.23	5	8.69	13.14–16.36	16.52	0.84	8	5.21	15.38–17.69
Foramen magnum height	6.81	0.47	5	7.18	6.23–7.34	7.06	0.79	8	11.59	6.09–8.59
Foramen magnum width	6.74	0.32	5	4.93	6.34–7.11	6.63	0.22	8	3.42	6.28–6.97
Length of M^1	4.28	0.33	5	8.13	3.90–4.70	4.24	0.39	8	9.39	3.47–4.67
Length of s M^2	3.52	0.20	5	6.16	3.37–3.82	3.49	0.31	8	9.10	3.09–3.87
Greatest cross–sectional crown width of M^1	4.39	0.16	5	3.84	4.21–4.54	4.37	0.30	8	7.17	3.94–4.77
Greatest cross–sectional crown width of M^2	3.74	0.20	5	5.64	3.52–3.92	3.59	0.30	8	8.71	3.31–4.22
Greatest mandible length	34.20	2.42	5	7.42	29.95–35.88	36.48	0.56	8	1.59	35.70–37.47
Mandible–ramus height	16.35	1.52	5	9.76	13.80–17.52	17.58	0.68	8	4.01	16.75–18.55
Mandibular condyle–angualr process distance	12.10	1.43	5	12.43	9.84–13.52	12.57	0.41	8	3.38	12.30–13.12
Mandibular toothrow length	18.29	0.95	5	5.47	17.38–19.73	18.94	0.68	8	3.70	17.91–19.73
Posterior incisor–third lower molar length	18.30	0.46	5	2.64	17.66–18.78	18.71	0.80	8	4.41	17.36–19.87
Length of M_1	4.52	0.23	5	5.30	4.13–4.70	4.13	0.42	8	10.58	3.47–4.77
Length of M_2	3.62	0.42	5	12.20	3.02–4.10	3.66	0.29	8	8.07	3.15–4.01
Greatest cross–sectional crown width of M_1	3.09	0.11	5	3.85	2.96–3.23	3.16	0.17	8	5.60	2.84–3.41
Greatest cross–sectional crown width of M_2	3.06	0.11	5	3.90	2.91–3.19	3.04	0.11	8	3.71	2.96–3.20

The multivariate analyses of age variation were particularly relevant in the present study since they included all the consecutive age classes I–IV and were therefore, instrumental in assessing patterns of age variation in the southern African hedgehog that included the individual of age classes I that was excluded in the univarite ANOVA. The scatterplot of the first two principal components of the PCA (Fig. 9) shows a progression of an age-related increase in size on the first PCA axis. Of particular importance, however, is that there are overlaps between individuals of age classes I and II as well as between individuals of age classes III and IV, with both overlapping groupings clearly separated from each other.

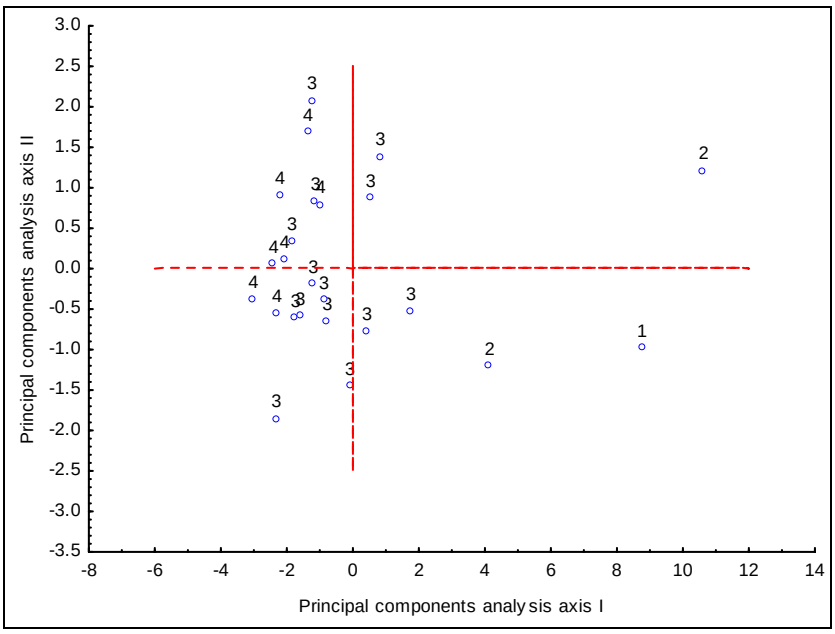

Figure 9. A scatterplot of the first two axes from a principal components analysis (PCA) of age classes I (1), II (2), III (3) and IV (4) of the southern African hedgehog, *Atelerix frontalis*.

The first principal component axis generally had high negative loadings on most measurements, with the first component contributing 71.13 % to the total variance (Table 4), suggesting a largely size-related age variation. The second PCA axis had measurement loadings of different signs and magnitude, suggesting some subtle shape-related age variation. Important measurements on the second PCA axis (8.21 % of the total variance) included length of M_1 and the greatest cross–sectional crown width of M_2 (Table 6).

TABLE 6. Loadings of measurements on the first two principal component axes from a principal component analysis (PCA) used to assess the nature and extent of age variation in the four age classes (I–IV) in the southern African hedgehog, *Atelerix frontalis*.

Measurement	PCA I	PCA II
Greatest length of skull	–0.98	0.08
Greatest length of nasals	–0.89	0.10
Zygomatic arch length	–0.96	0.17
Breadth of braincase width	–0.88	0.06
Least breadth of interorbital constriction	–0.88	0.08
Nasal width	–0.92	0.06
Incisor to condyle length	–0.96	0.10
Toothrow length	–0.84	–0.12
Greatest maxillary width between labial crown edges of M1	–0.91	0.04
Hard palate width at M^1	–0.86	0.37
Hard palate width at M^3	–0.79	0.37
Height of rostrum	–0.57	–0.03
Infraorbital–zygomatic plate distance	–0.68	–0.24
Foramen magnum–postorbital bar length	–0.97	0.08
Greatest height of skull	–0.95	0.18
Foramen magnum height	–0.94	0.20
Foramen magnum width	–0.88	–0.15
Length of M^1	–0.95	–0.12
Length of s M^2	–0.63	–0.38
Greatest cross–sectional crown width of M^1	–0.37	–0.84
Greatest cross–sectional crown width of M^2	–0.67	–0.29
Greatest mandible length	–0.74	–0.58
% trace	71.13%	8.21%

Because of the relatively low level of variation explained by successive principal components (e.g., 79.43 % of total variance by first two principal components axes), the sample was also assessed by UPGMA cluster analysis. The results of the UPGMA cluster analysis based on both Euclidean distances and correlation coefficients were similar. As exemplified by a Euclidean distance phenogram (Fig. 10), similar to the PCA of age variation based on traditional morphometric data above, the UPGMA cluster analysis also showed two discrete clusters, designated A and B. Cluster A included an assemblage of individuals of the older age classes III and IV, and cluster B comprised individuals of the younger age classes I and II.

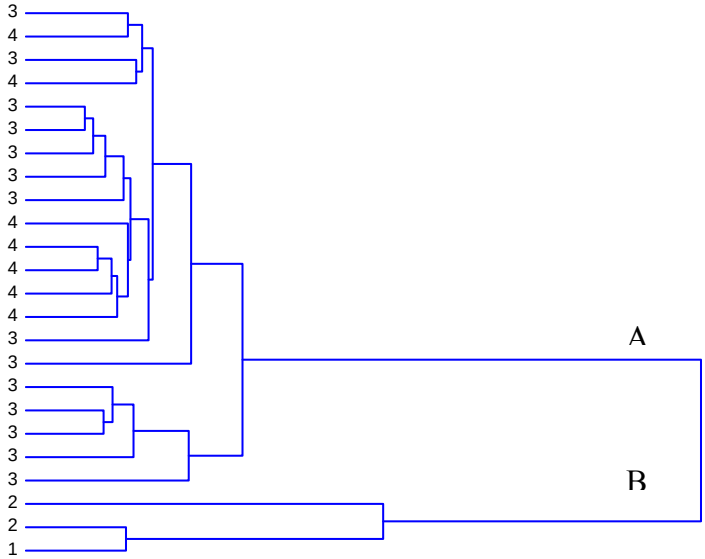

Figure 10. A Euclidean distance phenogram from an unweighted pair-group arithmetic averages (UPGMA) cluster analysis of age classes I (1), II (2), III (3) and IV (4) of the southern African hedgehog, *Atelerix frontalis*. Cluster A represents an assemblage of individuals of the older age classes III and IV, and cluster B comprised individuals of the younger age classes I and II.

3.2.2 *Geometric morphometric data*

The results of the geometric morphometric analyses of the dorsal, lateral and ventral views of the cranium, and the lateral view of the mandible to assess the nature and extent of age variation in *A. frontalis* were similar, and these results are best exemplified by those of the PCA (Fig. 11) and the UPGMA cluster analysis (Fig. 12) of the dorsal view of the cranium. The PCA scatterplot (Fig. 11) of the first relative warp (RW) explained 53.96 % of the total variance and the second RW accounts for 9.73 % of the total variance in the geometric morphometric data of individuals of age classes I–IV. Similar to the PCA and UPGMA cluster analysis of the traditional morphometric data, the PCA of the geometric data also showed overlaps between individuals of the younger age classes I and II, and between individuals of the older age classes III and IV, both of which were clearly separate from each other in multivariate space.

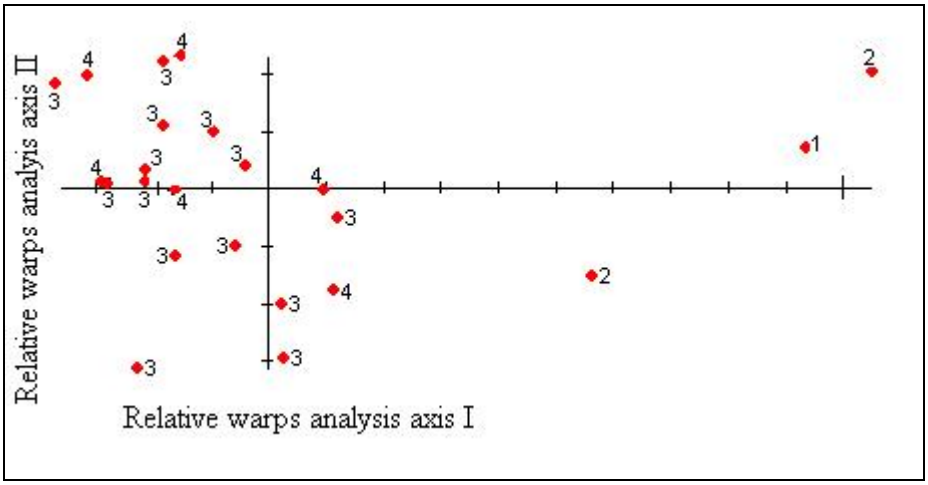

Figure 11. A scatterplot of relative warps (RW) I and II from a principal components analysis (PCA) of geometric morphometric data of the dorsal view of the cranium used to assess the nature and extent of age variation in individuals of age classes I (1), II (2), III (3) and IV (4) in the southern African hedgehog, *Atelerix frontalis*.

Similarly, the procrustes distance phenogram from the UPGMA cluster analysis (Fig. 12) showed two distinct clusters, designated A and B. Cluster A included an assemblage of individuals of the older age classes III and IV, and cluster B comprised individuals of the younger age classes I and II.

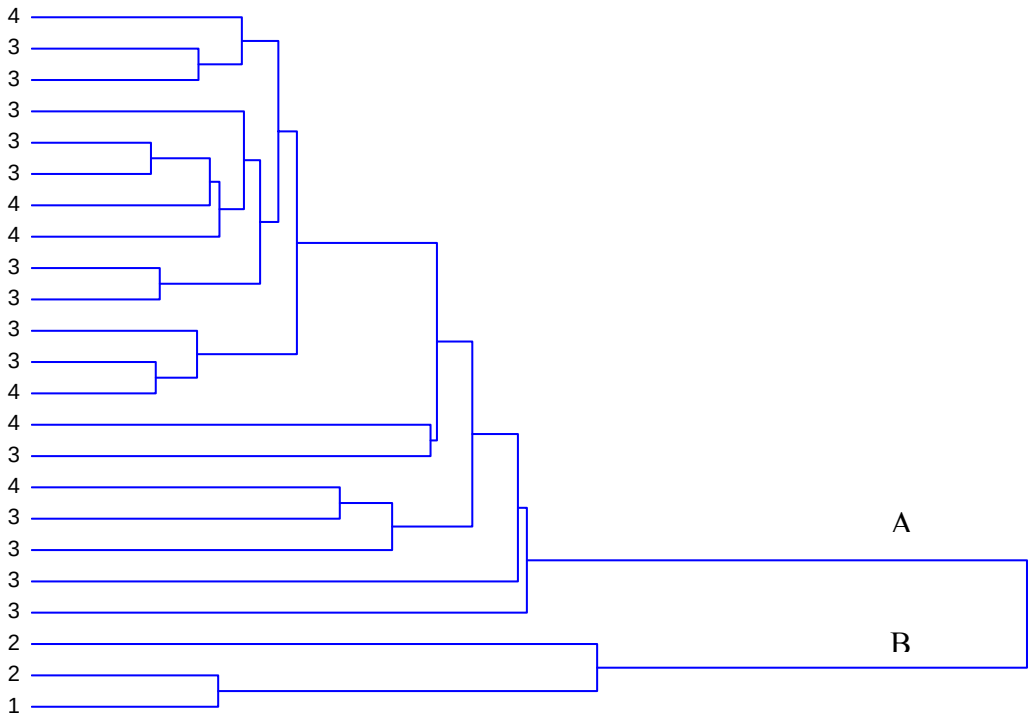

Figure 12. A procrustes distance phenogram from an Unweighted pair–group arithmetic averages (UPGMA) cluster analysis of geometric morphometric data of the dorsal view of the cranium used to assess the nature and extent of age variation in individuals of age classes I (1), II (2), III (3) and IV (4) of the southern African hedgehog, *Atelerix frontalis*. Cluster A represents an assemblage of individuals of the older age classes III and IV, and cluster B comprised individuals of the younger age classes I and II.

The morphological differences between individuals of the younger age classes I and II and the older age classes III and IV are shown by the changes in the position of landmarks for these age class groupings with reference to a consensus configuration of the dorsal view of the cranium derived from TPSSpline (Fig. 13 a & b). Differences in the dorsal view of the cranium between the younger (Fig. 13 c & d) and the older (Fig. 13 e & f) age classes are linked to both the anterior and dorsal parts of the cranium. Differences in the ventral view of the cranium (not illustrated) are linked to the posterior part of the cranium. Differences in the lateral view of the cranium (not illustrated) are linked to anterior part of the cranium, while differences in the lateral view of the mandible (not illustrated) are linked to the anterior and posterior part of the mandible.

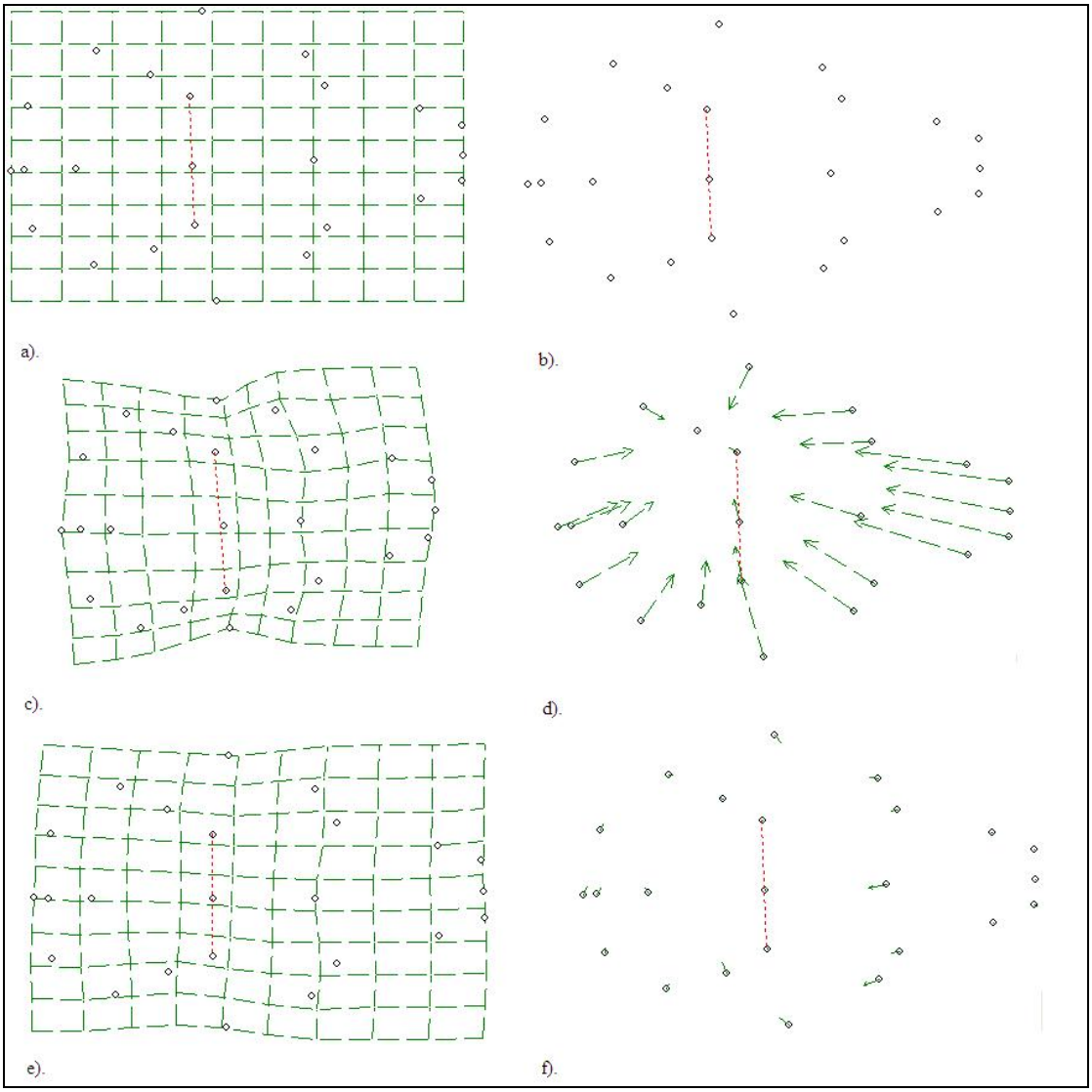

Figure 13. Changes in the position of landmarks with reference to a consensus configuration (splines) of the dorsal view of the cranium of the southern African hedgehog, *Atelerix frontalis* derived from TPSSpline (Rohlf 2004b) are indicated for: the consensus configuration (a & b), younger individuals of age classes I and II (c & d) and older individuals of age classes III and IV (e & f).

Similar to the traditional morphometric data, a CVA of the geometric morphometric data could not be undertaken because of *within*-cell sample size limitation. However, collation of all univariate and multivariate results of both traditional and geometric morphometric data in the present study strongly suggest the general lack of sexual dimorphism in the southern African hedgehog, but the presence of marked age variation in which the younger age classes I and II are shown to be morphologically different from the older age classes III and IV. All these results

justify the pooling of sexes and the recording and analysis of the older age classes III and IV in subsequent systematic revision of the southern African hedgehog.

[4] Discussion

The aim of this study was to determine age- and sex-related morphometric variation prior to a systematic revision of the southern African hedgehog, *Atelerix frontalis*. This was done with the primary objective of establishing criteria for the selection of specimens to consider for measurement recording and analysis and whether to analyse the sexes separately or together during the systematic revision. The study represents the first known analysis of non-geographic variation in the southern African hedgehog.

While all age classes were represented in the present analysis, there was only one individual of age class I that was available for analysis. It is possible that the lack of age class I individuals in museum collections may be related to the biology of hedgehogs in general and the southern African hedgehog in particular. Hedgehogs are known for their secretive behaviour as well as their secretive nursing behaviour therefore, reducing their chances of being captured. Apart from the lack of juvenile individuals in museum collections, the numbers of subadults and adults in museum collections are also limited. It is known that most hedgehogs do not survive the first few months after birth (Morris 1988). Those that survive past the first summer face the harsh physiological stress of hibernation in winter and if they die in their hibernation burrows, it would be difficult to obtain their samples (Morris 1988).

Collation of all univariate and multivariate results of both traditional and geometric morphometric data in the present study strongly suggest the general lack of sexual dimorphism in the southern African hedgehog. However, these analyses show the presence of marked age variation in which the younger age classes I and II are shown to be morphologically different from the older age classes III and IV. All these results justify the pooling of sexes and the recording and analysis of the older age classes III and IV in subsequent systematic revision of the southern African hedgehog. The general lack of sexual dimorphism and the presence of marked age variation have

been demonstrated in a range of small mammals that include bathyergid rodents such as the social mole-rats, *C. h. hottentotus* and *C. damarensis* (Bennett *et al.* 1990) and in murid rodents of the genus *Aethomys* (Chimimba & Dippenaar 1994).

Interestingly, in the solitary mole-rat, *Bathyergus suillus* (Hart *et al.* in press), sexual dimorphism has been shown to be absent in younger age classes, but present in older age classes, with reproductively mature males being larger than reproductively mature females. This has been attributed to the male-male interactions during the breeding season, where it would be more advantageous to be larger to secure a mating opportunity.

Ideally, a number of analyses should also have been used to address questions in the present study. These include *post hoc* analyses such as SNK tests (Gabriel & Sokal 1969; Sokal & Rohlf 1981), CVA (Sneath & Sokal 1973) and the partitioning of sum of squares (% SSQ) (Leamy 1983). However, these analyses could not be undertaken due to the lack of *within*-cell sample sizes. The latter series of analyses are particularly recommended because of their ability to partition sources of variation such as age and sexual dimorphism and their interaction. They are also able to identify the degree of variation that may be due to error (or residual variation) (Leamy 1983).

Nevertheless, the congruence between all univariate and multivariate results based on both traditional and geometric morphometric data strongly support the patterns of non-geographic variation delineated despite the small sample size. However, although the delineated patterns of non-geographic variation may be valid, these need to tested on other populations should additional samples become available for study. There may also be a need to increase the age categories assessed in order to be able to partition a potential growth curve for the southern African hedgehog which was not possible in the present study due to sample size limitations that precluded the categorization of more age categories. However, despite these constraints, studies of non-geographic variation are highly recommended prior to the analysis of geographic variation and systematic studies.

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Chapter 4

Geographic variation in the southern African hedgehog, *Atelerix frontalis* (Eulipotyphla: Erinaceidae): An analysis based on traditional morphometric data

Abstract

The southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831) is listed as near-threatened in the *Red Data Book of South African Mammals*. Despite reservations, its disjunct distribution of two allopatric populations has led to the recognition of two subspecies, namely, *A. f. frontalis* (A. Smith, 1831) and *A. f. angolae* (Thomas, 1918). While the former subspecies is confined to the eastern parts of southern African, the latter is restricted to the western parts of the subregion mostly in Namibia and extralimitally to south-western Angola. However, to date, the nature and extent of geographic variation in the species, that could allow an insight into the validity of the subspecies designations, remains virtually unknown. As part of a broader multidisciplinary characterization of the southern African hedgehog, a traditional morphometric study based on cranial and mandibular morphology was, therefore, conducted in order to assess the validity of the current subspecific taxonomy of the species. The results suggest a north-westerly–south-easterly clinal pattern of variation with cranial configuration being positively correlated with both latitude and longitude. No pronounced steps in the clinal pattern of variation were evident such that the recognition of subspecies within the southern African hedgehog may be untenable. It could, therefore, be argued that the disjunct distribution in the southern African hedgehog may represent a recent divergence event such that one disjunct population could act as a source population for the other leading to potential implications in conservation management strategies for the species. Although the suggested clinal pattern of variation may be valid, future studies should focus on comprehensive sampling as well as analyses involving a range of environmental parameters and/or climatic variables that may assist in identifying factors that may explain both the disjunct distribution and the delineated pattern of geographic variation within the southern African hedgehog.

[1] Introduction

The *Red Data Book of South African Mammals* (Friedman & Daly 2004) lists the southern African hedgehog, *Atelerix frontalis* (Thomas, 1918) of the family Erinaceidae as near-threatened and cautions that its suitable habitat is declining rapidly. This recent threat categorization is particularly relevant given the generally secretive nature of the species (Morris 1994) that renders the assessment of its conservation status difficult. This problem is exacerbated further by its disjunct distribution in southern Africa (Meester *et al.* 1986; Skinner and Chimimba 2005) that led to the taxonomic recognition of two subspecies *A. f. frontalis* (A. Smith, 1831) and *A. f. angolae* (Thomas 1918) that coincide with the two allopatric populations of the southern African hedgehog in the subregion (Meester *et al.* 1986; Skinner & Chimimba 2005).

Despite these subspecific designations not being rigorously tested by a range of systematic techniques, based on the complete isolation of the two disjunct populations, Rautenbach (1978) considered the recognition of the two subspecies within the southern African hedgehog justifiable. In these biogeographically-related taxonomic designations, the subspecies *A. f. frontalis* is considered to represent the form that occurs in the eastern parts of southern Africa ranging from eastern Botswana, western Zimbabwe and the Free State, Gauteng, and central parts of the Cape Provinces of South Africa (Meester *et al.* 1986; Skinner & Smithers 1990; Skinner & Chimimba 2005). The subspecies *A. f. angolae* is considered to represent the form that is restricted to the western parts of the subregion, mostly in Namibia but with a marginal extralimital occurrence in south-western Angola (Meester *et al.* 1986; Skinner & Smithers 1990; Skinner & Chimimba 2005).

However, there have been reservations on the validity of these subspecific taxonomic designations, particularly that of *A. f. angolae* (Corbet 1974; Gillies 1989; Skinner & Smithers 1990). To date, the nature and extent of geographic variation within *A. frontalis* that could allow an insight into the validity of the current subspecific taxonomic status of the southern African hedgehog, remains virtually unknown. Consequently, there is a critical need to further investigate the nature and extent of variation within this near-threatened species of hedgehog in an attempt to either confirm or refute the validity of its current subspecific taxonomy.

The present study, therefore, represents the first analysis of geographic variation in the southern African hedgehog, and includes the largest sample and widest geographical coverage than has hitherto been considered for the species, and is based on morphometric analysis of the cranium and mandible. Morphometric analysis allows the simultaneous assessment of joint relationships in character complexes by the reduction of large character sets to a few dimensions (James & McCulloch 1990) and is useful for quantifying morphological differences both *within* and *among* operational taxonomic units (OTUs; Sneath & Sokal 1973).

This can be undertaken using linear/orthogonal measurement-based traditional morphometrics and/or unit-free landmark/outline-based geometric morphometrics (Marcus 1990; Rohlf & Marcus 1993). By so doing, the generated data can in turn be subjected to a series of both univariate and multivariate statistical analyses. While these morphometric methods have been applied widely to a range of taxa, in mammals they are based on the cranium, mandible and teeth, and are similarly applied in the present study.

In the present study, linear/orthogonal measurement-based traditional morphometric analysis is used to assess geographic variation within the southern African hedgehog. The present study forms part of a broader multidisciplinary characterization of the southern African hedgehog that also included an analysis of geometric morphometric (Chapter 5) and molecular (Chapter 6) data.

[2] Materials and methods

2.1 *Specimens examined*

The analysis of intraspecific variation in the southern African hedgehog was based on 67 specimens from 43 localities in areas that represent the two disjunct distributions of the species in southern Africa where specimens from each of the 43 localities were pooled into operational taxonomic units (OTUs; Sneath and Sokal 1973). A list of all these specimens and geographic coordinates of their collecting localities are shown in Appendix I, while their collecting localities are presented in Fig. 1. Specimens examined came from the mammal collections of the Amathole Museum (KM), King William's Town, South Africa, the American Museum of Natural History (AMNH),

New York, U.S.A., the Durban Natural Science Museum (DM), Durban, South Africa, the National Museum, Bloemfontein (NMB), Bloemfontein, South Africa, and the Transvaal Museum (TM) of the Northern Flagship Institute, Pretoria, South Africa.

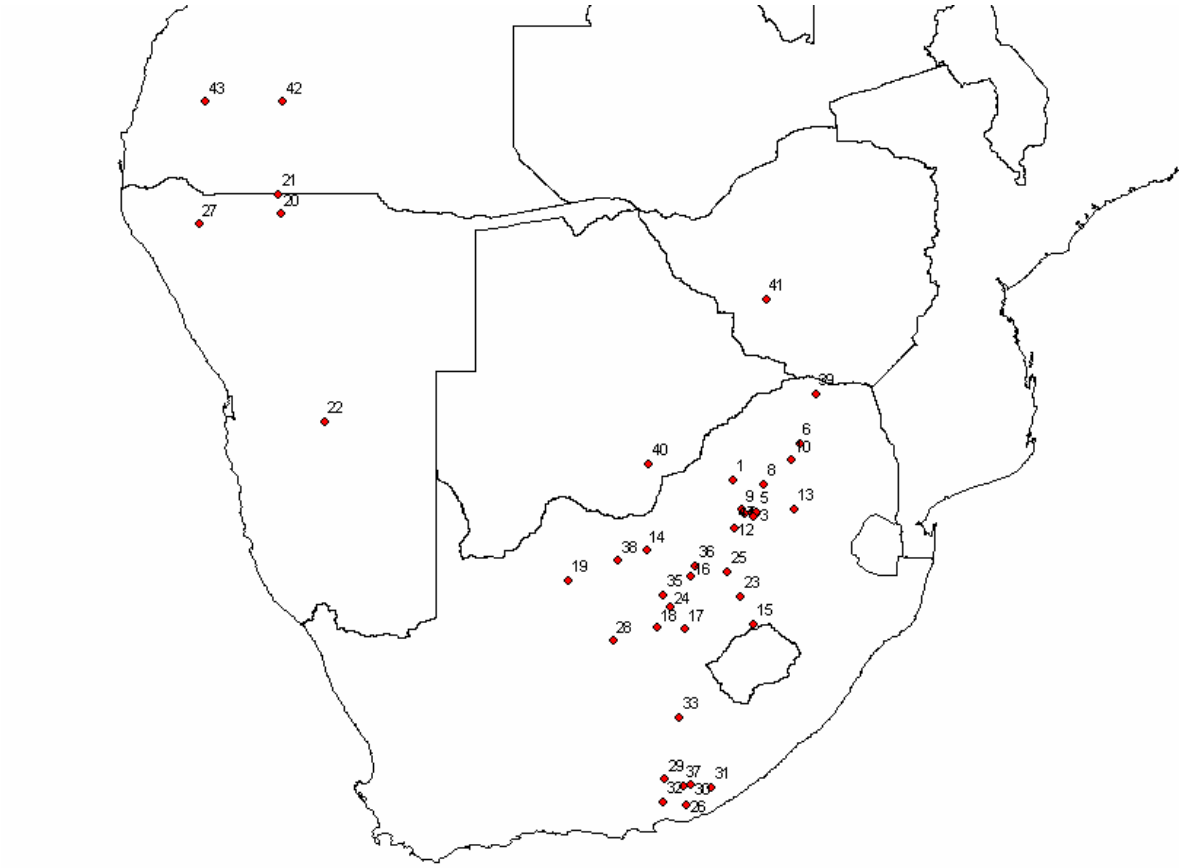


Figure 1. A map of southern Africa showing collection localities of *Atelerix frontalis* examined in this study: 1 = Rooiberg; 2 = Pretoria; 3 = Silverton, Pretoria; 4 = Hatfield, Pretoria; 5 = Derdepoort, Pretoria; 6 = Pietersberg; 7 = Waterberg; 8 = Settlers; 9 = De Wildt, Pretoria; 10 = Zebediela; 11.Waterkloof, Pretoria; 12 = Krugersdorp; 13 = Wonderboom, Pretoria; 14 = Delareyville; 15 = Ventersberg; 16 = Bothaville; 17 = Brandfort; 18 = Dealesville; 19 = Kuruman; 20 = Ondonga; 21 = Oshikango, 22 = Noates Rehoboth; 23 = Lindley; 24 = Bloemfontein; 25 = Koppies; 26 = Grahamstown; 27 = Okorosave; 28 = Modder river; 29 = Bedford; 30 = Fort Beaufort; 31 = Kaffaria; 32 = Somerset East; 33 = Burgersdorp; 34 = Kimberley; 35 = Hoopstad; 36 = Viljoenskroon; 37 = Adelaide, Waterfall; 38 = Vryburg; 39 = Mopani; 40 = Kweneng; 41 = Bulawayo; 42 = Humpata, Huila district; 43 = Lubango, Huila district.

2.2 *Morphometric measurements*

All traditional morphometric data analysed were based on 21 cranial and 9 mandibular measurements chosen from the character selection procedure described in Chapter 2 and are defined and illustrated in Fig. 2. The selections of these measurements were based on a character selection procedure applied by Chimimba and Dippenaar (1995) and were selected to adequately represent cranial and mandibular phenotypes of the southern African hedgehog. All measurements were recorded to the nearest 0.05 mm by one observer (LR) using a pair of Mitutoyo® digital callipers (Mitutoyo American Corporation, Aurora, Illinois, U.S.A).

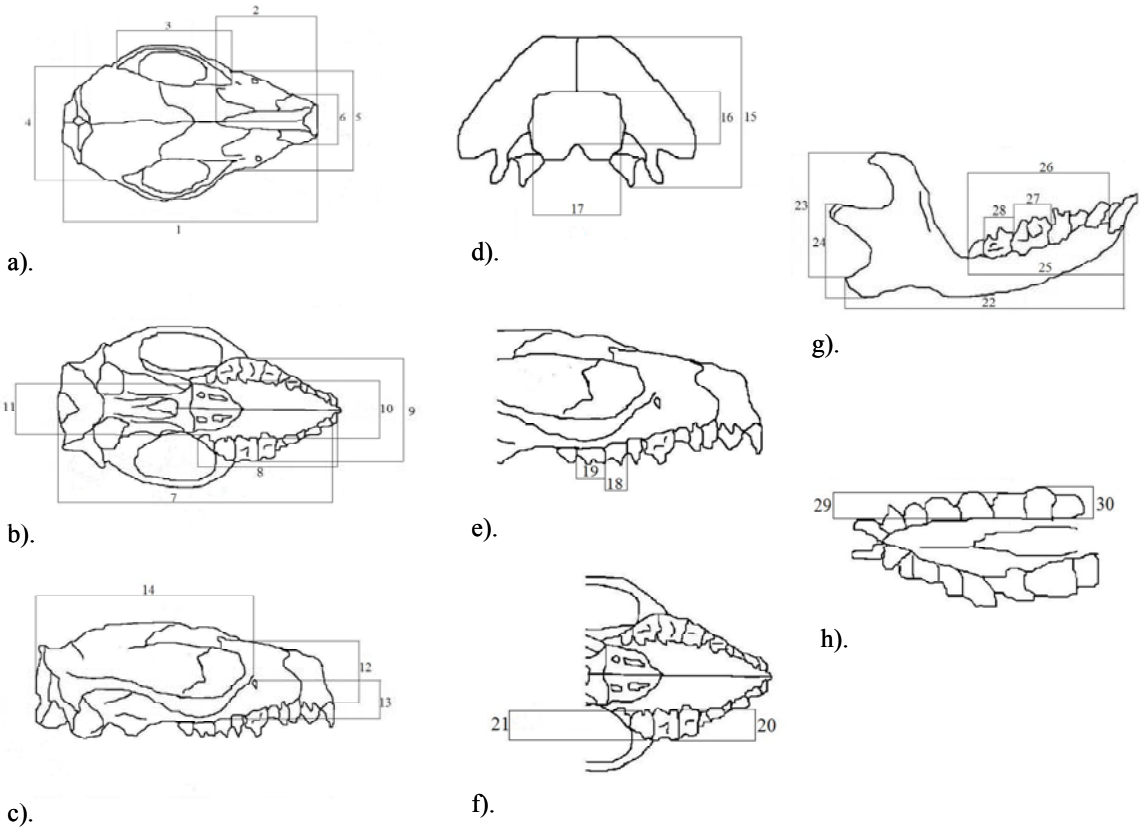


Figure 2. Cranial and mandibular measurements and their measuring points recorded in various views (a–h) of the southern African hedgehog, *Atelerix frontalis* in the present study: 1. GLS – greatest length of skull, from anterior edge of nasals to posterior edge of occipital condyle, along longitudinal axis of skull; 2. GLN – greatest length of nasals, from posterior projection of nasal wings to anterior-most edge of nasal bones; 3. ZAL – zygomatic arch length, from posterior-most part of anterior part of zygomatic arch to anterior-most part of posterior part of zygomatic arch; 4. BBC – breadth of braincase width at dorsal root of squamosals; 5. IOB – least breadth of interorbital constriction, least distance dorsally between orbits; 6. NAS – nasal width, at anterior-most point where nasals join premaxillae; 7. PIC – incisor to condyle length, from posterior surface of I^1 at alveolus to posterior-most projection of

occipital condyle; 8. TRL – toothrow length, from anterior alveolus to posterior surface of M^1 alveolus; 9. MAW – greatest maxillary width between labial crown edges of M^1; 10. PWM – hard palate width at M^1 measured on lingual side of teeth at alveolus; 11. PAC – hard palate width at point of constriction immediately posterior to M^3; 12. HOR – height of rostrum, perpendicularly from a point directly behind upper incisors; 13. IZD – infraorbital-zygomatic plate distance, from dorsal edge of infraorbital foramen to anterior base of zygomatic plate; 14. MPO – foramen magnum-postorbital bar length, from lateral edge of foramen magnum to anterior edge of postorbital bar; 15. GHS – greatest height of skull perpendicular to horizontal plane through bullae; 16. FMH – foramen magnum height, widest part of foramen in vertical plane; 17. FMW – foramen magnum width, widest part of foramen magnum in horizontal plane; 18. LFM – length of M^1 along cingulum; 19. LSM – length of M^2 along cingulum; 20. WFM – greatest cross-sectional crown width of M^1; 21. WSM – greatest cross-sectional crown width of M^2; 22. GML – greatest mandible length, in a straight line from anterior edge of I_1 alveolus to posterior surface of angular process; 23. MRH – mandible-ramus height, from dorsal edge of coronoid process to ventral edge of angular process; 24. MCA – mandibular condyle-angular process distance, in straight line from dorsal edge of mandibular condyle to ventral edge of angular process; 25. MTL – mandibular toothrow length, from anterior edge of I_1 alveolus to posterior edge of M_3 alveolus; 26. IML – posterior incisor- M_3 length, in a straight line from posterior edge of I_1 alveolus to posterior edge of M_3 alveolus; 27. LLM – length of M_1 along cingulum; 28. LMS – length of M_2 along cingulum; 29. WLM – greatest cross-sectional crown width of M_1, 30. WMS – greatest cross-sectional crown width of M_2.

2.3 *Ageing of specimens and sexual dimorphism*

To reduce the effect of age variation, measurement recording and analyses were based on adult specimens of toothwear classes III and IV as defined and illustrated in Chapter 2. The absence of sexual dimorphism in the southern African hedgehog as demonstrated in Chapter 2, justified the pooling of sexes in all analyses in the present study.

2.4 *Multivariate analyses*

The generated traditional morphometric data were subjected to a series of multivariate morphometric analyses to identify phenetic groupings in which no *a priori* sub-divisions of samples were presumed based on Unweighted pair-group arithmetic average (UPGMA) cluster analysis and principal components analysis of standardized variables (Sneath & Sokal 1973; Marcus 1990). The UPGMA cluster analysis was based on both Euclidean distances and correlation coefficients among groups (Sneath & Sokal 1973), while the PCA was based on product-moment correlation coefficients among variables (Sneath & Sokal 1973). The rationale behind the use of UPGMA

cluster analysis and PCA in the analysis of morphometric data is reviewed in Chapter 3. Although analyses were based on samples pooled on a per locality basis, the observed major patterns of variation were always verified by analyses of all sampled individuals from the two disjunct populations of the southern African hedgehog.

2.5 *Univariate analyses*

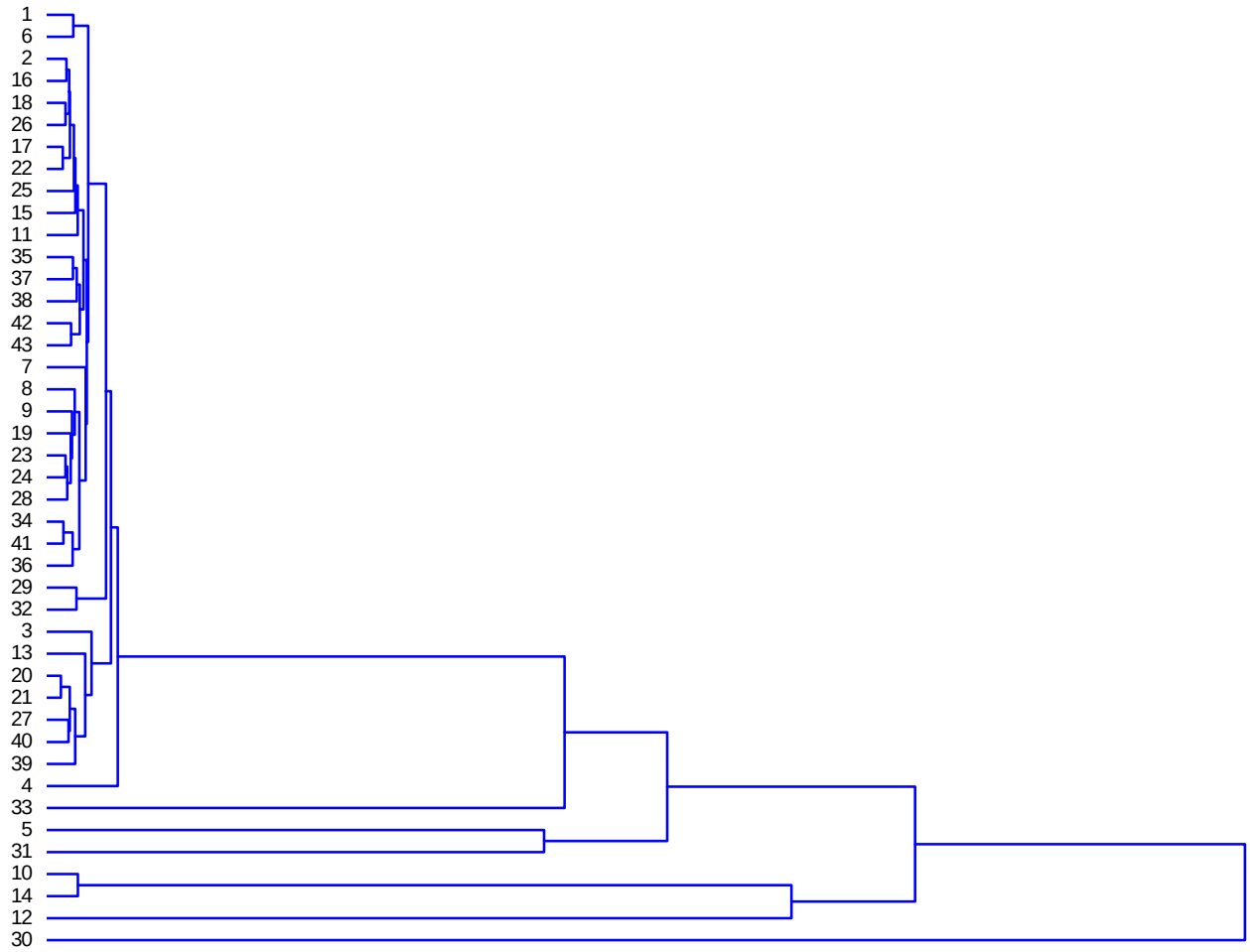
Univariate analyses included one-way analysis of variance (ANOVA; Zar 1996). Where significant differences were detected, maximally non-significant subsets were derived by the *a posteriori* Tukey's test (Sokal and Rohlf 1981) using ranked means. Patterns of variation were also evaluated by regression analysis (Zar 1996) of samples as well as PCA scores of OTUs, with longitude and latitude as independent variables. Geographic coordinates for localities with samples pooled on a per locality basis were based on mean latitude and longitude calculated from the coordinates of composite localities.

Other analyses in the study included the generation of relevant standard univariate descriptive statistics for each of the 43 localities where samples were pooled on a per locality basis as OTUs. All statistical procedures were accomplished using algorithms available in STATISTICA version 7.0 (StatSoft, Inc. 2004). All morphometric analyses were based on the 21 cranial and 9 mandibular measurements.

[3] Results

3.1 *Multivariate analyses*

Neither the Euclidean distance (Fig. 3a) nor the correlation (Fig. 3b) phenograms revealed geographically discernible patterns among the 43 OTUs analyzed.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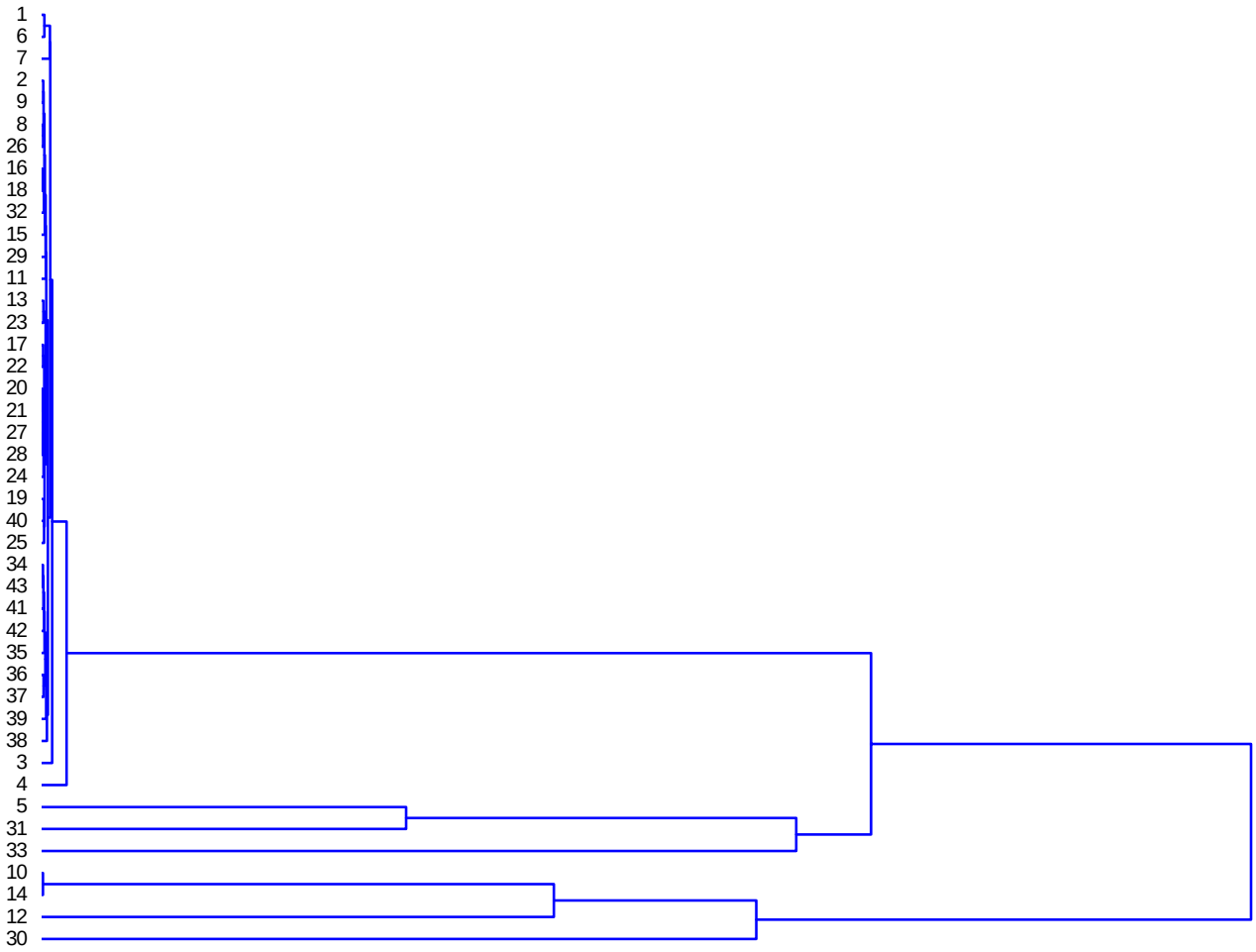

b).

Figure 3. Euclidean distance (a) and correlation (b) phenograms from an unweighted pair-group arithmetic average (UPGMA) cluster analysis of 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intra-specific variation in the southern African hedgehog, *Atelerix frontalis* based on traditional morphometric data. The OTU numbers correspond to those illustrated in Fig. 1.

The lack of an apparent geographic structure observed in the UPGMA cluster analysis above was also evident in the PCA, scatterplot of the first two principle components (Fig. 4). However, there is a tendency for OTU scores along the first PCA axis to increase with increasing longitude (Fig. 4). Similarly, there are indications for PCA scores of OTUs along the second PCA axis to increase with increasing latitude (Fig. 4).

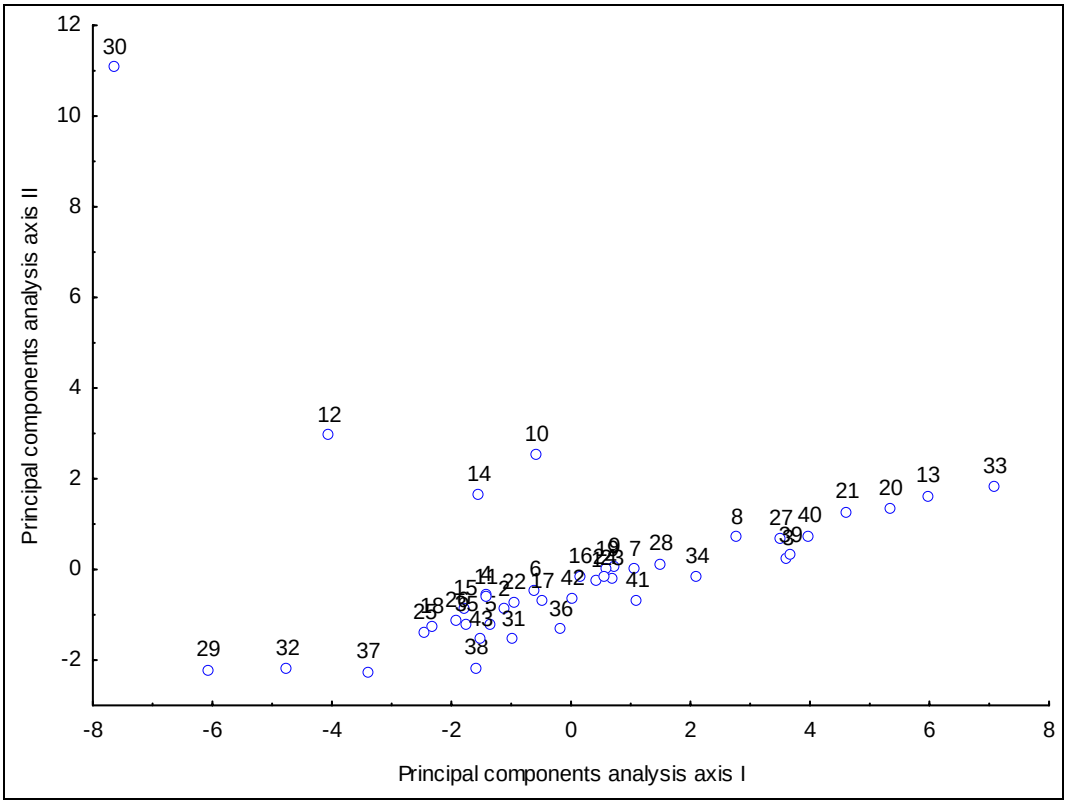

Figure 4. A scatterplot of the first two principle components axes from a principal components analysis (PCA) of 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on traditional morphometric data. The OTU numbers correspond to those illustrated in Fig. 1.

The first component, which accounts for 31.24 % of the total variance, has most measurements with relatively high negative loadings. As is usually the case, the first PCA axis with largely relatively high loadings of the same mathematical sign generally represents a size vector. The second component, which accounts for 15.07 % of the variance (Table 1), has measurements that are also intra-specifically important in the southern African hedgehog. It has measurements that have different magnitudes, and is dominated by six measurements, namely: greatest maxillary width between labial crown edges of M^1, hard palate width at M^1, height of rostrum, infraorbital-zygomatic plate distance, Length of M_1, and greatest cross-sectional crown width of M_1. As is usually the case, PCA axes subsequent to the first PCA axis with loadings of different mathematical signs and different magnitudes generally represent shape axes.

TABLE 1. Loadings of measurements on principal components I and II from a principle component analysis (PCA) of 43 localities with samples being pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on traditional morphometric data. Measurements are defined and illustrated in Fig. 2.

Measurement	PCA I	PCA II
Greatest length of skull	-0.91	-0.25
Greatest length of nasals	-0.69	-0.15
Zygomatic arch length	-0.74	-0.36
Breadth of braincase width	-0.77	-0.10
Least breadth of interorbital constriction	-0.76	-0.27
Nasal width	-0.69	-0.36
Incisor to condyle length	-0.91	-0.24
Greatest maxillary width between labial crown edges of M^1	-0.47	0.78
Hard palate width at M^1	-0.46	0.79
Height of rostrum	-0.43	0.79
Infraorbital-zygomatic plate distance	-0.43	0.78
Length of M^1	-0.31	-0.35
Greatest cross-sectional crown width of M^1	-0.24	0.22
Greatest mandible length	-0.61	-0.18
Mandible-ramus height	-0.57	0.25
Mandibular condyle-angular process distance	0.20	0.03
Mandibular toothrow length	-0.09	-0.15
Posterior incisor-M_3 length	-0.21	0.22
Length of M_1	-0.37	0.69
Length of M_2	-0.21	0.22
Greatest cross-sectional crown width of M_1	-0.37	0.69
Greatest cross-sectional crown width of M_2	-0.85	-0.23
% trace	31.24%	15.07%

To ascertain whether there was any geographic directionality in patterns of variation in the 43-OTU PCA, regressions were performed on OTU scores of the 30 derived principal component axes, with latitude and longitude as independent variables. All regressions of principal components axes scores with latitude revealed positive relationships in all 30 principal components derived from the initial PCA (Table 1) in which PC I (r = 0.43) was highly significant at $P < 0.001$ (Table 2) and PC X (r = 0.30) was statistically significant at $P < 0.05$ (Table 2), with PC scores generally suggesting an increase with increasing latitude (Fig. 5).

TABLE 2. Results of regressions of principal component (PC) scores with latitude and longitude for 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on traditional morphometric data.

	Correlation coefficient (*r*)	
Dependent variable	Latitude	Longitude
PCA 1	0.43^{***}	0.18^{ns}
PCA 2	0.01^{NS}	0.02^{ns}
PCA 3	0.11^{NS}	0.14^{ns}
PCA 4	0.02^{NS}	0.05^{ns}
PCA 5	0.23^{NS}	0.33^{*}
PCA 6	0.21^{NS}	0.06^{ns}
PCA 7	0.02^{NS}	0.10^{ns}
PCA 8	0.26^{NS}	0.03^{ns}
PCA 9	0.09^{NS}	0.05^{ns}
PCA 10	0.30^{*}	0.20^{ns}
PCA 11	0.14^{NS}	0.23^{ns}
PCA 12	0.11^{NS}	0.06^{ns}
PCA 13	0.08^{NS}	0.19^{ns}
PCA 14	0.02^{NS}	0.08^{ns}
PCA 15	0.16^{NS}	0.10^{ns}
PCA 16	0.16^{NS}	0.12^{ns}
PCA 17	0.07^{NS}	0.02^{ns}
PCA 18	0.24^{NS}	0.05^{ns}
PCA 19	0.13^{NS}	0.09^{ns}
PCA 20	0.18^{NS}	0.11^{ns}
PCA 21	0.00^{NS}	0.03^{ns}
PCA 22	0.22^{NS}	0.29^{ns}
PCA 23	0.09^{NS}	0.24^{ns}
PCA 24	0.03^{NS}	0.03^{ns}
PCA 25	0.07^{NS}	0.11^{ns}
PCA 26	0.25^{NS}	0.18^{ns}
PCA 27	0.09^{NS}	0.10^{ns}
PCA 28	0.07^{NS}	0.01^{ns}
PCA 29	0.02^{NS}	0.26^{ns}
PCA 30	0.16^{NS}	0.03^{ns}

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$; ^{ns} = not statistically significant.

This positive relation between longitude and PC I and PC X is best exemplified by the former PC axis that had a higher correlation coefficient ($r = 0.43$) (Fig. 5).

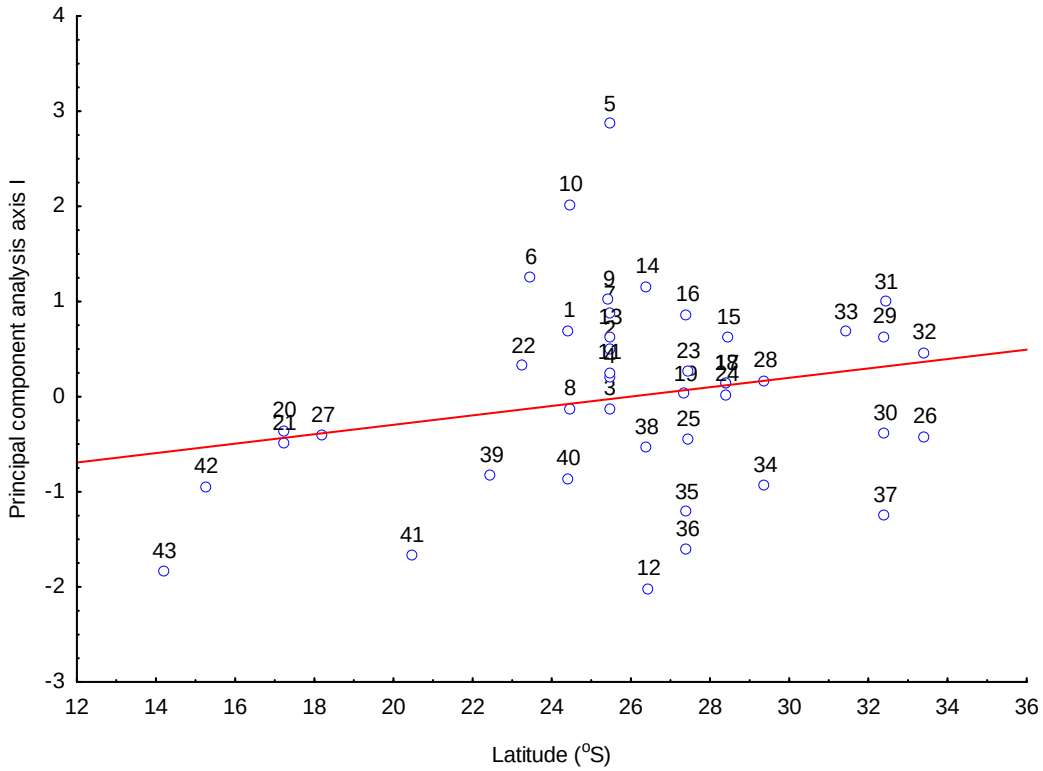

Figure 5. Regressions of principal components (PC) I scores with latitude for 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on traditional morphometric data. The OTU numbers correspond to those illustrated in Fig. 1. Regression equation: y = 1.2855 + 0.0494*x.

Similarly, all regressions of PC axis scores with longitude revealed positive relationships in all 30 PC axes derived from the initial PCA (Table 2) in which PC V (r = 0.33) was statistically significant at $P < 0.05$ (Table 2), with PC scores generally suggesting an increase with increasing longitude (Fig. 6).

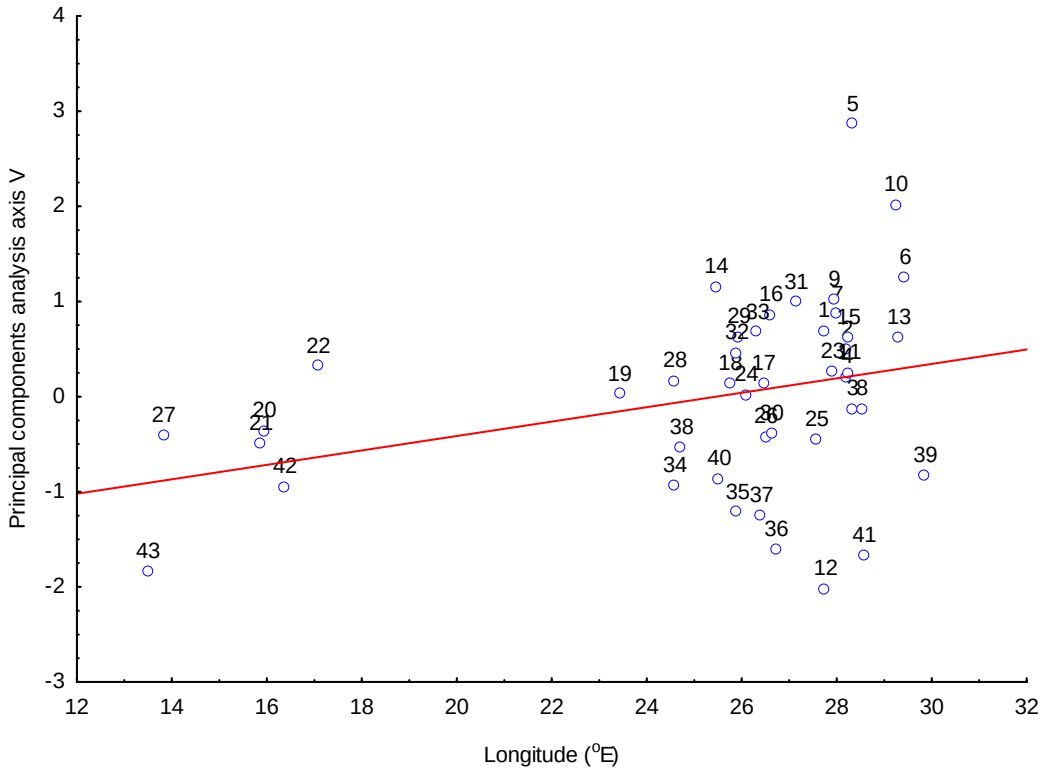

Figure 6. Regressions of principal components (PC) V scores with longitude for 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on traditional morphometric data. The OTU numbers correspond to those illustrated in Fig. 1. Regression equation: y = 1.929 + 0.0757*x.

Collation of all the results of the regression analyses involving longitude and latitude as independent variables, suggest cranial configuration being positively correlated with both latitude and longitude. These results suggest a cranial size/shape cline of a morphometric character complex, with north-western OTUs being on average smaller than south-eastern OTUs and of particular relevance is that no pronounced steps in the clinal pattern of variation was evident in all the analyses.

Although the north-westerly–south-easterly clinal pattern of variation in the southern African hedgehogs was evident in the analyses of the 43 localities where samples were pooled on a per locality basis as OTUs, the same trend was also evident in regressions of single measurements. All regressions of single measurements with latitude also revealed positive relationships in all 30 measurements in which nine of these measurements were statistically significant (Table 3). These measurements

include: 1) greatest length of skull ($r = 0.35$; $P < 0.05$); 2) greatest length of nasals ($r = 0.47$; $P < 0.001$); 3) zygomatic arch length ($r = 0.36$; $P < 0.05$); 4) breadth of braincase ($r = 0.40$; $P < 0.01$); 5) least breadth of interorbital constriction ($r = 0.41$; $P < 0.01$); 6) nasal width ($r = 0.41$; $P < 0.01$); 7) incisor to condyle length ($r = 0.31$; $P < 0.05$); 8) mandibular toothrow length ($r = 0.46$; $P < 0.001$); and 9) posterior incisor–M_3) length ($r = 0.42$; $P < 0.01$) (Table 3), with individual measurements generally suggesting an increase with increasing latitude.

TABLE 3. Results of regressions of individual measurements with latitude and longitude for specimens of the southern African hedgehog, *Atelerix frontalis* used to assess intraspecific variation based on traditional morphometric data. Measurements are defined and illustrated in Fig. 2.

	Correlation coefficient (r)	
Measurement	Latitude	Longitude
Greatest length of skull	0.35^{*}	0.13^{ns}
Greatest length of nasals	0.47^{***}	0.27^{ns}
Zygomatic arch length	0.36^{*}	0.08^{ns}
Breadth of braincase width	0.40^{**}	0.20^{ns}
Least breadth of interorbital constriction	0.41^{**}	0.28^{ns}
Nasal width	0.41^{**}	0.10^{ns}
Incisor to condyle length	0.31^{*}	0.07^{ns}
Toothrow length	0.24^{ns}	0.05^{ns}
Greatest maxillary width between labial crown edges of M^1	0.25^{ns}	0.05^{ns}
Hard palate width at M^1	0.24^{ns}	0.04^{ns}
Hard palate width at point of constriction of M^3	0.24^{ns}	0.05^{ns}
Height of rostrum	0.05^{ns}	0.06^{ns}
Infraorbital-zygomatic plate distance	0.23^{ns}	0.38^{**}
Foramen magnum-post orbital bar length	0.29^{ns}	0.15^{ns}
Greatest height of skull	0.16^{ns}	0.12^{ns}
Foramen magnum height	0.13^{ns}	0.10^{ns}
Foramen magnum width	0.15^{ns}	0.12^{ns}
Length of M^1	0.02^{ns}	0.09^{ns}
Length of M^2	0.10^{ns}	0.14^{ns}
Greatest cross-sectional crown width of M^1	0.02^{ns}	0.09^{ns}
Greatest cross-sectional crown width of M^2	0.11^{ns}	0.14^{ns}
Greatest mandible length	0.19^{ns}	0.03^{ns}
Mandible-ramus height	0.33^{*}	0.22^{ns}
Mandibular condyle-angular process distance	0.13^{ns}	0.14^{ns}
Mandibular toothrow length	0.46^{***}	0.04^{ns}
Posterior incisor- M_3 length	0.42^{**}	0.19^{ns}
Length of M_1	0.00^{ns}	0.10^{ns}
Length of M_2	0.18^{ns}	0.24^{ns}
Greatest cross-sectional crown width of M_1	0.18^{ns}	0.03^{ns}
Greatest cross-sectional crown width of M_2	0.01^{ns}	0.09^{ns}

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$; ^{ns} = not statistically significant.

This positive relation between latitude and single measurements is best exemplified by the greatest length of nasals that had the highest correlation coefficient (r = 0.47) (Fig. 7).

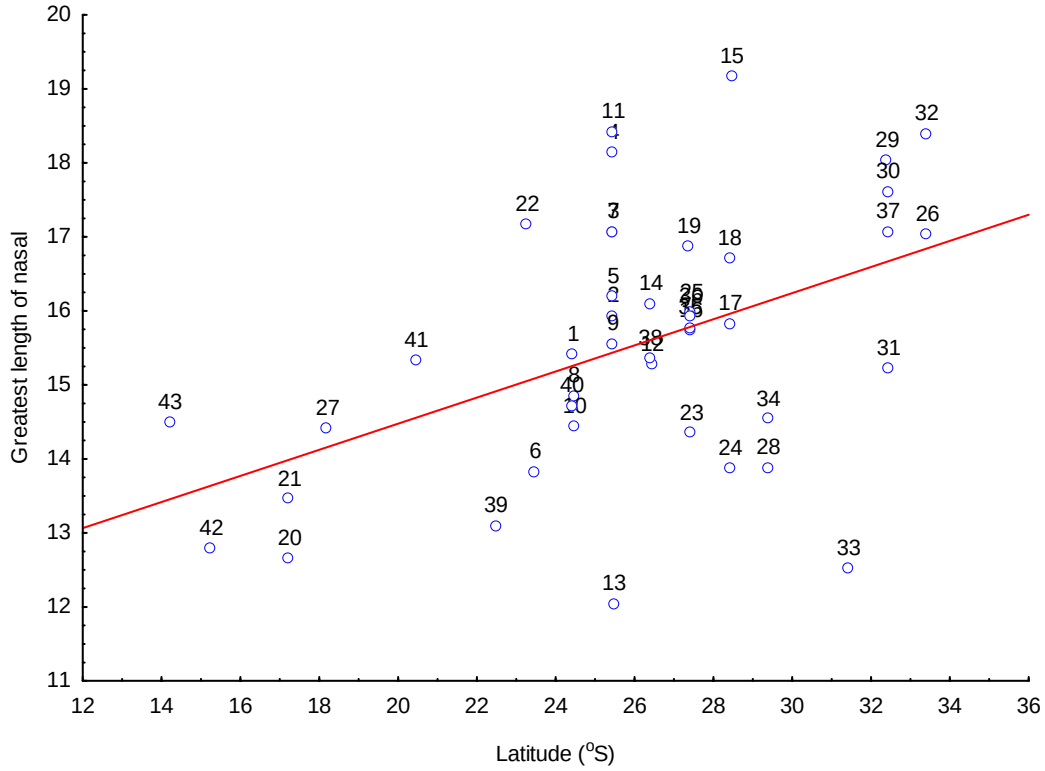

Figure 7. Regressions of the greatest length of nasals with latitude for single measurements of specimens of the southern African hedgehog, *Atelerix frontalis* used to assess intraspecific variation based on traditional morphometric data. The numbers correspond to the 43 localities illustrated in Fig. 1 from which specimens examined emanated from. Regression equation: y = 10.9481 + 0.1764*x.

Similarly, all regressions of single measurements with longitude also revealed positive relationships in all 30 measurements in which infraorbital-zygomatic plate distance was statistically significant (r = 0.38; P < 0.01; Table 4.3), with measurements generally suggesting an increase with increasing longitude (Fig. 8). This pattern of variation in individual measurements was also apparent in a scatterplot of individual-level analyses rather than the analyses that were based on the 43 localities where samples were pooled on a per locality basis (results not illustrated), with latitude and longitude as independent variables.

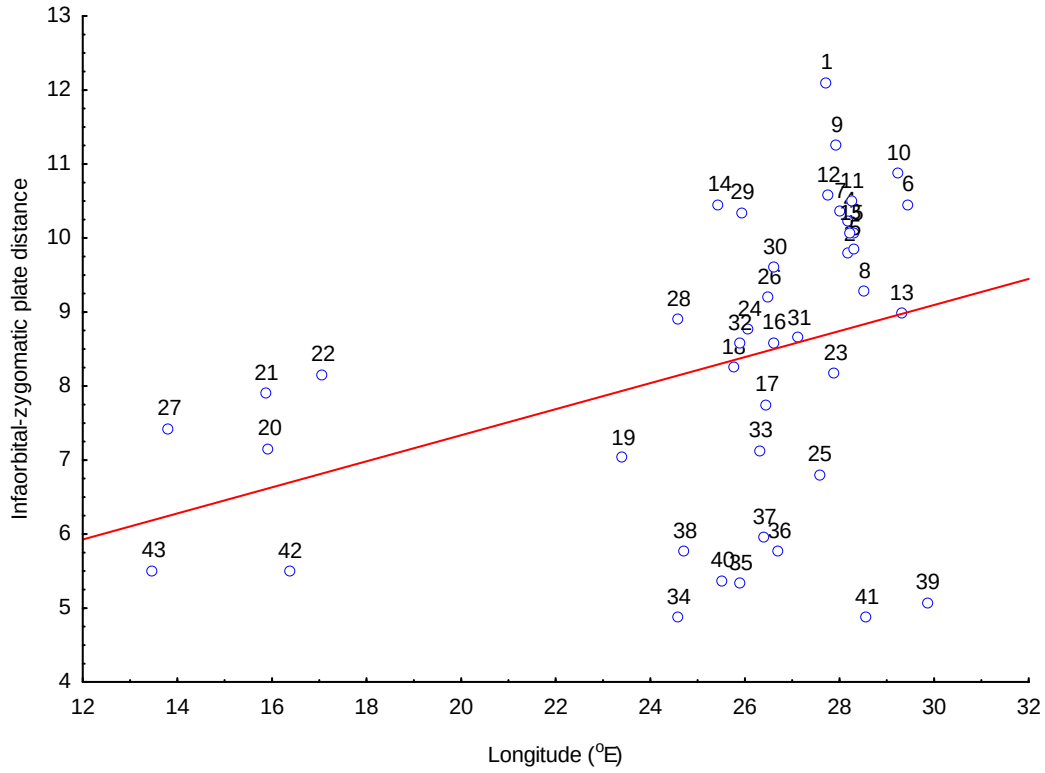

Figure 8. Regressions of infraorbital-zygomatic plate distance with longitude for single measurements of specimens of the southern African hedgehog, *Atelerix frontalis* used to assess intraspecific variation based on traditional morphometric data. The numbers correspond to the 43 localities illustrated in Fig. 1 from which specimens examined emanated from. Regression equation: y = 3.8131 + 0.1761*x.

3.2 *Univariate analyses*

In the ANOVA of the 43 OTUs, statistically significant differences ($P < 0.05$) were detected in 10 of the 30 measurements examined (Table 4). This suggests some differences in measurement magnitudes among OTUs examined that may reflect the latitudinal and longitudinal clinal pattern of variation evident in the regression analyses.

Table 4. *F*-values from a one-way analysis of variance (ANOVA) of 43 localities where samples were pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on traditional morphometric data. Measurements defined and illustrated in Fig. 1.

Measurement	*F*-value
Greatest length of skull	0.16^{ns}
Greatest length of nasals	1.42^{ns}
Zygomatic arch length	1.76^{ns}
Breadth of braincase width	2.04^{*}
Least breadth of interorbital constriction	1.94^{*}
Nasal width	0.68^{ns}
Incisor to condyle length	0.16^{ns}
Toothrow length	84.97^{***}
Greatest maxillary width between labial crown edges of M^1	145.88^{***}
Hard palate width at M^1	327.09^{***}
Hard palate width at point of constriction of M^3	189.71^{***}
Height of rostrum	1.66^{ns}
Infraorbital-zygomatic plate distance	2.97^{***}
Foramen magnum-post orbital bar length	0.14^{NS}
Greatest height of skull	0.91^{ns}
Foramen magnum height	0.96^{ns}
Foramen magnum width	0.96^{ns}
Length of M^1	1.08^{ns}
Length of M^2	9245.90^{***}
Greatest cross-sectional crown width of M^1	1.09^{ns}
Greatest cross-sectional crown width of M^2	2748.09^{***}
Greatest mandible length	1.17^{ns}
Mandible-ramus height	1.56^{ns}
Mandibular condyle-angular process distance	1.50^{ns}
Mandibular toothrow length	1.59^{ns}
Posterior incisor-M_3 length	1.50^{ns}
Length of M_1	0.78^{ns}
Length of M_2	2.36^{**}
Greatest cross-sectional crown width of M_1	1.06^{ns}
Greatest cross-sectional crown width of M_2	1.66^{ns}

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$ and ^{ns} = not statistically significant.

Because of limited intra-OTU sample sizes among the 43 OTUs examined, Tukey's *post-hoc* tests could not be undertaken. However, the overall north-westerly–south-easterly clinal pattern of variation is reflected in the order of ranked means of greatest nasal width and infraorbital-zygomatic plate distance, the two measurements

that had the highest and positive correlation coefficients in their regressions with latitude and longitude (Table 2) as independent variables, respectively (Table 5).

TABLE 5. Means of each of the 43 OTUs in ascending order (i.e., from smallest to largest) of greatest nasal width (a) and infraorbital-zygomatic arch distance (b) respectively for the southern African hedgehog, *Atelerix frontalis* used to illustrate the overall north-westerly–south-easterly clinal pattern of variation at the individual measurement level. The 43 OTUs are defined and illustrated in Fig. 1.

a). Greatest length of nasals			b). Infraorbital-zygomatic arch distance		
Locality code	*n*	$\bar{X}$	Locality code	*n*	$\bar{X}$
13	1	12.04	34	1	4.87
33	1	12.51	41	1	4.87
20	1	12.64	39	1	5.06
42	1	12.78	35	1	5.32
39	1	13.09	40	5	5.36
21	1	13.45	42	1	5.48
6	1	13.82	43	1	5.50
24	1	13.86	36	1	5.75
28	2	13.87	38	1	5.76
23	1	14.34	37	1	5.95
27	4	14.41	25	2	6.79
10	1	14.43	19	2	7.02
43	1	14.48	33	1	7.12
34	1	14.53	20	1	7.14
40	5	14.71	27	4	7.42
8	1	14.85	17	2	7.74
31	1	15.21	21	1	7.89
12	1	15.26	22	1	8.15
41	1	15.32	23	1	8.16
38	1	15.35	18	2	8.24
1	1	15.40	32	1	8.57
9	2	15.55	16	1	8.58
16	1	15.73	31	1	8.64
35	1	15.77	24	1	8.76
17	2	15.83	28	2	8.89
36	1	15.92	13	1	8.97
2	10	15.92	26	3	9.19
25	2	15.97	8	1	9.27
14	1	16.09	30	1	9.59
5	1	16.19	2	10	9.77
18	2	16.70	5	1	9.85
19	2	16.87	15	1	10.05
26	3	17.03	3	1	10.06
3	1	17.06	4	1	10.23
7	1	17.06	29	1	10.33
37	1	17.06	7	1	10.35
22	1	17.16	6	1	10.44
30	1	17.60	14	1	10.44
29	1	18.02	11	1	10.49
4	1	18.13	12	1	10.57
32	1	18.39	10	1	10.87
11	1	18.42	9	2	11.24
15	1	19.16	1	1	12.09

n = sample size; $\bar{X}$ = arithmetic mean

[4] Discussion

The subspecies concept is highly debatable (Mayr 1982). While some have restricted the recognition of subspecies to strictly allopatric distributions (Mayr 1982), others have argued that allopatric distributions may be due to temporal factors (Wilson & Brown 1953; Inger 1961; Van Devender *et al.* 1992). It is for the former reason that Rautenbach (1978) argued, despite reservations (Corbet 1974; Gillies 1989; Skinner & Smithers 1990)), for the recognition of two subspecies (*A. f. frontalis* and *A. f. angolae*) within the near-threatened (Friedmann & Daly 2004) southern African hedgehog based on its disjunct distribution of two allopatric populations.

However, the recognition of subspecies without having an insight into the species' nature and extent of geographic variation is considered inappropriate. In the case of the southern African hedgehog, its nature and extent of geographic variation remains virtually unknown to date. It is for this reason and as part of a multidisciplinary characterization of the southern African hedgehog that also included the analysis of geometric morphometric (Chapter 4) and molecular data (Chapter 5), that the present study that involves traditional morphometric data of the cranium and mandible was initiated. This study represents the first attempt to assess the nature and extent of geographic variation in *A. frontalis*.

All the results in the present study suggest a north-westerly–south-easterly clinal pattern of variation with cranial and mandibular configurations being positively correlated with both latitude and longitude. More specifically, the north-western populations (representing the currently recognized *A. f. angolae*) are narrower in cranial and mandibular configuration, while the south-eastern populations (representing the currently recognized *A. f. frontalis*) are broader. In addition, the delineated pattern of variation is reflected at both the locality- (represented by mean values) as well as the individual-level analyses and is also reflected by both the geometric (Chapter 5) and molecular (Chapter 6) analyses.

Of particular relevance is that the analyses in the present study showed no evidence of pronounced steps in the cline. Given the consensus of not splitting a cline into subspecies unless there is evidence of pronounced steps (James 1970; Mayr and

Ashlock 1991), the results in the present study suggest that the recognition of subspecies within the southern African hedgehog may be untenable.

In southern Africa, clinal patterns of variation have also been reported in other small mammals such as the murid rodents *Aethomys granti* (Chimimba *et al.* 1998) and *A. ineptus* (Chimimba 2001). Although a clinal pattern of variation in hometherms has often been interpreted with reference to Bergmann's (1874) rule, as has been reported in other studies (e.g., Sokal and Rinkel 1963; Rising 1970; Gould and Johnston 1972; Elder 1977; Ellison *et al*. 1993), the pattern of variation within the southern African hedgehog may also be due to a complex combination of interdependent climatic factors.

Consequently, there may be a need to re-asses the nature and extent of variation within the southern African hedgehog, but with special reference to environmental parameters and/or climatic variables that may assist in identifying factors that may explain both the disjunct distributions and clinal pattern of variation in this near-threatened species. Such studies could involve comprehensive sampling of the southern African hedgehog as well as being extended to other southern African small mammals.

Of particular significance in the present study is that the delineated clinal pattern of variation rather than representing distinct morphological gaps between the north-western and south-eastern populations, is a continuum and suggestive of a recent divergence event. If this argument is valid, then the results in the present study may have implications in the conservation management strategies for the near-threatened southern African hedgehogs, in that one disjunct population could act as a source population for the other. Nevertheless, it is highly recommended that prior to the formulation of conservation management strategies for the near-threatened southern African hedgehogs, additional studies involving a wide range of alternative systematic techniques need to be undertaken first in order to gain a better understanding of the nature and extent of geographic variation within *A. frontalis*.

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Appendix I

A gazetteer and geographic coordinates of sampled localities and specimens of the southern African hedgehog, *Atelerix frontalis* examined in the present study. Museum number denoted as :TM – Northern Flagship Institute (Transvaal museum), Pretoria; KM - Kaffrarian Museum, King William's Town; DM - Durban Natural Science Museum, Durban; NMB - National Museum, Bloemfontein; and AMNH - American Museum of Natural History, New York. Locality numbers correspond to those in Fig. 1.

Locality	Locality code	Geographic co-ordinates	Museum number	No. of samples
Rooiberg	1	24º 50'S; 27º 44'E	TM 749	1
Pretoria	2	25º 42'S; 28º 13'E	TM 2857; 4113; 5686; 7375; 16603; 16611; 27406; 40314; AMNH 54366; 90723	10
Pretoria, Silverton	3	25º 43'S; 28º 20'E	TM 27408	1
Pretoria, Hatfield	4	25º 44'S; 28º 13'E	TM 1830	1
Pretoria, Derdepoort	5	25º 40'S; 28º 20'E	TM 27684	1
Pietersburg	6	23º 54'S; 29º 27'E	TM 12470	1
Waterberg	7	25º 44'S; 28º 01'E	TM 1570	1
Settlers	8	24º 57'S; 28º 32'E	TM 28496	1
Pretoria, De Wildt	9	25º 37'S; 27º 57'E	TM 5554; 5687	2
Zebediela	10	24º 18'S; 29º 15'E	TM 12203	1
Pretoria, Waterkloof	11	25º 47'S; 28º 16'E	TM 15504	1
Krugersdorp	12	26º 06'S; 27º 46'E	TM 27409	1
Pretoria, Wonderboom	13	25° 36'S; 29° 19'E	TM 25942	1
Delareyville	14	26º 41'S; 25º 28'E	TM 23439	1
Ventersberg	15	28° 36'S; 28° 15'E	TM 751	1
Bothaville	16	27° 22'S; 26° 37'E	TM 4961	1
Brandfort	17	28° 42'S; 26° 28'E	TM 6220; NMB 1683	2
Dealesville	18	28° 40'S; 25° 46'E	TM 7587; NMB 1707	2
Kuruman	19	27° 27'S; 23° 26'E	TM 28209; 28210	2
Ondonga	20	17° 55'S; 15° 57'E	TM 7586	1
Oshikango	21	17° 24'S; 15° 53'E	TM 8019	1
Noates rehoboth	22	23° 19'S; 17° 05'E	TM 8732	1
Lindley	23	27° 52'S; 27° 55'E	NMB3497	1
Bloemfontein	24	28° 09'S; 26° 06'E	NMB 3631	1
Koppies	25	27° 14'S; 27° 35'E	KM 519; NMB 1665	2
Grahamstown	26	33° 18'S; 26° 31'E	KM 26283; 32311; 32316	3
Okorosave	27	18° 11'S; 13° 50'E	KM 526; 527; 528	3
Modder river	28	29° 02'S; 24° 36'E	KM 516; 517	2
Bedford	29	32° 38'S; 25° 57'E	KM 31729	1
Fort Beaufort	30	32° 47'S; 26° 38'E	KM 513	1
Kaffaria	31	32° 50'S; 27° 09'E	KM 15957	1
Somerset East	32	33° 13'S; 25° 54'E	KM 31968	1
Burgersdorp	33	31° 01'S; 26° 20'E	KM 514	1
Kimberley	34	29° 02'S; 24° 36'E	KM 513	1
Hoopstad	35	27° 50'S; 25° 55'E	KM 521	1
Viljoenskroon	36	27° 05'S; 26° 44'E	KM 518	1
Adelaide, Waterfall	37	32° 48'S; 26° 25'E	KM 34106	1
Vryburg	38	26° 57'S; 24° 44'E	DM 589	1
Mopani	39	22° 37'S; 29° 52'E	DM 609	1
Kweneng, Molepolole	40	29° 09'S; 25° 32'E	AMNH 167978; 167979; 167980; 167981; 168257	5
Bulawayo	41	20° 09'S; 28° 35'E	AMNH 207247	1
Huila district, Humpata	42	15° 01'S; 16° 23'E	AMNH 87640	1
Huila district, Lubango	43	14° 55'S; 13° 30'E	AMNH 87639	1

Chapter 5

Geographic variation in the southern African hedgehog, *Aterlerix frontalis* (Eulipotyphla: Erinaceidae): An analysis based on geometric morphometric data

Abstract

The near-threatened southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831) has a disjunct distribution of two allopatric populations in southern Africa. This disjunct distribution coincides with subspecific taxonomic designations within the species, with the subspecies *A. f. frontalis* (A. Smith, 1831) being restricted to the eastern parts of southern Africa and the subspecies *A. f. angolae* (Thomas, 1918) being confined to the western parts of the subregion mostly in Namibia, and extralimitally in south-western Angola. However, there have been reservations on the validity of these subspecific taxonomic designations. Consequently, the present study is an attempt to assess intra-specific variation in *A. frontalis* over the largest geographic coverage than has previously been considered for the species, in an attempt to assess the validity of the current subspecific taxonomic status of the southern African hedgehog using geometric morphometric data of the cranium and mandible. The results of this analysis of geographic variation in the southern African hedgehog suggest a north-westerly–south-easterly clinal pattern of variation with cranial configuration being positively correlated with longitude. These results are supported by traditional morphometric data. No pronounced steps in the clinal pattern of variation were evident which supports the present recognition of subspecies within the southern African hedgehog. Instead the disjunct distribution of the southern African hedgehog may represent a recent divergence event, and it could be argued that one disjunct population could be a source population for the other. These results may have implications in conservation management strategies for the southern African hedgehog.

[1] Introduction

The naming of subspecies was popular in the 20th century until the 1950s (Mayr 1982) with minor morphological dissimilarities among a few specimens being considered sufficient to warrant subspecific taxonomic status. In addition, allopatric distributions and disjunct patterns of morphological variation where poor geographic sampling masked patterns of smooth clinal variation also warranted the recognition of

subspecies (Montanucci 1992). Furthermore, in some cases, localities among known clines were arbitrarily chosen as subspecies boundaries (Mayr 1982; Frost & Hillis 1990).

A typical example relating to the recognition of subspecies in the southern African subregion includes the near-threatened (Friedman & Daly 2004) southern African hedgehog, *Atelerix frontalis* (Smith, 1831) of the family Erinaceidae that has a disjunct distribution of two allopatric populations in the southern African subregion (Skinner & Smithers 1990; Mills & Hes 1997; Skinner & Chimimba 2005). This disjunct distribution coincides with subspecific taxonomic designations within the species (Rautenbach 1978; Skinner & Chimimba 2005). The subspecies *A. f. frontalis* is restricted to the eastern parts of southern Africa that include eastern Botswana, western Zimbabwe and the Free State, Gauteng, and the central parts of the Cape Provinces of South Africa (Rautenbach 1978). The subspecies *A. f. angolae* is confined to the western parts of the subregion, mostly in Namibia, but with an extralimital occurrence in south-western Angola (Rautenbach 1978).

Although Rautenbach (1978), based on the complete isolation of the two populations, considered the recognition of the two subspecies within the southern African hedgehog justifiable, reservations have been expressed on the validity of these subspecific taxonomic designations, particularly that of *A. f. angolae* (Corbet 1974; Gillies 1989; Skinner & Smithers 1990). To date, very little is known about patterns of intra-specific variation in *A. frontalis* that would either confirm or refute the validity of the current subspecific taxonomic status of the southern African hedgehog.

In an attempt to assess the validity of the current subspecific taxonomic status of the southern African hedgehog, the present study represents the first attempt to assess intra-specific variation within the species over a broader geographical area than has previously been considered for the species, and is based on geometric morphometric data of the cranium and mandible. Morphometrics is useful for assessing joint relationships in character complexes that are assessed simultaneously by the reduction of large character sets to a few dimensions (James & McCulloch 1990). This can be achieved by linear/orthogonal measurement-based traditional morphometrics and/or

unit-free landmark/outline-based geometric morphometrics (Marcus 1990; Rohlf & Marcus 1993), where the generated data are in turn subjected to a series of both univariate and multivariate statistical analyses.

These morphometric methods are useful systematic tools for quantifying morphological differences both *within* and *among* operational taxonomic units (OTUs; Sneath & Sokal 1973). In mammals these morphometric methods are based on the cranium, mandible and teeth, and are similarly applied in the present study. Consequently, the present study is aimed at assessing intra-specific variation in *A. frontalis* and is based on geometric morphometric data in an attempt to assess the subspecific taxonomic status of the species. This part of the study forms part of a broader multidisciplinary characterization of the southern African hedgehog that also includes traditional morphometrics (Chapter 4) and molecular (Chapter 6) data.

[2] Materials and methods

2.1 *Specimens examined*

The analysis of intra-specific variation in the southern African hedgehog was based on 66 specimens from 43 localities in areas that represent the two disjunct distributions of the species in southern Africa where specimens from each of the 43 localities were pooled into operational taxonomic units (OTUs; Sneath and Sokal 1973). A list of all these specimens and geographic coordinates of their collecting localities are shown in Appendix I, while the collecting localities are presented in Fig. 1. Specimens examined came from the mammal collections of the Amathole Museum (KM), King William's Town, South Africa, the American Museum of Natural History (AMNH), New York, U.S.A., the Durban Natural Science Museum (DM), Durban, South Africa, the National Museum, Bloemfontein (NMB), Bloemfontein, South Africa, and the Transvaal Museum (TM) of the Northern Flagship Institute, Pretoria, South Africa.

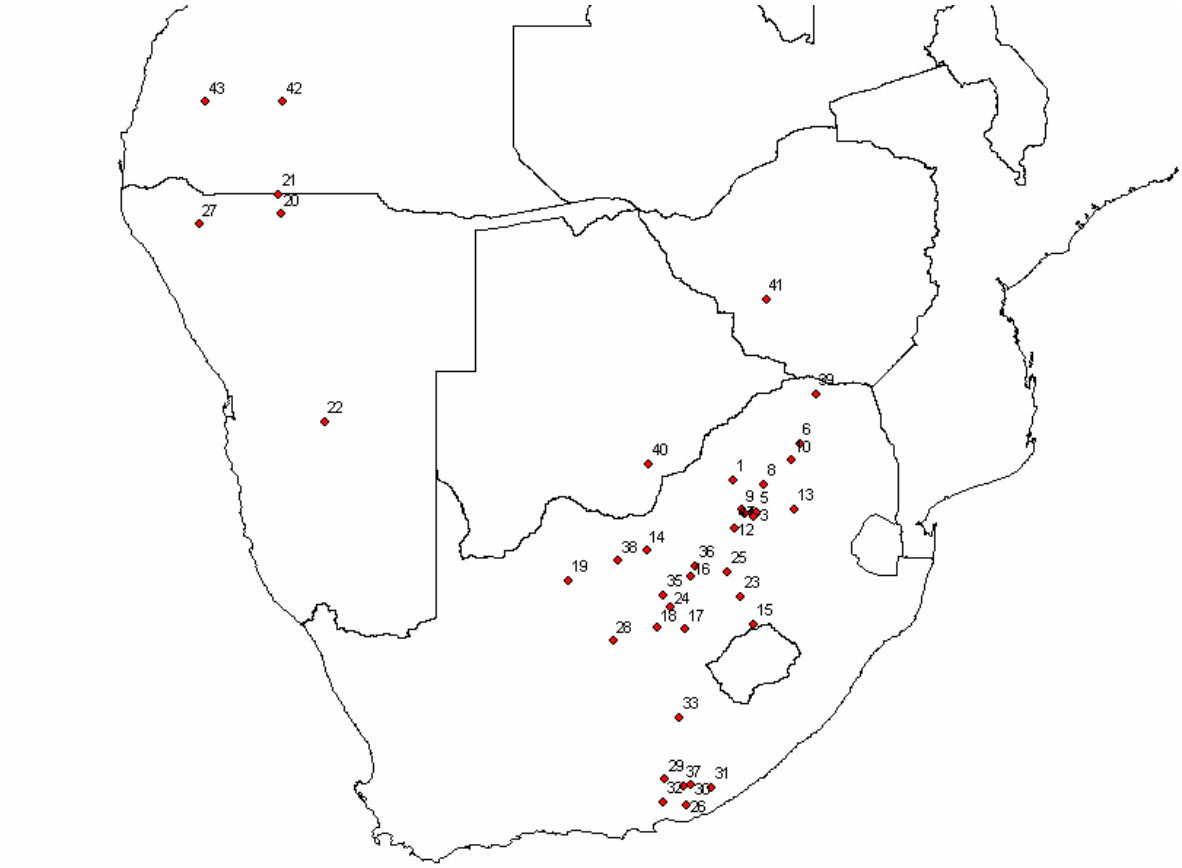

Figure 1. A map of southern Africa showing collection localities of *Atelerix frontalis* examined in this study: 1 = Rooiberg; 2 = Pretoria; 3 = Silverton, Pretoria; 4 = Hatfield, Pretoria; 5 = Derdepoort, Pretoria; 6 = Pietersberg; 7 = Waterberg; 8 = Settlers; 9 = De Wildt, Pretoria; 10 = Zebediela; 11.Waterkloof, Pretoria; 12 = Krugersdorp; 13 = Wonderboom, Pretoria; 14 = Delareyville; 15 = Ventersberg; 16 = Bothaville; 17 = Brandfort; 18 = Dealesville; 19 = Kuruman; 20 = Ondonga; 21 = Oshikango, 22 = Noates Rehoboth; 23 = Lindley; 24 = Bloemfontein; 25 = Koppies; 26 = Grahamstown; 27 = Okorosave; 28 = Modder river; 29 = Bedford; 30 = Fort Beaufort; 31 = Kaffaria; 32 = Somerset East; 33 = Burgersdorp; 34 = Kimberley; 35 = Hoopstad; 36 = Viljoenskroon; 37 = Adelaide, Waterfall; 38 = Vryburg; 39 = Mopani; 40 = Kweneng; 41 = Bulawayo; 42 = Humpata, Huila district; 43 = Lubango, Huila district.

2.2 *Geometric morphometric data*

Geometric morphometric (Marcus & Corti 1996) data, which is considered to be more superior in assessing organismal shape differences in morphology than traditional morphometrics (Marcus & Corti 1996), was used to assess intra-specific cranial and mandibular variation in the southern African hedgehog. A Pentax® Opti 33I digital camera attached to a tripod stand was used to capture images of the dorsal, ventral, and lateral views of the cranium, as well as lateral views of the mandible of each

specimen (Fig. 2). To standardize the image capturing procedure, each specimen was placed on a fixed piece of marked graph paper. All images were captured by one observer (LR).

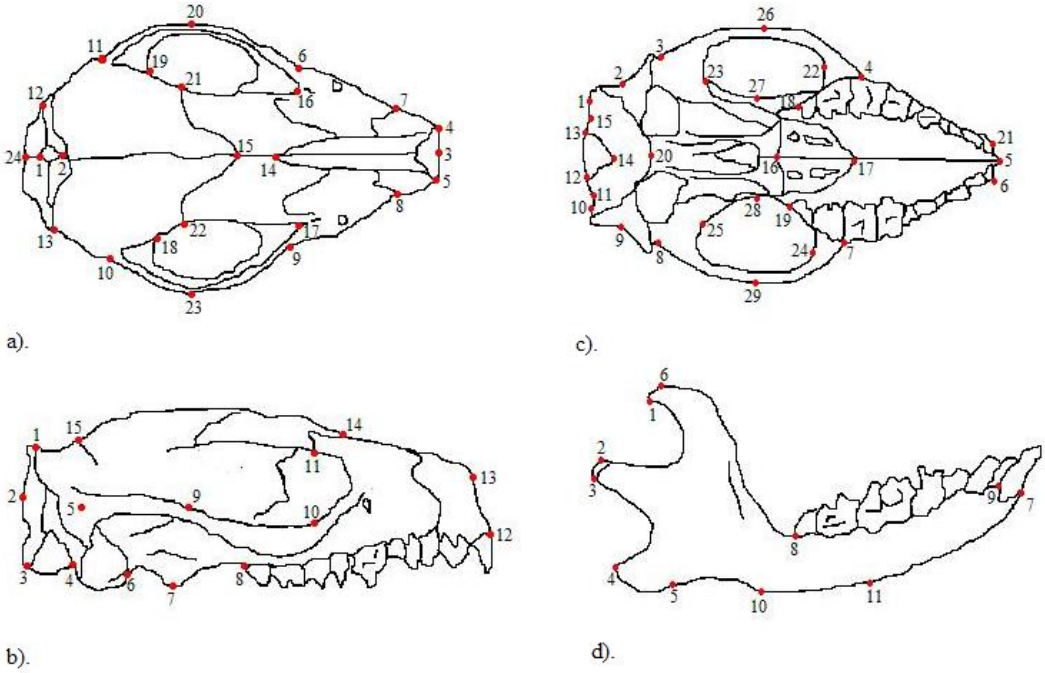

Figure 2. Landmarks of the dorsal (a) lateral (b), ventral (c) views of the cranium and the lateral view of the mandible (d) used in the geometric morphometric analyses of intra-specific variation in the southern African hedgehog, *Atelerix frontalis* in the present study.

2.3 *Digitizing error*

A Thin Plate Spline (TPS) sub-routine, TPSDig (Rohlf 2004a) was used to digitize landmarks, each with an (*x*,*y*) coordinate, on each of the four views for each specimen. Landmarks captured included 24, 15, 29 landmarks of the dorsal, lateral and ventral views of the cranium, respectively, and 12 landmarks of the lateral view of the mandible (Fig. 3). In order to assess the degree of landmark digitizing error (DE), the degree of error was expressed as a percentage (% DE) of the total variability due to *within*-individual variation (Pankakoski *et al.* 1987; Bailey & Byrnes 1990). The % DE analysis was based on three independent datasets of repeated digitized landmarks on the sample derived by LR on three separate occasions. Because the analyses revealed very low % DE values, averages of landmarks were computed and used in all subsequent geometric morphometric analyses. A TPS sub-routine,

TPSSpline (Rohlf 2004b), was used to compute splines in order to compare each specimen to a consensus configuration in order to detect any subtle differences in cranial and mandibular morphology (Marcus & Corti 1996) with reference to intraspecific variation in the southern African hedgehog.

2.4 *Ageing of specimens and sexual dimorphism*

To reduce the effect of age variation, image scanning and analyses were based on adult specimens of toothwear classes III and IV as defined and illustrated in Chapter 2. The absence of sexual dimorphism in the southern African hedgehog as demonstrated in Chapter 2, justified the pooling of sexes in all analyses in the present study.

2.5 *Geometric morphometric analysis*

All generated geometric morphometric data were subjected to a series of analyses to identify phenetic groupings in which no *a priori* sub-divisions of samples were presumed using principal components analysis (PCA; Jolliffe 1986) and an Unweighted-pair group arithmetic average (UPGMA) cluster analysis of standardized data (Sneath & Sokal 1973). The PCA of the geometric morphometric data was based on a weighted matrix generated from the TPS sub-routine TPSRelw (Rohlf 2004c) that was used to perform a relative warps analysis, which is equivalent to a PCA. The UPGMA cluster analysis of geometric morphometric data was based on procrustes distances generated from the TPS sub-routine, TPSSmall (Rohlf 2004d). The rationale behind the use of UPGMA cluster analysis and PCA in the analysis of morphometric data is reviewed in Chapter 3.

Patterns of variation were also evaluated by regression analysis (Zar 1996) of RW scores of OTUs, with longitude and latitude as independent variables. Geographic coordinates for localities with samples pooled on a per locality basis were based on mean latitude and longitude calculated from the coordinates of composite localities. Although all analyses in the present study were based on localities with samples pooled on a per locality basis, the observed major patterns of variation were always verified by analyses of all sampled individuals from the two disjunct populations of the southern African hedgehog.

All analyses in the present study were accomplished using algorithms in Statistica version 6.0 (StatSoft Inc. 2004) and/or sub-routines in the TPS (Rohlf 2004a-d) series of programmes. All geometric morphometric analyses were based on the 24, 15, 29 landmarks of the dorsal, lateral and ventral views of the cranium, respectively, and 12 landmarks of the lateral view of the mandible.

[3] Results

The results of the geometric morphometric analyses of the dorsal, lateral, and ventral views of the cranium, and the lateral view of the mandible to assess intraspecific variation in *A. frontalis* were broadly similar, and these results are best exemplified by those of the UPGMA cluster analysis (Fig. 3) and the PCA (Fig. 4) of the dorsal view of the cranium. The procrustes distance phenogram from the UPGMA cluster analysis (Fig. 3) showed no geographically discernible pattern of variation within the southern African hedgehog.

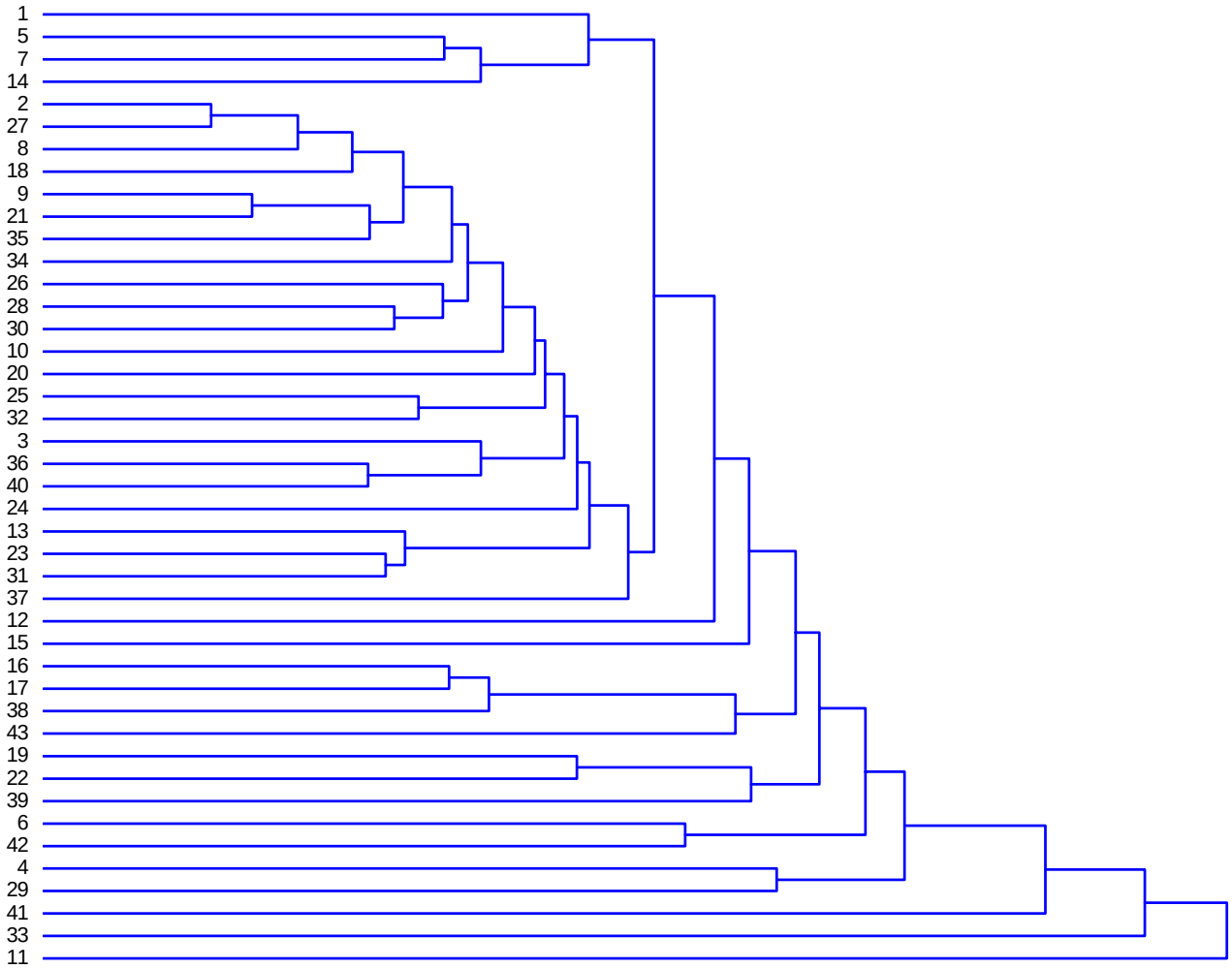

Figure 3. A procrustes distance phenogram from an Unweighted-pair group arithmetic average (UPGMA) cluster analysis of 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) used to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on geometric morphometric data of the dorsal view of the cranium. The OTU numbers correspond to those illustrated in Fig. 1.

In the PCA scatterplot (Fig. 4) of the first relative warp (RW) that explained 17.69 % of the total variance and the second RW that accounts for 15.32 % of the total variance in the geometric morphometric data of the 43 localities with samples pooled on a per locality basis, however, there is a tendency for RW scores of OTUs along the first RW axis to increase with increasing longitude (Fig 4). Similarly, there are indications for RW scores of OTUs along the second RW axis to increase with increasing latitude (Fig. 4). Similar indications of longitudinal and latitudinal

geographic patterns of morphometric variation were also evident in the results of the PCA of the traditional morphometric data (Chapter 4).

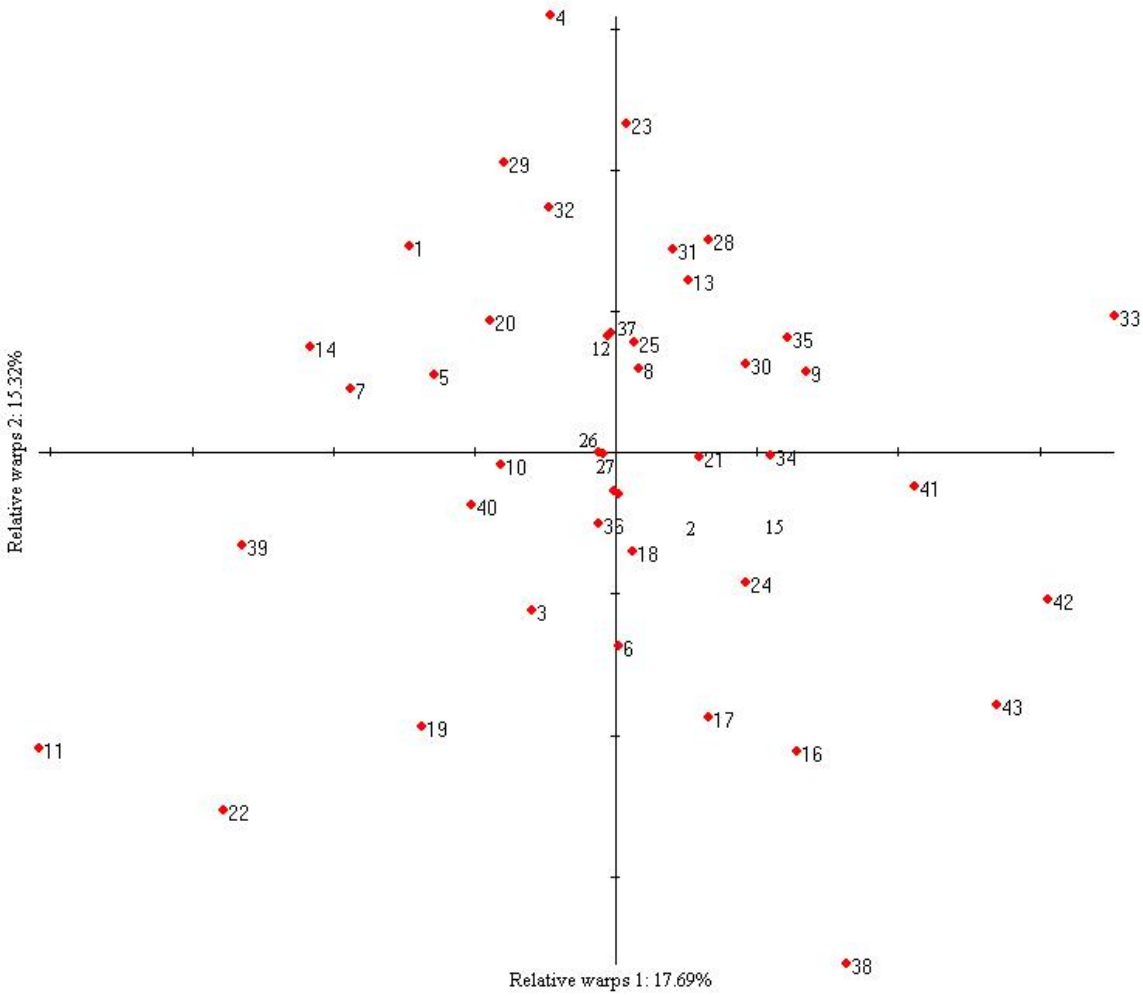

Figure 4. A scatterplot of relative warps (RW) I and II from a principal components analysis (PCA) of 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) used to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on geometric morphometric data of the dorsal view of the cranium. The OTU numbers correspond to those illustrated in Fig. 1.

To ascertain whether there was any geographical directionality in the patterns of variation in the 43-OTU PCA, regressions were performed on OTU scores of the 42 derived RW axes, with longitude and latitude as independent variables. All regressions of RW axis scores with latitude revealed positive relationships in all 42

RWs derived from the initial PCA in which RW V ($r = 0.48$) was highly statistically significant at $P < 0.001$ (Table 1), with RW scores generally suggesting an increase with increasing latitude (Fig. 5).

TABLE 1. Results of regressions of 42 derived Relative Warp (RW) scores with latitude and longitude for 43 localities with samples pooled on a per locality basis as operational taxonomic units (OTUs; Sneath & Sokal 1973) used to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on geometric morphometric data of the dorsal view of the cranium.

	Correlation coefficient (r)			Correlation coefficient (r)	
Dependent variable	Latitude	Longitude	Dependent variable	Latitude	Longitude
RW 1	0.06^{NS}	0.20^{NS}	RW 22	0.04^{NS}	0.01^{NS}
RW 2	0.29^{NS}	0.24^{NS}	RW 23	0.17^{NS}	0.15^{NS}
RW 3	0.15^{NS}	0.15^{NS}	RW 24	0.01^{NS}	0.10^{NS}
RW 4	0.03^{NS}	0.23^{NS}	RW 25	0.12^{NS}	0.01^{NS}
RW 5	0.48^{***}	0.06^{NS}	RW 26	0.19^{NS}	0.32^{*}
RW 6	0.08^{NS}	0.05^{NS}	RW 27	0.13^{NS}	0.14^{NS}
RW 7	0.03^{NS}	0.12^{NS}	RW 28	0.10^{NS}	0.20^{NS}
RW 8	0.18^{NS}	0.01^{NS}	RW 29	0.01^{NS}	0.04^{NS}
RW 9	0.17^{NS}	0.03^{NS}	RW 30	0.19^{NS}	0.18^{NS}
RW 10	0.22^{NS}	0.07^{NS}	RW 31	0.07^{NS}	0.06^{NS}
RW 11	0.03^{NS}	0.09^{NS}	RW 32	0.24^{NS}	0.07^{NS}
RW 12	0.10^{NS}	0.23^{NS}	RW 33	0.13^{NS}	0.09^{NS}
RW 13	0.21^{NS}	0.23^{NS}	RW 34	0.10^{NS}	0.15^{NS}
RW 14	0.16^{NS}	0.24^{NS}	RW 35	0.24^{NS}	0.33^{*}
RW 15	0.07^{NS}	0.01^{NS}	RW 36	0.09^{NS}	0.15^{NS}
RW 16	0.21^{NS}	0.14^{NS}	RW 37	0.14^{NS}	0.16^{NS}
RW 17	0.14^{NS}	0.22^{NS}	RW 38	0.10^{NS}	0.15^{NS}
RW 18	0.00^{NS}	0.12^{NS}	RW 39	0.19^{NS}	0.02^{NS}
RW 19	0.02^{NS}	0.16^{NS}	RW 40	0.08^{NS}	0.12^{NS}
RW 20	0.01^{NS}	0.12^{NS}	RW 41	0.09^{NS}	0.08^{NS}
RW 21	0.06^{NS}	0.16^{NS}	RW 42	0.11^{NS}	0.10^{NS}

* = $P < 0.05$; *** = $P < 0.001$; ^{NS} = Not statistically significant

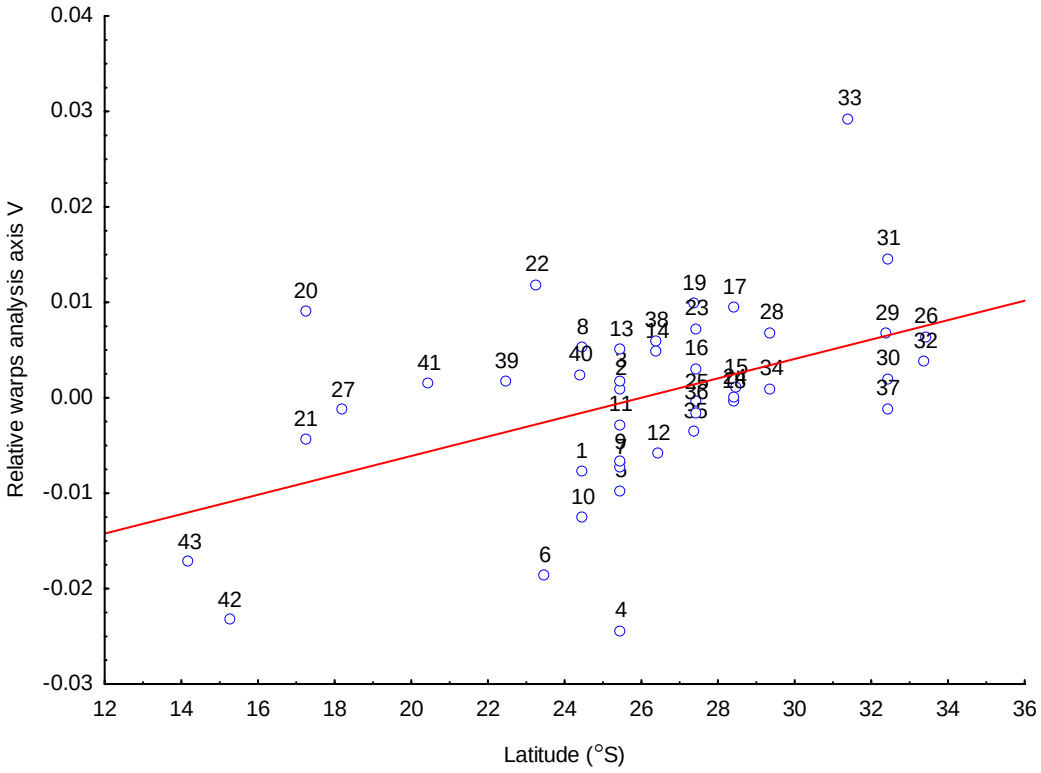

Figure 5. Regressions of Relative Warp (RW) V scores with latitude, for 43 localities with specimens from each locality being pooled as operational taxonomic units (OTUs; Sneath & Sokal 1973) in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on geometric morphometric data of the dorsal view of the cranium. The OTU numbers correspond to those illustrated in Fig. 1. Regression equation: y = 0.0264 + 0.001*x.

Similarly, all regressions of RW axis scores with longitude revealed positive relationships in all 42 RWs derived from the initial PCA (Table 1) in which RW XXVI (r = 0.32) and RW XXXV (r = 0.33) were statistically significant both at $P <$ 0.05 (Table 1), with RW scores generally suggesting an increase with increasing longitude. This positive relation between longitude and RW XXVI and RW XXXV is best exemplified by the latter RW that had a slightly higher correlation coefficient (r = 0.33) (Fig. 6).

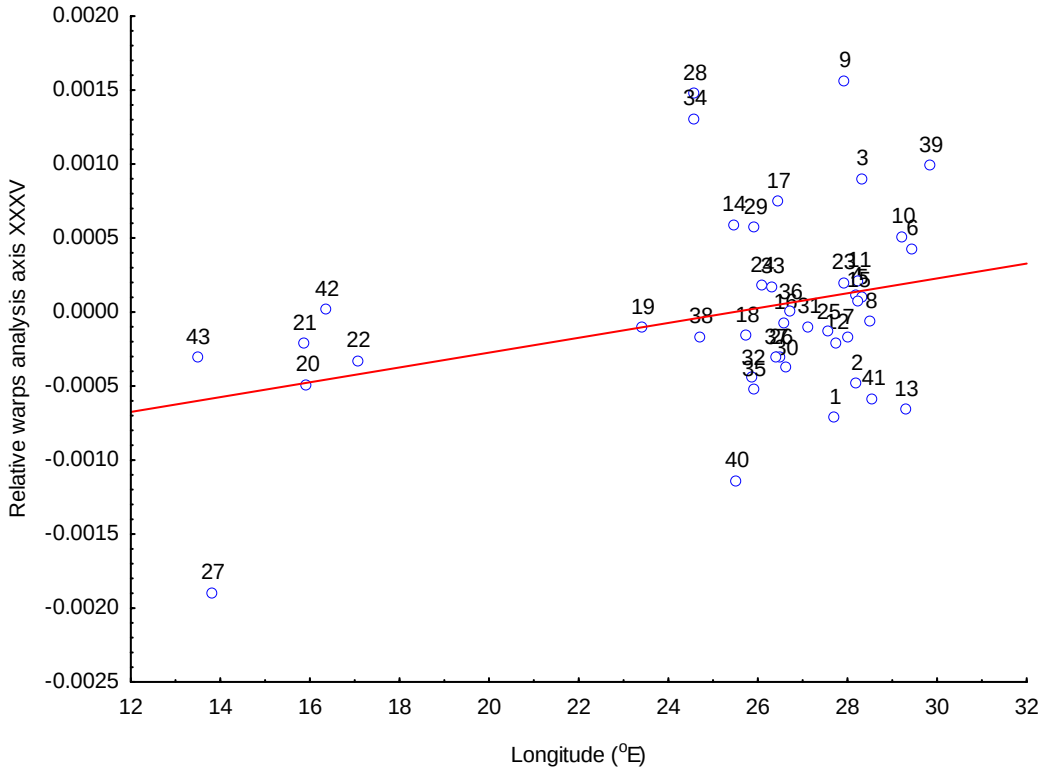

Figure 6. Regressions of Relative Warp (RW) XXXV scores with latitude, for specimens from 43 localities that were pooled as operational taxonomic units (OTUs; Sneath & Sokal 1973) on a per locality basis in order to assess intraspecific variation in the southern African hedgehog, *Atelerix frontalis* based on geometric morphometric data of the dorsal view of the cranium. The OTU numbers correspond to those illustrated in Fig. 1. Regression equation: y = 0.0013 + 5.0152E-5*x.

Collation of all the results of the regression analyses involving longitude and latitude as independent variables suggest cranial configuration being positively correlated with both longitude and latitude. These results suggest a cranial size/shape cline of a morphometric character complex, with north-western OTUs being on average smaller than south-eastern OTUs, and with no evidence of steps in the clines. Of particular importance is that similar indications of a north-westerly–south-easterly clinal pattern of morphometric geographic variation were also evident in the results of the traditional morphometric analyses (Chapter 3). Furthermore, this pattern of variation is also evident in the geometric morphometric results of the lateral and ventral views of the cranium, and the lateral view of the mandible.

Although the north-westerly–south-easterly clinal pattern of variation in the southern African hedgehogs was evident in the analyses of the 43 localities where

samples were pooled on a per locality basis as OTUs, the same trend was also evident in individual-level analyses. All regressions of RW axis scores in individual-level analyses with latitude also revealed positive relationships in all 44 RWs derived from the initial PCA in which RW IV (r = 0.30), RW VII (r = 0.33), and RW XXXVIII were statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively (Table 2), with RW scores generally suggesting an increase with increasing latitude at the individual-level.

TABLE 2. Results of regressions of 44 generated Relative Warp (RW) scores with latitude and longitude for individual specimens of the southern African hedgehog, *Atelerix frontalis* used to assess intraspecific variation based on geometric morphometric data of the dorsal view of the cranium.

	Correlation coefficient (r)			Correlation coefficient (r)	
Dependent variable	Latitude	Longitude	Dependent variable	Latitude	Longitude
RW 1	0.19^{ns}	0.20^{ns}	RW 23	0.10^{ns}	0.01^{ns}
RW 2	0.08^{ns}	0.24^{*}	RW 24	0.08^{ns}	0.10^{ns}
RW 3	0.06^{ns}	0.08^{ns}	RW 25	0.10^{ns}	0.14^{ns}
RW 4	0.30^{*}	0.03^{ns}	RW 26	0.13^{ns}	0.01^{ns}
RW 5	2.61^{ns}	0.06^{ns}	RW 27	0.20^{ns}	0.09^{ns}
RW 6	0.02^{ns}	0.18^{ns}	RW 28	0.08^{ns}	0.02^{ns}
RW 7	0.33^{**}	0.09^{ns}	RW 29	0.06^{ns}	0.04^{ns}
RW 8	0.19^{ns}	0.04^{ns}	RW 30	0.10^{ns}	0.22^{ns}
RW 9	0.02^{ns}	0.15^{ns}	RW 31	0.07^{ns}	0.15^{ns}
RW 10	0.12^{ns}	0.11^{ns}	RW 32	0.12^{ns}	0.13^{ns}
RW 11	0.09^{ns}	0.10^{ns}	RW 33	0.08^{ns}	0.03^{ns}
RW 12	0.07^{ns}	0.25^{*}	RW 34	0.13^{ns}	0.15^{ns}
RW 13	0.03^{ns}	0.02^{ns}	RW 35	0.15^{ns}	0.29^{*}
RW 14	0.00^{ns}	0.04^{ns}	RW 36	0.00^{ns}	0.01^{ns}
RW 15	0.21^{ns}	0.33^{**}	RW 37	0.18^{ns}	0.08^{ns}
RW 16	0.01^{ns}	0.06^{ns}	RW 38	0.25^{*}	0.17^{ns}
RW 17	0.18^{ns}	0.30^{*}	RW 39	0.16^{ns}	0.11^{ns}
RW 18	0.06^{ns}	0.03^{ns}	RW 40	0.01^{ns}	0.09^{ns}
RW 19	0.01^{ns}	0.11^{ns}	RW 41	0.10^{ns}	0.11^{ns}
RW 20	0.06^{ns}	0.16^{ns}	RW 42	0.04^{ns}	0.24^{ns}
RW 21	0.14^{ns}	0.01^{ns}	RW 43	0.05^{ns}	0.16^{ns}
RW 22	0.01^{ns}	0.08^{ns}	RW 44	0.18^{ns}	0.09^{ns}

Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ^{ns} = no statistically significant differences.

This positive relation with latitude at the individual-level is best exemplified by RW VII that had the highest positive relationship with latitude ($r = 0.33$; $P < 0.01$; Table 2) (Fig. 7).

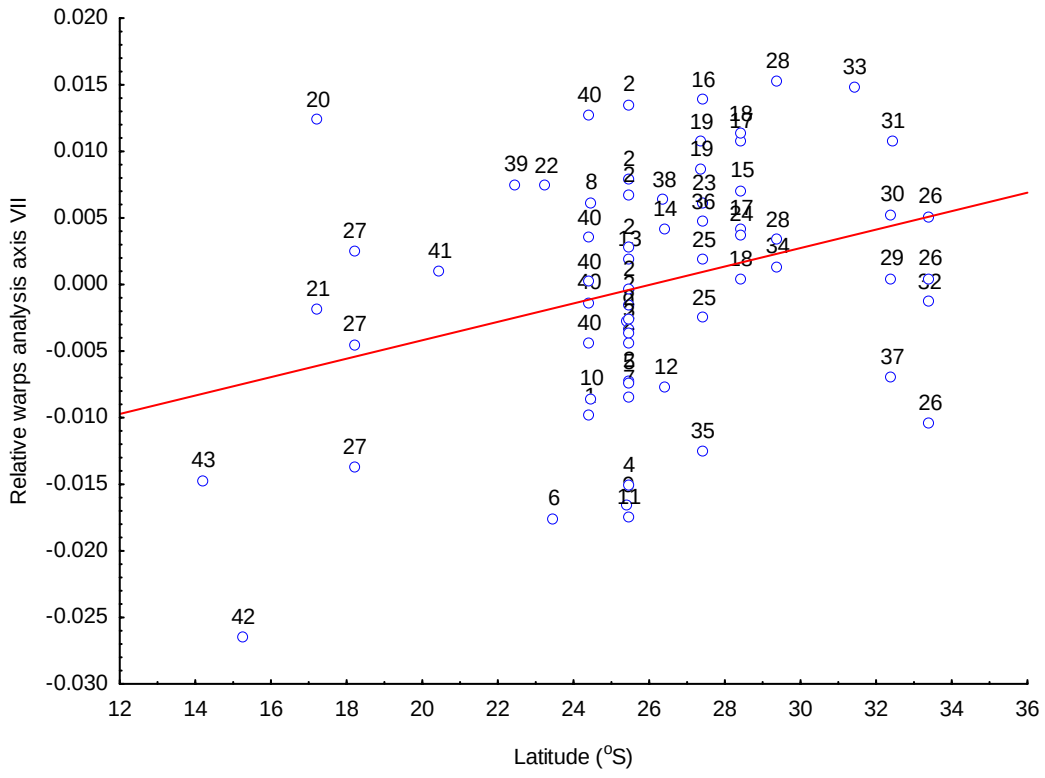

Figure 7. Regressions of Relative Warp (RW) VII scores with latitude for individual specimens of the southern African hedgehog, *Atelerix frontalis* used to assess intraspecific variation based on geometric morphometric data of the dorsal view of the cranium. The numbers correspond to the 43 localities illustrated in Fig. 1 from which individual specimens emanated from. Regression equation: y = 0.018 + 0.0007*x.

Similarly, all regressions of RW axis scores in individual-level analyses with longitude also revealed positive relationships in all 44 RWs derived from the initial PCA in which RW II ($r = 0.25$), RW XII ($r = 0.25$), RW XV ($r = 0.33$), RW XVII ($r = 0.33$), and RW XXXV ($r = 0.25$) were all statistically significant at $P < 0.05$ except for RW XXV that was statistically significant at $P < 0.01$ (Table 2), and best exemplifies the positive relation between RWs and longitude (Fig. 8).

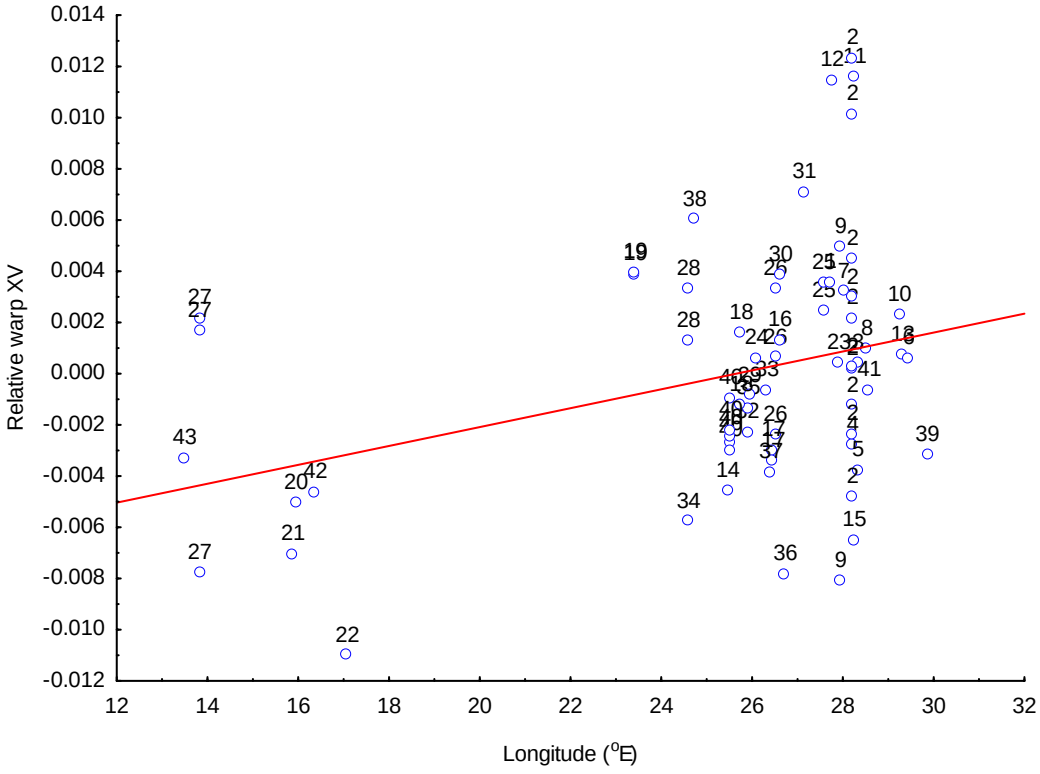

Figure 8. Regressions of Relative Warp (RW) XV scores with longitude for individual specimens of the southern African hedgehog, *Atelerix frontalis* used to assess intraspecific variation based on geometric morphometric data of the dorsal view of the cranium. The numbers correspond to the 43 localities illustrated in Fig. 1 from which individual specimens emanated from. Regression equation: y = 0.0095 + 0.0004*x.

The changes in the position of landmarks with reference to a consensus configuration (splines and vectors) of the dorsal view of the cranium are shown in Fig. 9. These configurations of the cranium show that if the configuration of the cranium from the western population were to attain that of the consensus (or average) configuration (Figs. 9a & b), they have to broaden as shown by the outward-pointing vectors (Figs. 9c & d) and vice versa for the eastern population with inward-pointing vectors (Figs. 9e & f). These results suggest that the cranium of the eastern population is much broader than that of the western population, and broadly similar results are reflected in the results of the lateral and ventral views of the cranium, and the lateral view of the mandible (not illustrated), and in traditional morphometric analysis (Chapter 4).

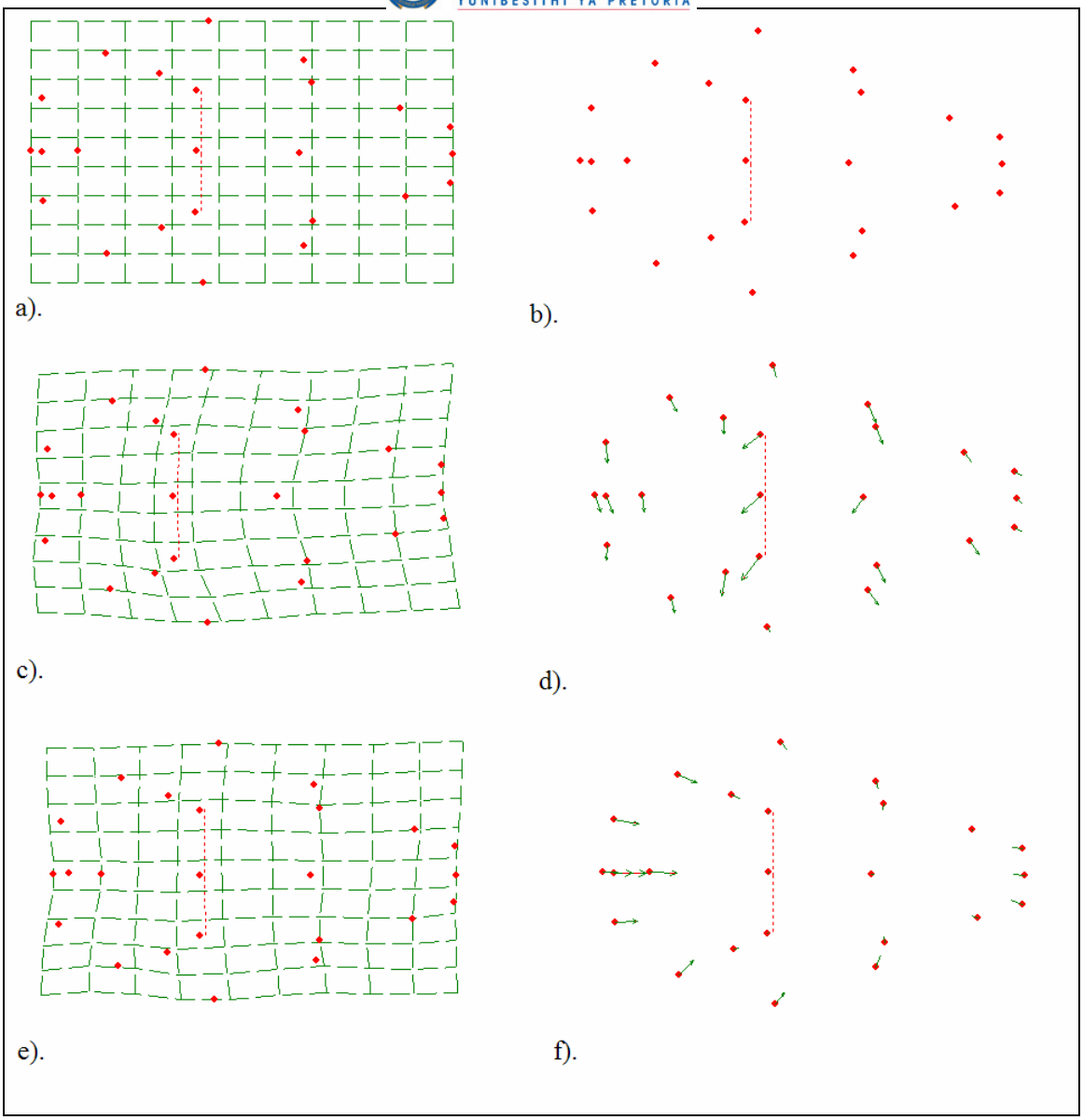

Figure 9. Changes in the position of landmarks with reference to a consensus configuration (splines) (a & b) of the dorsal view of the cranium derived from TPSSpline (Rohlf 2004a) are indicated for the specimens of the southern African hedgehog, *Atelerix frontalis* from the eastern (c & d) and the western part of its disjunct distributional range (e & f).

[4] Discussion

The subspecies category in systematics has been a subject of much debate (Mayr 1982) and one of the arguments in the 1950s was that the subspecies should be restricted to populations with strictly allopatric distributions (Mayr 1982). It was for this reason that Rautenbach (1978), despite reservations, argued for the recognition of two subspecies

within the southern African hedgehog that has a disjunct distribution of two allopatric populations in the southern African subregion.

However, the criterion of allopatry to recognize subspecies has been contested on the grounds that geographic separation of populations may be due to temporal factors (Wilson & Brown 1953; Inger 1961; Van Devender *et al.* 1992). In other words, geographic distributions may fluctuate leading to allopatric distribution that is transient at a geological scale (Manier 2004).

It is for these reasons that the present study was initiated in an attempt to gain an insight into the nature and extent of geographic variation in the near-threatened southern African hedgehog with a view to either confirm or refute the subspecific status of the two currently recognized subspecies within the southern African hedgehog, namely, *A. f. frontalis* and *A. f. angolae* using geometric morphometric (Rohlf & Marcus 1993) data of the cranium and mandible. The results suggest a north-westerly–south-easterly clinal pattern of variation with cranial configuration being positively correlated with longitude. These results are supported by traditional morphometric data.

A north-westerly–south-easterly clinal continuum of cranial and mandibular configuration with north-western populations (representing the currently recognized *A. f. angolae*) being narrower in cranial and mandibular configuration and the south-eastern populations (representing the currently recognized *A. f. frontalis*) being broader in cranial and mandibular configuration, was observed. Of particular relevance is that no pronounced steps in the clinal pattern of variation were detected in the present analysis. Given the consensus against splitting a cline into subspecies unless there is evidence of pronounced steps (James 1970; Mayr and Ashlock 1991), the results in the present study suggest that that the recognition of subspecies within the southern African hedgehog may be untenable.

Clines in homeotherms have often been interpreted in terms of Bergmann's (1874) rule. However, given that other studies (Sokal and Rinkel 1963; Rising 1970; Gould and Johnston 1972; Endler 1977; Ellison et al 1993) have suggested that clines may be a function of a complex combination of interdependent climatic factors, further

investigation in the case of the southern African hedgehog is required. It is particularly relevant in southern Africa that other small mammals have also been shown to exhibit clinal patterns of variation. More recently, these include the murid rodents *Aethomys granti* (Chimimba et al. 1998) and *A. ineptus* (Chimimba 2001). Future small mammal studies in the southern African subregion should perhaps focus on comprehensive sampling as well as analyses involving a range of environmental parameters and/or climatic variables that may assist in identifying factors that may explain both the disjunct distributions and clinal pattern of variation in the subregion.

Of particular relevance in the present study is that the delineation of a clinal pattern of variation suggests that the disjunct distribution of the southern African hedgehog may represent a recent divergence event. If this is indeed the case, then it may be argued that with additional multidisciplinary supporting evidence, one disjunct population of the southern African hedgehog could be a source population for the other, leading to implications in conservation management strategies for the southern African hedgehog.

Of particular importance is that geometric morphometric (Marcus & Corti 1996) data as applied in the present study is considered to be more superior in assessing organismal shape differences in morphology than traditional morphometrics (Marcus & Corti 1996). Nevertheless, the clinal pattern of variation was also detected by the traditional morphometric analyses therefore, providing additional support for the robustness of the delineated pattern of variation. While these analyses are phenetic in nature (Rohlf 1998), they all provide evidence for the lack of morphological discontinuities, and more importantly are well-supported by molecular data (Chapter 6).

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Appendix I

A gazetteer and geographic coordinates of sampled localities and specimens of the southern African hedgehog, *Atelerix frontalis* examined in the present study. Museum numbers denoted as: TM - Northern Flagship Institute (Transvaal museum), Pretoria; KM - Kaffrarian Museum, King William's Town; DM - Durban Natural Science Museum, Durban; NMB - National Museum, Bloemfontein; and AMNH - American Museum of Natural History, New York. Locality numbers correspond to those in Fig. 1.

Locality	Locality code	Geographic co-ordinates	Museum number	No. of samples
Rooiberg	1	24º 50'S; 27º 44'E	TM 749	1
Pretoria	2	25º 42'S; 28º 13'E	TM 2857; 4113; 5686; 7375; 16603; 16611; 27406; 40314; AMNH 54366; 90723	10
Pretoria, Silverton	3	25º 43'S; 28º 20'E	TM 27408	1
Pretoria, Hatfield	4	25º 44'S; 28º 13'E	TM 1830	1
Pretoria, Derdepoort	5	25º 40'S; 28º 20'E	TM 27684	1
Pietersburg	6	23º 54'S; 29º 27'E	TM 12470	1
Waterberg	7	25º 44'S; 28º 01'E	TM 1570	1
Settlers	8	24º 57'S; 28º 32'E	TM 28496	1
Pretoria, De Wildt	9	25º 37'S; 27º 57'E	TM 5554; 5687	2
Zebediela	10	24º 18'S; 29º 15'E	TM 12203	1
Pretoria, Waterkloof	11	25º 47'S; 28º 16'E	TM 15504	1
Krugersdorp	12	26º 06'S; 27º 46'E	TM 27409	1
Pretoria, Wonderboom	13	25° 36'S; 29° 19'E	TM 25942	1
Delareyville	14	26º 41'S; 25º 28'E	TM 23439	1
Ventersberg	15	28° 36'S; 28° 15'E	TM 751	1
Bothaville	16	27° 22'S; 26° 37'E	TM 4961	1
Brandfort	17	28° 42'S; 26° 28'E	TM 6220; NMB 1683	2
Dealesville	18	28° 40'S; 25° 46'E	TM 7587; NMB 1707	2
Kuruman	19	27° 27'S; 23° 26'E	TM 28209; 28210	2
Ondonga	20	17° 55'S; 15° 57'E	TM 7586	1
Oshikango	21	17° 24'S; 15° 53'E	TM 8019	1
Noates rehoboth	22	23° 19'S; 17° 05'E	TM 8732	1
Lindley	23	27° 52'S; 27° 55'E	NMB3497	1
Bloemfontein	24	28° 09'S; 26° 06'E	NMB 3631	1
Koppies	25	27° 14'S; 27° 35'E	KM 519; NMB 1665	2
Grahamstown	26	33° 18'S; 26° 31'E	KM 26283; 32311; 32316	3
Okorosave	27	18° 11'S; 13° 50'E	KM 526; 527; 528	3
Modder river	28	29° 02'S; 24° 36'E	KM 516; 517	2
Bedford	29	32° 38'S; 25° 57'E	KM 31729	1
Fort Beaufort	30	32° 47'S; 26° 38'E	KM 513	1
Kaffaria	31	32° 50'S; 27° 09'E	KM 15957	1
Somerset East	32	33° 13'S; 25° 54'E	KM 31968	1
Burgersdorp	33	31° 01'S; 26° 20'E	KM 514	1
Kimberley	34	29° 02'S; 24° 36'E	KM 513	1
Hoopstad	35	27° 50'S; 25° 55'E	KM 521	1
Viljoenskroon	36	27° 05'S; 26° 44'E	KM 518	1
Adelaide, Waterfall	37	32° 48'S; 26° 25'E	KM 34106	1
Vryburg	38	26° 57'S; 24° 44'E	DM 589	1
Mopani	39	22° 37'S; 29° 52'E	DM 609	1
Kweneng, Molepolole	40	29° 09'S; 25° 32'E	AMNH 167978; 167979; 167980; 167981; 168257	5
Bulawayo	41	20° 09'S; 28° 35'E	AMNH 207247	1
Huila district, Humpata	42	15° 01'S; 16° 23'E	AMNH 87640	1
Huila district, Lubango	43	14° 55'S; 13° 30'E	AMNH 87639	1

Chapter 6

Mitochondrial DNA sequence phylogeny of the southern African hedgehog, *Atelerix frontalis* (Eulipotyphla: Erinaceidae)

Abstract

The near-threatened southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831) has a disjunct distribution, occurring over the southern African subregion and extralimitally into Angola. Consequently, the species has taxonomically been allocated to two subspecies namely, *A. f. frontalis* (A. Smith, 1831) and *A. f. angolae* (Thomas, 1918). A molecular study was therefore conducted to assess the validity of the current subspecific status of the southern African hedgehog using three genes, namely, Cyt-*b*, ND2 and the control region that are all located within the mitochondrial genome. Cyt-*b* and ND2 revealed no variation across the 377 bp and 1034 bp region sequenced for each gene, respectively, whilst the 377 bp control region sequenced revealed low levels of variation between representatives of the two currently recognized subspecies (0.54 % pairwise sequence divergence). Together, these results indicate that there may be no support at the molecular level for assigning sub-specific status to the two disjunct populations of the southern African hedgehog suggesting that the disjunct distribution in this species is a very recent divergence event. These results are congruent with both traditional and geometric morphometric analyses that showed no morphological discontinuities between the two disjunct populations but rather showed evidence of a northwesterly-southeasterly clinal pattern of variation in morphology, providing further support that subspecies may be untenable. It could therefore be argued that one disjunct population could act as a source population for the other. These results may have implications for nature conservation authorities within the southern African subregion in formulating conservation management strategies for the southern African hedgehog.

[1] Introduction

The southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831) belongs to the family Erinaceidae and has a disjunct distribution in southern Africa occurring in two discrete parts of the subregion, namely: (i) South Africa, Botswana and Zimbabwe, and (ii) Namibia and Angola. The current subspecies classification coincides with this disjunct

distribution with the subspecies *A. f. frontalis* (A. Smith, 1831) restricted to the eastern part of the species-range from the Cape Province, the Free State Province, Gauteng, western Zimbabwe and eastern Botswana and the subspecies *A. f. angolae* (Thomas, 1918) occurring over most of Namibia, and extralimitally into Angola (Meester *et al.* 1986).

However, although Rautenbach (1978), based on the complete isolation of the two populations, considered the recognition of the two subspecies within the southern African hedgehog justifiable, there have been reservations on the validity of these subspecific taxonomic designations, particularly that of *A. f. angolae* (Corbet 1974, Gillies 1989, Skinner & Smithers 1990). To date, very little is known about patterns of intraspecific variation in *A. frontalis* that could either confirm or refute the validity of the current subspecific taxonomic status of the southern African hedgehog. The present study therefore, represents the first attempt to assess molecular variation within the southern African hedgehog, and is based on mitochondrial DNA (mtDNA) sequence data. This part of the study complements traditional (Chapter 4) and geometric (Chapter 4) morphometric aspects of a multidisciplinary characterization of the southern African hedgehog.

The analysis of mtDNA sequence variation has been used for more than two decades to understand the phylogeny of species as well as the geographical distribution of intra-specific genetic variation (Brown & Wright 1979; Gemmel *et al.* 1996; Stanley *et al.* 1996; Talbot & Shields 1996; Vilà *et al.* 1997; Avise 2000). The main advantage of using mtDNA for phylogenetic inference is that it is believed to represent a single, non-recombining locus so that relationships among variants may be constructed and traced back to an ancestral type.

Uniparental inheritance of mtDNA means that only one copy of the DNA molecule should be present in each individual. If multiple copies of mtDNA are present, it is termed heteroplasmy and can arise as a result of mutations in somatic or oocyte cells (Petri *et al.* 1996). Another potentially confounding problem is that translocated non-functional copies of mtDNA are known to occur in the nuclear genome (Bensasson *et al.* 2001). After transposition into the nucleus, these nuclear insertions or 'numts' evolve

independently as paralogous copies of the original mtDNA segments (Smith *et al.* 1992; Arctander 1995), but at a slower rate of sequence evolution due to their location in the nucleus. These nuclear insertions may be amplified inadvertently by PCR in addition to or even instead of authentic target mtDNA when using universal or conserved primers, leading to incorrect inferences (Sorenson & Fleischer 1996; Zhang & Hewitt 1996; Mirol *et al.* 2000; Thalmann *et al.* 2004).

Primers targeting the mtDNA control region have been shown to primarily amplify numts when the DNA is extracted from epithelial cells of proboscid hair samples as opposed to being extracted from either tissue or blood samples of the same animals (Greenwood & Pääbo 1999). This is of great concern as many studies have been conducted using hair because it is a non-invasive sampling method for the study animal. Even when the samples used are of the same tissue type, as was the case in a study on great apes (Thalmann *et al.* 2004), different targets can be amplified. Authentic mtDNA of hypervariable region 1 (HVR-1) was obtained from the bonobo, human and two orangutan liver samples, whereas multiple sequences were obtained for the HVR-1 gene in the chimpanzee and gorilla, due to the presence and amplification of nuclear copies of this mitochondrial genome segment (Thalmann *et al.* 2004).

Molecular studies are useful when morphological and anatomical information is limited, or extant species are threatened. In these cases, DNA from museum specimens can provide powerful molecular evidence to examine the genetic changes and phylogenetic relationships between taxa. However, as museum material contains highly degraded DNA, studies of this nature are often confounded with problems that lead to a decrease in efficiency of PCR amplification due to the presence of chemical inhibitors (Yang *et al.* 1997) and to the low number of copies of target DNA.

In the present study, three gene regions of medium to high levels of variation were targeted to investigate the sub-specific status of the southern African hedgehog. As the *Red Data Book of South African Mammals* lists the status of the southern African hedgehog as near-threatened (Friedman & Daly 2004) and as there is currently a decrease in its suitable habitat (Friedman & Daly 2004), material was limited for the molecular part of the multidisciplinary characterization of the southern African hedgehog. This is

because of the paucity of samples that is also due to the general secretive nature of hedgehogs, and there was therefore a need to mainly use samples obtained from museums and augment this with opportunistically-obtained fresh material for the molecular part of the study.

Samples from museum specimens (for localities outside of South Africa) as well as fresh material obtained from live animals being treated for injuries, or from roadkills from within South Africa were sourced for DNA extraction. In this manner, the possibility of inadvertent amplification of 'numts' from different tissue sources could be assessed, whilst the sample size, which unavoidably remained small, could be increased in order to be representative of the geographical distributional range of the southern African hedgehog.

[2] Materials and methods

2.1 *DNA extraction*

A total of 20 *A. frontalis* samples from 7 localities across the distributional range of the species were analyzed (Appendix I). Tissue, blood or hair samples were collected from roadkills, and from live hedgehogs of known origin taken to the Bryanston Animal Hospital (Johannesburg, Gauteng Province, South Africa) for treatment following an accident, whilst epithelial cells from hair follicles were used for DNA extraction from museum specimens.

DNA was extracted using the Roche extraction kit for fresh specimens. For museum specimens, the hair follicles were placed in ddH_20 and incubated at 55ºC. The water was changed each day, over a 3-day sample incubation period (Pääbo *et al.* 1988), prior to extraction by means of the Roche DNA extraction kit. Filter tips were used throughout the extraction process so as to reduce the chance of sample contamination.

2.2 *Evaluation and selection of mitochondrial DNA genes*

Complete mtDNA sequences of related species were obtained from Genbank and aligned using the DAPSA programme version 4.91 (Harley 2001). The sequences were: AB099481 – long-eared hedgehog, *Hemiechinus auritus*; X88898 – European hedgehog, *Erinaceus europaeus*; AB099484 – small Madagascar hedgehog, *Echinops telfairi*; and

AF348079 – moonrat, *Echinosorex gymnura*. MEGA version 3.1 (Kumar *et al.* 2005) was used to obtain sequence divergences on a per gene basis, and the three genes with the highest levels of sequence divergence were selected for characterization in this study. In this manner, three target genes, Cyt-*b*, ND2 and the control region (D-loop) were selected for this study.

2.3 *Genomic amplification of itochondrial DNA genes*

Targeted fragments were amplified by means of the polymerase chain reaction (PCR). A typical reaction comprised of 1U of BioTools *Taq* DNA polymerase, 0.4µM of each primer and 1X reaction buffer in a final volume of 50µl. Usually, 2µl of DNA from a fresh sample and 5µl of DNA from a museum sample was added to each PCR reaction tube, and contamination was controlled for by the inclusion of a DNA-free negative control. The primers used for each gene are summarized in Table 1.

TABLE 1. A list of primers used in the mitochondrial DNA (mtDNA) analysis of the southern African hedgehog, *Atelerix frontalis*, indicating the oligonucleotide sequence, orientation (F/R), gene target, melting temperature ™ (ºC) as well as the reference where the primers were obtained from. Melting temperature Tm was calculated by the equation: [69.3 + (0.41* %GC)] – 650/primer length.

Name	Sequence	Orientation	Gene target	Tm (ºC)	Reference
L14724	TGAYATGAAAAAYCATCGTTG	Forward	Cyt-b	51.88	Irwin *et al*. 1991
Mus-IR	AATGATATTTGTCCTCATG	Reverse	Cyt-b	48.21	Bastos *unpub*.
H15915LR	CTCATTTTTGGTTTACAAGA	Reverse	Cyt-b	49.10	This study
Mus-IF	AATGACAAACATCCGA	Forward	Cyt-b	46.72	Bastos *unpub*.
H15915-Mus	CATTTCAGGTTTACAAGAC	Reverse	Cyt-b	50.26	Russo 2003
Univ-R	TGTTCTACGGGTTGTCCTCCRATTCA	Reverse	Cyt-*b*	50.00	Bastos *unpub*.
VMet2	GCTAAACAAGCTTTCGGGCCCATACC	Forward	ND2	66.44	Cunningham & Cherry 2004
VTrp	CTCCTGCTTCGGGCTTTGAAGGC	Reverse	ND2	66.05	Cunningham & Cherry 2004
ND2F-LR	GGCCCATACCCCGAAAATGTT	Forward	ND2	59.67	This study
ND2R-LR	CTTAGRGCTTTGAAGGCTCT	Reverse	ND2	55.25	This study
L15925	TACACTGGTCTTGTAAACC	Forward	D-loop	49.00	Modified from Kocher *et al.* 1989
L16499	CTTGAAGTAGGAACCAGAT	Reverse	D-loop	49.00	Modified from Kocher *et al.* 1989
ProL-He	ATACTCCTACCATCAACACCCAAAG	Forward	D-loop	61.34	Seddon *et al*. 2001
DLH-He	GTCCTGAAGAAAGAACCAGATGTC	Reverse	D-loop	61.08	Seddon *et al*. 2001
LR-ForwD	CCTGAATAAACATGTATATGCATAT	Forward	D-loop	54.78	This study
LR-F58	GATATTCTRCTTAAACTATTCCCTGA	Forward	D-loop	58.00	This study
LR-F56	GATATTCTATTTTAAACTACTCCYTG	Forward	D-loop	56.00	This study
LR-RevD	GATGTCTTGTGAAATACAAGGTTA	Reverse	D-loop	54.11	This study
LR-RevD60	GGCGAGGAGAGGGATACTGT	Reverse	D-loop	60.00	This study

2.4 *Optimization and amplification conditions for museum specimens*

ß-mercaptoethonal, bovine serum albumin (BSA) and high concentrations of Biotools *Taq* DNA polymerase were used to overcome inhibitory factors present in museum samples (Pääbo *et al.* 1988). The effect of ß-mercaptoethonal, BSA and Bio tools *Taq* DNA polymerase was investigated by testing a concentration range of one of these reagents whilst maintaining a constant concentration for the remaining two. Primers L14724 and Mus-IR, which were previously determined to effectively amplify the 5' terminal end of the cyt-b gene of southern African hedgehog DNA extracted from fresh samples, were used when assessing the effect of these PCR reagents. This was done to establish the optimal concentration that achieves the best amplification from museum samples and was used in all subsequent PCR reactions. Following this, PCRs were optimised further on a per-gene basis by adjusting the annealing temperatures either up or down by a few degrees from the initially determined annealing temperatures listed in Table 2.

2.5 *Controls for amplification bias in different tissue samples*

To determine if there was any difference in the target sequence amplified from different sample types, DNA was extracted from hair follicles and tissue samples of animals from the Free State and Gauteng Provinces, South Africa and used as template for PCR.

2.6 *Nucleotide sequencing and analysis*

The amplified fragments were purified using the Roche High-Pure purification kit. Cycle sequencing was preformed at primer-specific annealing temperatures (Table 2) using Big-dye version 3.1 with all unincorporated nucleotides and primers being removed by sodium acetate DNA precipitation. The sequences were run on an ABI377 automated sequencer (Applied Biosystems, California), and were viewed with CHROMAS (version 1.43). Sequences edited in CHROMAS were exported as text files to the DAPSA programme version 4.91 (Harley 2001) and aligned.

TABLE 2. The different primer combinations used for mitochondrial DNA (mtDNA) analysis of the southern African hedgehog, *Atelerix frontalis*. The initial annealing temperature (Ta) calculated as 4ºC below the Tm of the primer with the lowest Tm of a particular primer pair, gene target and the expected amplicon size are given. Amplicon sizes were estimated from primer alignment to the complete mtDNA genome of the European hedgehog, *Erinaceus europaeus* (Genbank no. X88898).

Primer 1	Primer 2	Gene target	Amplicon size	Ta (ºC)
ProL-He	DLH-He	D-loop	451bp	60
L15925	H16499	D-loop	584bp	49
ProL-He	LR-RevD	D-loop	439bp	54
ProL-He	LR-RevD60	D-loop	383bp	58
DLH-He	LR-Fwd	D-loop	545bp	54
LR-Fwd	LR-RevD	D-loop	413bp	52
LRF-58	DLH-He	D-loop	476bp	58
LRF-58	LR-RevD	D-loop	458bp	58
LRF-58	LR-RevD60	D-loop	408bp	58
LRF-56	DLH-He	D-loop	424bp	56
LRF-56	LR-RevD	D-loop	406bp	56
LRF-56	LR-RevD60	D-loop	356bp	56
L14724	Mus-IR	Cyt-*b*	1432bp	46
L14724	H15915-Mus	Cyt-*b*	1194bp	48
L14724	H15915LR	Cyt-*b*	1191bp	48
Mus-IF	H15915-Mus	Cyt-*b*	~ 420bp	45
Mus-IF	H15915LR	Cyt-*b*	~ 420bp	46
L14724	Univ-R	Cyt-*b*	~ 420bp	46
vMet2	vTrp	ND2	1154bp	58
ND2F-LR	ND2R-LR	ND2	1125bp	47

MEGA version 3.1 (Kumar *et al.* 2005) was used to obtain basic sequence statistics and to infer a preliminary phylogeny. Due to the sequences having both base composition and transition:transversion bias, the Tamura-Nei model of sequence evolution was used to infer a phylogeny with the neighbor-joining (NJ) algorithm in MEGA version 3.1 (Kumar *et al.* 2005). Non-parametric bootstrap resampling was used to assess nodal support and all trees were mid-point rooted. Maximum parsimony (MP) analyses were performed in PAUP* version 4.0 (Swofford 2002). Equal weighting and

successive weighting schemes were investigated prior to resampling by 10000 bootstrap replicates.

Model Test (Posada & Crandall 1998) was used to select the model that best fitted each dataset, and these model parameters were subsequently used for maximum likelihood (ML) analysis in PAUP* version 4.0 (Swofford 2002). Differences in the relative rate of mutations among lineages was assessed for the Cyt-*b* dataset, by comparing the likelihood scores obtained with and without a molecular clock enforced. In the absence of statistically significant differences a molecular clock can be enforced.

[3] Results

3.1 *Optimization and amplification of museum specimens*

ß-mercaptoethonal, BSA and increased amounts of Bio tools *Taq* DNA polymerase were used to overcome inhibitory factors found in the extractions of museum specimens. The optimum amounts for each were 2.5µl of a 100mM ß-mercaptoethonal solution (freshly prepared), 1.5µl of a 2mg/ml solution of BSA (diluted with ddH_2O) and 4U of *Taq* per PCR reaction tube (Fig. 1), using primers L14724 and *Mus*-IR.

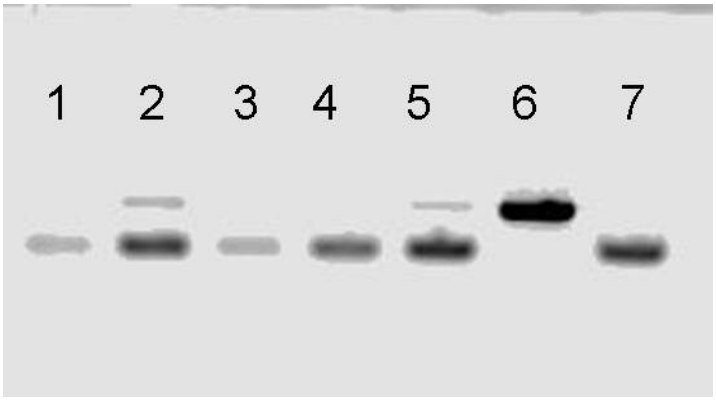

Figure 1. A gel illustrating the trial of optimization conditions for museum specimens of the southern African hedgehog, *Atelerix frontalis*. Lane 1: 1µl BSA, 2.5µl ß-mercaptoethonal and 4µl of *Taq* DNA polymerase. Lane 2: 1.5µl BSA, 2.5µl ß-mercaptoethonal and 4µl of *Taq* DNA polymerase. Lane 3: 0.5µl BSA, 2.5µl ß-mercaptoethonal and 4µl of *Taq* DNA polymerase. Lane 4: 0.5µl BSA, 2.5µl ß-mercaptoethonal and 7.5µl of *Taq* DNA polymerase. Lane 5: 1µl BSA, 5µl ß-mercaptoethonal and 8µl of *Taq* DNA polymerase. Lane 6: Positive control. Lane 7: Negative control. Primers used were L14724 and Mus-IR.

A PCR preformed after the optimization trial illustrated that the genomic amplicons are obtained even in the absence of ß-mercaptoethonal (Fig. 2), when using primers LRF-58 and DLH-He that target the D-loop. The results obtained with the control region mirrored those of the Cyt-*b* region with museum sample 1 (Lane 1, Fig. 2) not amplifying with or without ß-mercaptoethonal.

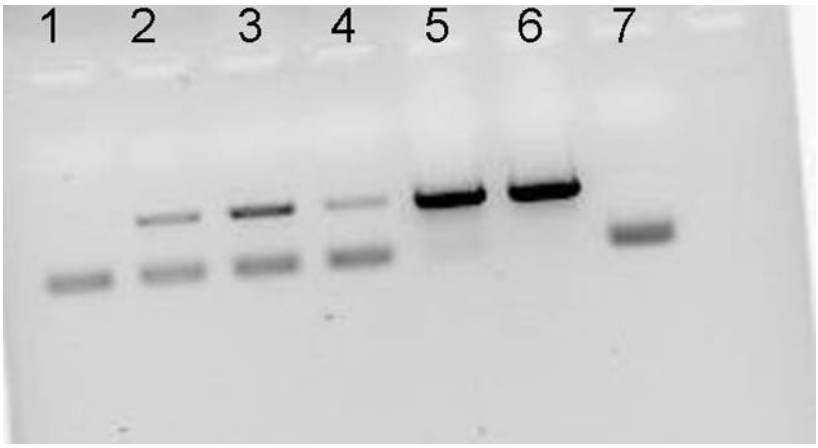

Figure 2. The gel of D-loop PCR products obtained with primers LRF-58 and DLH-He and without the addition of ß-mercaptoethonal. Lanes 1 – 3 are the museum specimens, whilst lane 4 is a hair sample for a fresh Free State province specimen and 5 is a liver sample for the fresh Free State province specimen of the southern African hedgehog, *Atelerix frontalis*. Lane 6 is the positive control obtained from an ear clipping of a fresh specimen collected in Bryanston. Lane 7 is the negative control.

3.2 *Controls for amplification bias*

A test for amplification bias using hair and tissue samples from two of the individuals used in the present study (MID and FS) revealed no difference between the sequences of the different DNA sources used for PCR with the same primers, namely LRF-58 and DLH-He (Appendix II).

3.3 *Differential amplification success with different primers*

Published primer sets were initially used in this study. However as many of the primer combinations failed to amplify the target gene there was a need to design primers

specifically for the present study. Table 3 summarises all primer combinations investigated as well as the results obtained with each primer set.

3.4 *Nucleotide sequence analysis*

Sequence data from the Cyt-*b* gene comprised a homologous dataset of 377 bp, of which 116 sites were variable and 60 were parsimony informative. There was base composition bias (A-T-rich), with base composition being: T = 37.9 %, C = 18.7 %, A = 30.7 %, G =12.6 %. The transition:transversion ratio was biased towards transitions with R = 2.0.

For the control region, the homologous dataset comprised 377 bp of which 103 sites were variable and 43 were parsimony informative. This gene region also displayed A-T bias, with proportions of each base being as follows: T = 34.1 %, C = 19.9 %, A = 39.1 %, G = 7.0 %. The transition:transversion ratio was slightly biased towards transversions with an R = 0.9.

TABLE 3. Primer combinations used in the mitochondrial DNA (mtDNA) analysis of the southern African hedgehog, *Atelerix frontalis* to amplify gene targets from DNA extracted from different sources. This included fresh material from the Gauteng and Free State Provinces, South Africa, museum specimens from Angola and a fresh liver sample from Tanzania thought to be *A. albiventris*.

Primer 1	Primer 2	Gene target	DNA source	No. of samples tested	Results
ProL-He	DLH-He	D-loop	Fresh & museum samples, Tanzanian sample	6 fresh, 4 museum 1 Tanzanian	Amplification but no readable sequence
L15925	H16499	D-loop	Fresh samples	2	No amplification
ProL-He	LR-RevD	D-loop	Fresh & museum samples, Tanzanian sample	1 fresh, 2 museum, 1 Tanzanian	No amplification
ProL-He	LR-RevD60	D-loop	Fresh & museum samples, Tanzanian sample	4 fresh, 2 museum, 1 Tanzanian	Amplification, double sequence
DLH-He	LR-Fwd	D-loop	Fresh & museum samples, Tanzanian sample	4 fresh, 2 museum, 1 Tanzanian	No amplification
LR-Fwd	LR-RevD	D-loop	Fresh & museum samples, Tanzanian sample	4 fresh, 2 museum, 1 Tanzanian	Only the Tanzanian sample amplified
LRF-58	DLH-He	D-loop	Fresh samples, Tanzanian sample	4 fresh, 1 Tanzanian	Only Gauteng samples amplified
LRF-58	LR-RevD	D-loop	Fresh & museum samples, Tanzanian sample	4 fresh, 2 museum, 1 Tanzanian	All amplified and sequenced
LRF-58	LR-RevD60	D-loop	Fresh samples, Tanzanian sample	4 fresh, 1 Tanzanian	Amplification and sequenced
LRF-56	DLH-He	D-loop	Fresh samples, Tanzanian sample	4 fresh, 1 Tanzanian	No amplification
LRF-56	LR-RevD	D-loop	Fresh samples, Tanzanian sample	4 fresh, 1 Tanzanian	No amplification
LRF-56	LR-RevD60	D-loop	Fresh samples, Tanzanian sample	4 fresh, 1 Tanzanian	No amplification
L14724	Mus-IR	Cyt-*b*	Fresh & museum samples, Tanzanian sample	8 fresh, 7 museum, 1 Tanzanian	Amplification and readable sequence
L14724	H15915-Mus	Cyt-*b*	Fresh samples, Tanzanian sample	2 fresh, 1 Tanzanian	No amplification
L14724	H15915LR	Cyt-*b*	Fresh samples, Tanzanian sample	2 fresh, 1 Tanzanian	No amplification
Mus-IF	H15915-Mus	Cyt-*b*	Fresh samples, Tanzanian sample	2 fresh, 1 Tanzanian	No amplification
Mus-IF	H15915LR	Cyt-*b*	Fresh samples, Tanzanian sample	2 fresh, 1 Tanzanian	No amplification
L14724	Univ-R	Cyt-*b*	Fresh samples, Tanzanian sample	2 fresh, 1 Tanzanian	No amplification
vMet2	vTrp	ND2	Fresh samples, Tanzanian sample	2 fresh, 1 Tanzanian	Only fresh samples amplified and sequenced
ND2F-LR	ND2R-LR	ND2	Fresh samples, Tanzanian sample	2 fresh, 1 Tanzanian	Only fresh samples amplified and sequenced

Neither Cyt-*b* nor ND2 showed any sequence variation between specimens from the southern African subregion, over the 377 and 1034 nucleotides (Appendices III & IV), respectively determined for each gene region. In the control region (D-loop) data, only a slight difference in sequences between the eastern and western populations of the southern African hedgehog was observed (Appendix V). However, both Cyt-*b* and D-loop gave considerable variation between the sample collected in Tanzania and the samples from the southern African subregion. For Cyt-*b*, the sequence divergence was 13 % and for D-loop, the sequence divergence was 8 % confirming that the sample collected from Tanzania is representative of a species distinct from that occurring in southern Africa. The sample collected from Tanzania failed to amplify with the ND2 primers despite testing over a wide range of annealing temperatures.

The GTR+I and TVM+G models of sequence evolution were selected for Cyt-*b* and the control region, respectively in Model Test under the Akaike Information criterion (AIC). The tree generated by MEGA version 3.1 (Kumar *et al.* 2005) (Fig. 3) for Cyt-*b*, using midpoint rooting, indicates that the Angolan sample does not differ from the South African samples and that these samples form a single cluster that has 100 % bootstrap support. Sequences obtained from Genebank (two European hedgehogs, euro – AF379791, conc – AF379803 and the long-eared hedgehog –AB099481) were also included in the analysis. The maximum parsimony and Maximum likelihood trees had similar topology to the neighbor-joining tree. Parsimony analysis resulted in one most parsimonious tree for Cyt-*b*, 148 in length and with a retention index (RI) = 0.829, consistency index (CI) = 0.878 and rescaled consistency index (RC) = 0.728. As the ND2 gene tree was similar to that obtained following Cyt-*b* gene analysis it is not illustrated.

By comparing the maximum likelihood scores with a clock enforced and without a clock enforced, using PAUP (version 4.0), it was found that there was no statistically significant difference (likelihood = 0.43) and that a molecular clock could be imposed. A rate of 2 % per million years was used (Avise *et al.* 1987), however, this may be an under-estimation as the p-distance table (Appendix VI) of full-length Cyt-*b* sequences versus the shorter 5' region sequenced in this study, indicated that the latter has a lower rate of mutation presumably due to the more stringent functional constraints in the latter region.

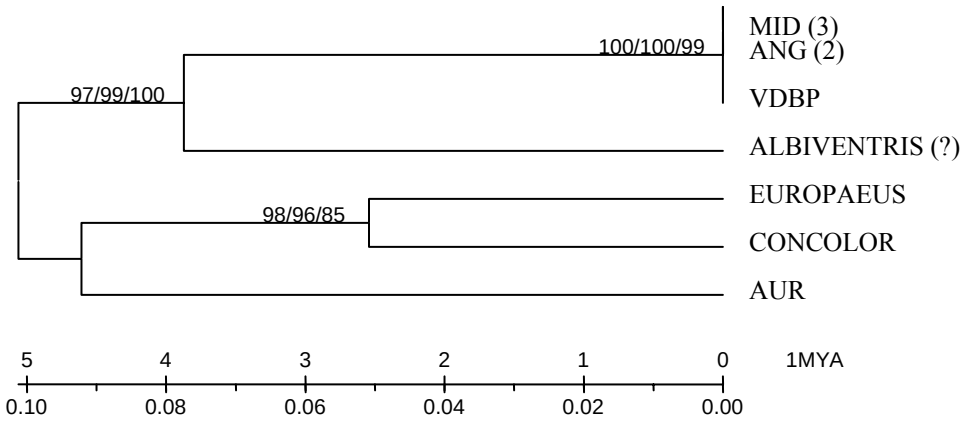

Figure 3. The neighbor-joining tree constructed in MEGA (version 3.1) using 377 bp southern African hedgehog, *Atelerix frontalis* sequences that correspond to the 5' end of Cyt-*b*. The abbreviations used for the specimens are as follows: VDBP-Vanderbijlpark, MID-Bryanston, ANG-Angola, ALBIVENTRIS (?)-Tanzanian sample, *A. albiventris*, CONCOLOR-European hedgehog, *Erinaceus concolor*, EUROPAEUS-Western European hedgehog, *Erinaceus europaeus*, AUR-long-eared hedgehog, *Hemichinus auritus*. A molecular clock was imposed in MEGA (version 3.1) using 2 % divergence per million years. Bootstrap values on each node correspond to those obtained from neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) and that were >50 % are indicated in the order NJ/MP/ML next to each relevant node. The number of samples sequenced per locality is given in brackets next to the locality abbreviation.

Figure 4 shows the neighbor-joining tree generated by MEGA version 3.1 (Kumar *et al.* 2005) for the HVR-1 portion of the control region (D-loop) illustrating that the Angolan sample differs slightly, forming a separate lineage which is distinct from the remaining southern African samples. All samples collected within South Africa were identical to each other and clustered together with the Angolan sample with between 93 to 100 % bootstrap support. The distance between the Angolan and South African samples is, however, less than the distance between two eastern European hedgehogs of the same species (*E. concolor*) as shown in Fig. 4. The genetic diversity between the two European hedgehog species could not be assessed as the single western European hedgehog, *E. europaeus* sequence in the Genbank database was found to be identical to an eastern European hedgehog (Appendix VII) and therefore of questionable authenticity.

The long-eared hedgehog (AB099481) reference sequence is basal to all other clades in the midpoint rooted tree.

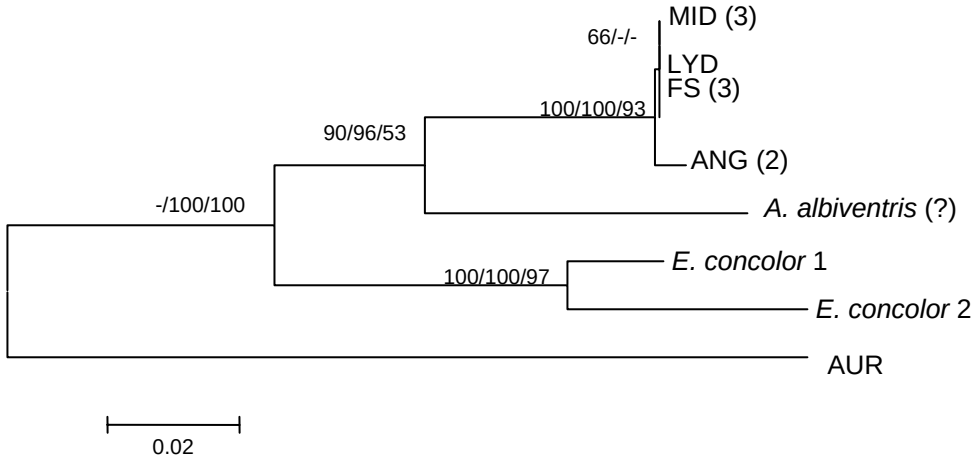

Figure 4. The neighbor-joining tree constructed in MEGA (version 3.1) using 377nt of the HVR-I portion of the control region (D-loop). The abbreviations used for the specimens are as follows: MID-Bryanston, FS-Free State, LYD-Lydenburg, ANG-Angola, *A. albiventris* (?)-Tanzanian sample, CONCOLOR-eastern European hedgehog, *Erinaceus concolor*, AUR-long-eared hedgehog, *Hemichinus auritus*. Bootstrap values > 50 % that were obtained from NJ, MP and ML are given in that order with a slash between, with dashes denoting bootstrap support values below 50 %. The number of samples sequenced per locality is given in brackets next to the locality abbreviation.

The sequence divergence between the South African specimens and the Angolan specimen was 0.54 %. If nodes with less than 50 % support are collapsed, then only three well-supported clades are retained (Fig. 4), namely: (i) the southern African and Angolan *Atelerix* clade, (ii) the Tanzanian *Atelerix* lineage and (iii) the European hedgehog clade (Genbank Accession numbers for *E. concolor* 1 – AF379761 and *E. concolor* 2 – AF379765). These same three clades are recovered with parsimony analysis.

Likelihood scores obtained with and without a molecular clock enforced revealed no significant rate heterogeneity within the control region but a clock was not imposed as the control region is hypervariable within species (Larizza *et al.* 2002), and therefore less likely to result in reliable divergence estimates. The derived Maximum parsimony and Maximum likelihood bootstrap values supported the phylogenetic tree inferred from

neighbor-joining. The maximum parsimony D-loop tree had a retention index of 0.847, a consistency index of 0.915 and a rescaled consistency index of 0.775. The Maximum likelihood gave a likelihood score of 1047.88.

[4] Discussion

Currently, the southern African hedgehog, *Atelerix frontalis*, is split into two subspecies namely, *A. f. frontalis* and *A. f. angolae* (Rautenbach 1978). However, reservations have been expressed on the validity of these subspecific desiginations, particularly that of *A. f. angolae* (Corbet 1974, Gillies 1989, Skinner & Smithers 1990). This taxonomic uncertainty prompted the present study into the taxonomic status of the species. Due to the near-threatened listing of the southern African hedgehog in the *Red Data Book for South African Mammals* (Friedman & Daly 2004), and the limited material available for the molecular study due to the paucity of samples that is also due to the general secretive nature of hedgehogs, there was a need to use samples obtained from museums and augmented by opportunistically-obtained fresh material.

The use of museum material in the present study necessitated that the PCR had to be optimized in order to overcome inhibitory substances. It was found that the addition of 2.5µl of a 100mM ß-mercaptoethonal solution (freshly prepared), 1.5 µl of a 2mg/ml solution of BSA (diluted with ddH_2O), and four times the amount of *Taq* DNA polymerase (4U instead of 1U), was required to ensure amplification from museum samples (Fig. 1). However, as the inadvertent exclusion of ß-mercaptoethonal made little difference to the results obtained with the D-loop primers (Fig. 2), it was subsequently excluded from PCRs directed at this gene target.

Greenwood and Pääbo (1999) suggested that the type of material used in a study could cause a bias in amplification of nuclear insertions or numts. This was found to be the case for elephants where hair samples resulted in preferential amplification of numts whilst authentic mitochondrial DNA sequences were obtained from blood samples. Due to the need to use museum specimens in the present study, and specifically hair samples to extract DNA, it was considered necessary to test for amplification bias between hair and tissue samples in fresh specimens.

In the analysis of the sequences obtained in the present study, a perfect alignment was found between hair and tissue samples. It was therefore, concluded that no amplification bias was present, based on the type of material used, and that sequences obtained from blood or tissue of fresh specimens could be safely compared with sequences obtained from the hair of museum specimens.

The length of the complete mitochondrial genome sequence of the European hedgehog (*E. europaeus*) is 17422 nucleotides (Genbank accession number X888898), with the control region comprising about 1988 bp of this total. This is notably longer that the control region of most other eutherian mammals, which is only ~1000 bp (Nikaido *et al.* 2003). The increased mtDNA genome length of hedgehogs is mainly due to the longer control region which contains a number of repeated motifs at two different positions in the 3' end.

However, the length of the control region is not absolute due to pronounced heteroplasmy caused by variable numbers of the motif TACGCA in one of the repetitive regions. The sequence presented includes 46 repeats of this type whilst the other repeat region is composed of different A-T-rich repeats (Krettek *et al.* 1995).

All southern African hedgehog sequences were identical across the Cyt-*b* and ND2 gene regions sequences. Minor sequence variation was observed for the control region. The Angolan sequence differed slightly and formed a separate clade in the control region analysis but no separation was observed for either the Cyt-*b* or the ND2 gene regions. Because bootstrap values over 70 percent usually correspond to a 95 % probability that the corresponding clade is meaningful (Hillis & Bull 1993), the clustering of the Angolan sample with and within the clade of South African samples (100 % support) was considered significant (Fig. 4). Similar results from maximum parsimony and the maximum likelihood analyses confirm that the data are not sensitive to the underlying assumptions of each method of analysis and support the conclusion that the current sub-specific status may not be valid.

The use of museum specimens in the present study precluded the generation of a full-length sequences for Cyt-*b*, due to the degraded state of the DNA, but also because of the low amplification success obtained with published universal Cyt-*b* primers (Table 3). Future studies should be directed at developing *Atelerix*-specific cyt-b primers that will permit the generation of complete gene sequences and a more accurate estimate of divergence of this species from its Tanzanian con-generic, which from this study was estimated to have occurred more than 1.5 million years earlier than the European hedgehog species split. The marked difference between the Tanzanian and southern African samples at both the Cyt-*b* and D-loop regions (p-distances of 0.127 and 0.078, respectively) indicates a more ancient divergence than that observed for the European species. From the conservative Cyt-*b* gene molecular clock imposed in this study it was estimated that the East African hedgehog last shared a common ancestor with the southern African hedgehog, approximately 4 MYA.

Forced movements due to climatic fluctuations result in differences in dispersal conditions, which may influence the consequent genetic diversity (Seddon *et al.* 2001). This may explain the slight separation of the Angolan sample from the South African samples in the phylogeny presented in Fig. 4. The sequence divergence obtained between the South African samples and the Angolan sample was 0.54 %. The rate of mitochondrial DNA differentiation in mammals is about 2 % sequence divergence per million years (Avise *et al.* 1987) and as much as 7 % per million years for the primate control region (Avise *et al. 1987*) pointing to the split between the Angolan and the South African hedgehogs and hence the disjunct distribution to be a very recent divergence event.

The phylogenetic results, together with the low levels of sequence divergence (0 % for Cyt-*b* and 0.54 % for D-loop) indicate that the current subspecific classification may be untenable, and may need to be revised. These results are congruent with both traditional and geometric morphometric analyses that showed no morphological discontinuities between the two disjunct populations but rather showed evidence of a northwesterly-southeasterly clinal pattern of variation in morphology further supporting the recognition of subspecies to be untenable. It could therefore be argued that one disjunct population could act as a source population for the other. These results may have implications for

nature conservation authorities within the southern African subregion in formulating conservation management strategies for the southern African hedgehog.

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Appendix I

A list of specimens of the southern African hedgehog, *Atelerix frontalis* used in the present study, as well as the type of material used. AMNH is the abbreviation for the American Museum of Natural History, New York, U.S.A.

Specimen number	Source	Type of material
TM 1830	Gauteng	Hair
TM 749	Gauteng	Hair
Hedge 1	Gauteng	Extracted DNA
Hedge 2	Gauteng	Extracted DNA
AMNH 167980	Botswana	Hair
AMNH 167979	Botswana	Hair
AMNH 207247	Zimbabwe	Hair
AMNH 167981	Botswana	Hair
AMNH 168257	Botswana	Hair
AMNH 87639	Angola	Hair
AMNH 87640	Angola	Hair
LY 23	Lydenburg	Ear tissue
FS 1	Free State	Hair
FS 2	Free State	Hair
FS 3	Free State	Hair, liver tissue and ear tissue
HM baby	Gauteng	Ear tissue
HM 2 months	Gauteng	Liver tissue, hair, ear tissue
HedgeG	Gauteng	Blood
HT-liver	Tanzania	Liver tissue

Appendix II

The sequence alignment for the Gauteng and FS samples of the southern African hedgehog, *Atelerix frontalis* used to determine if amplification bias occurred, depending on DNA source. Aligned at a stringency of 10. Asterisks indicate 100% base pair match at a given site.

```
                                                                      60
MID tissue TATAATCAAC ATTATTTAAT TACCACATAA TGATATGCAC TTAAATATTA AATAATACAA
MID hair   TATAATCAAC ATTATTTAAT TACCACATAA TGATATGCAC TTAAATATTA AATAATACAA
FS hair    TATAATCAAC ATTATTTAAT TACCACATAA TGATATGCAC TTAAATATTA AATAATACAA
FS tissue  TATAATCAAC ATTATTTAAT TACCACATAA TGATATGCAC TTAAATATTA AATAATACAA
           ********** ********** ********** ********** ********** **********
                                                                     120
MID tissue AGACATTAAA TTAATATTTA CTATAAATTT ATGTAAAACT AGCATATAAG CATGTACATT
MID hair   AGACATTAAA TTAATATTTA CTATAAATTT ATGTAAAACT AGCATATAAG CATGTACATT
FS hair    AGACATTAAA TTAATATTTA CTATAAATTT ATGTAAAACT AGCATATAAG CATGTACATT
FS tissue  AGACATTAAA TTAATATTTA CTATAAATTT ATGTAAAACT AGCATATAAG CATGTACATT
           ********** ********** ********** ********** ********** **********
                                                                     180
MID tissue AAATCTTAAT TATTACATAA TACATTAAAT TATCTCACAA CTTTAAAATA AATAACAATA
MID hair   AAATCTTAAT TATTACATAA TACATTAAAT TATCTCACAA CTTTAAAATA AATAACAATA
FS hair    AAATCTTAAT TATTACATAA TACATTAAAT TATCTCACAA CTTTAAAATA AATAACAATA
FS tissue  AAATCTTAAT TATTACATAA TACATTAAAT TATCTCACAA CTTTAAAATA AATAACAATA
           ********** ********** ********** ********** ********** **********
                                                                     240
MID tissue CGAATATCTA AATCAATTAT AATTTATTAA TATTACATAG TACATATTAA TATTAATCGT
MID hair   CGAATATCTA AATCAATTAT AATTTATTAA TATTACATAG TACATATTAA TATTAATCGT
FS hair    CGAATATCTA AATCAATTAT AATTTATTAA TATTACATAG TACATATTAA TATTAATCGT
FS tissue  CGAATATCTA AATCAATTAT AATTTATTAA TATTACATAG TACATATTAA TATTAATCGT
           ********** ********** ********** ********** ********** **********
                                                                     300
MID tissue ACATAGCGCA TTCTATTAAT AAATTTTCTC TACCACCCGC ATATCACCTC CATTAGGTTA
MID hair   ACATAGCGCA TTCTATTAAT AAATTTTCTC TACCACCCGC ATATCACCTC CATTAGGTTA
FS hair    ACATAGCGCA TTCTATTAAT AAATTTTCTC TACCACCCGC ATATCACCTC CATTAGGTTA
FS tissue  ACATAGCGCA TTCTATTAAT AAATTTTCTC TACCACCCGC ATATCACCTC CATTAGGTTA
           ********** ********** ********** ********** ********** **********
                                                                     360
MID tissue TTTCTTAATC TACCAACTCA CGTGAAACCA ACAACCCTTG TGAACAGTAT CCCTCTCCTC
MID hair   TTTCTTAATC TACCAACTCA CGTGAAACCA ACAACCCTTG TGAACAGTAT CCCTCTCCTC
FS hair    TTTCTTAATC TACCAACTCA CGTGAAACCA ACAACCCTTG TGAACAGTAT CCCTCTCCTC
FS tissue  TTTCTTAATC TACCAACTCA CGTGAAACCA ACAACCCTTG TGAACAGTAT CCCTCTCCTC
           ********** ********** ********** ********** ********** **********

MID tissue GCCCCGGGCC CAT
MID hair   GCCCCGGGCC CAT
FS hair    GCCCCGGGCC CAT
FS tissue  GCCCCGGGCC CAT
           ********** ***
```

Appendix III

Cyt-*b* alignment of the southern African hedgehog, *Atelerix frontalis* from MEGA version 3. No difference was found in any of the specimens studied. Specimens were sampled from the eastern and western populations of the species with VDBP and MIDRAND from the eastern population and the Angolan samples from the western population. The European hedgehogs, *Erinaceus europaeus* and *E. concolor*, the long-eared hedgehog, *Hemichinus auritus* were also included.

```
VDBP    TAATAAAAAT TGTTAATGAA TCTTTCATTG ACTTACCTAC CCCGCTAAAT ATCTCATCTT
MIDR    .......... .......... .......... .......... .......... ..........
TANZ    .......... .......... .......... .......... T..AT..... ..T.....A.
ANGO    .......... .......... .......... .......... .......... ..........
CONCOL  .......... .A.....A.T .....T.... .T..G..A.. T..ATCC... ..T..T....
EURO    .......... .A.....A.T .....T.... .T.....A.. ...ATCT... ..T..T....
AUR     .......G.. CA.....A.T .......... ..C.C..... ...ATC.... ..T..T....

VDBP    GATGAAATTT TGGTTCTTTA TTAGGCCTAT GCTTAATTAT ACAAATTATT ACAGGACTAT
MIDR    .......... .......... .......... .......... .......... ..........
TANZ    .......... .......C.. ......T... .......... ...G....CC ......T...
ANGO    .......... .......... .......... .......... .......... ..........
CONCOL  ....G..... .......... C.....T... C.C....... C......... .....TT...
EURO    .......... ......A... C......... ..C....A.. C..G...... .....CT...
AUR     .......... ......A... .....T.... .T.......C T.....C... .....TT...

VDBP    TCCTAGCCAT ACATTATTCA TCAGATACAA TTACAGCATT CTCATCAATT AACCATATTT
MIDR    .......... .......... .......... .......... .......... ..........
TANZ    .T.....T.. .......... .......... .CTT...... .......... .GT.......
ANGO    .......... .......... .......... .......... .......... ..........
CONCOL  .TT....T.. ...C...... .........C .......... ......T... .CA.......
EURO    .TT....T.. ...C..CA.. .........C .......... T.....C... .CT.......
AUR     .T.....T.. ...C...A.. .....C...C .......... ...T...G.. .CA..C..C.

VDBP    GTCGAGACGT GAATTATGGT TGACTAATCC GCTATATACA TGCTAATGGT GCCTCAATAT
MIDR    .......... .......... .......... .......... .......... ..........
TANZ    .......T.. A..C.....C .....T..T. .T........ C..C..C... ..A.......
ANGO    .......... .......... .......... .......... .......... ..........
CONCOL  .C.....T.. A..C...... ........T. .T....C... ...C...... .....C....
EURO    .C.....T.. A.....C... .......... .T....C... C..C.....C ..........
AUR     .C........ A......... ...T...... .A...T.... C.....C... ..........

VDBP    TCTTTATATG CATATTCTTA CATATTGGAC GAGGCATTTA CTACGGTTCA TATTTATTTA
MIDR    .......... .......... .......... .......... .......... ..........
TANZ    .T..C..... T.....T... ........T. ....T..C.. T......... ..C.......
ANGO    .......... .......... .......... .......... .......... ..........
CONCOL  .T........ .T....TC.C .....C..C. .....C.... T..T..A... ..C.......
EURO    .T........ .C....T... .....C..C. .....C.... T.....A... ..C......T
AUR     .T........ .C.T..TC.. ..C..C..C. .......... T.....A..C ..C.......

VDBP    AAGAAACATG AAACATTGGT ATTATTCTTC TACTTATCAC TATAGCCACA GCTTTTATGG
MIDR    .......... .......... .......... .......... .......... ..........
TANZ    .......... ...T.....A ........A. .G.....A.. ......T... ..C.......
ANGO    .......... .......... .......... .......... .......... ..........
CONCOL  .T........ ...TG....A .....AT.AT ..A....T.. G.....T... ..........
EURO    .T..G..... ...T.....A .....C..A. ..A....T.. .......... .......A.
AUR     .......... ...T..A..G .....CT.A. ..A...C... .........C ..C.....A.

VDBP    GTTACGTCCT ACCATGA
MIDR    .......... .......
TANZ    ....T..AT. .......
ANGO    .......... .......
CONCOL  .......... G......
EURO    .......... .......
AUR     ....T..T.. .......
```

Appendix IV

ND2 alignment of the southern African hedgehog, *Atelerix frontalis* from DAPSA, preformed at a stringency of 10. The samples tested were from Gauteng and the Free State (FS) Provinces, South Africa.

```
                                                                                                                    60
Gauteng                                    TA GCATTATTCA TTTACTTTAT ATTAACAATA
FS                                         TA GCATTATTCA TTTACTTTAT ATTAACAATA
                                           ** ********** ********** **********

                                                                                                                   120
Gauteng     GGTACAATCA TAGTATTAAT TAGCTCGCAT TGACTTTTAA TTTGAGTAGG TTTTGAAGTT
FS          GGTACAATCA TAGTATTAAT TAGCTCGCAT TGACTTTTAA TTTGAGTAGG TTTTGAAGTT
            ********** ********** ********** ********** ********** **********

                                                                                                                   180
Gauteng     AATTTAATAG CAATAATTCC TATTATAATT AATAAGCACA ATCCTCGATC TACAGAATCC
FS          AATTTAATAG CAATAATTCC TATTATAATT AATAAGCACA ATCCTCGATC TACAGAATCC
            ********** ********** ********** ********** ********** **********

                                                                                                                   240
Gauteng     GCAATTAAGT ATTTTTTAGT CCAATCAATA GCCTCAATCG TATTTATAAT ATCTATTTCA
FS          GCAATTAAGT ATTTTTTAGT CCAATCAATA GCCTCAATCG TATTTATAAT ATCTATTTCA
            ********** ********** ********** ********** ********** **********

                                                                                                                   300
Gauteng     ACTAACATAA TATTAACGGG TCAATGAACC ATATTATATA TTGACAATAA TATTGTATCT
FS          ACTAACATAA TATTAACAGG TCAATGAACC ATATTATATA TTGACAATAA TATTGTATCT
            ********** ********** ********** ********** ********** **********

                                                                                                                   360
Gauteng     TCTATCATTA CAGTTTCAAT AATAATAAAA ATTGGAACAG CCCCCTTCCA CATATGACTC
FS          TCTATCATTA CAGTTTCAAT AATAATAAAA ATTGGAACAG CCCCCTTCCA CATATGACTC
            ********** ********** ********** ********** ********** **********

                                                                                                                   420
Gauteng     CCTGAAGTAA CTCAAGGGTT ACCATTAAAT TCTAGTATAA TTCTTCTCAC CTGACAAAAA
FS          CCTGAAGTAA CTCAAGGGTT ACCATTAAAT TCTAGTATAA TTCTTCTCAC CTGACAAAAA
            ********** ********** ********** ********** ********** **********

                                                                                                                   480
Gauteng     ATTGCTCCAT TATCAATTTT ATACTCACTA TATTATTCTC TTAATCCCAA TATTATGTTT
FS          ATTGCTCCAT TATCAATTTT ATACTCACTA TATTATTCTC TTAATCCCAA TATTATGTTT
            ********** ********** ********** ********** ********** **********

                                                                                                                   540
Gauteng     ATCTCAGCCC TCTTATCTAT TATACTAGGC GGATGAGGAG GCCTAAATCA AACTCAATTA
FS          ATCTCAGCCC TCTTATCTAT TATACTAGGC GGATGAGGAG GCCTAAATCA AACTCAATTA
            ********** ********** ********** ********** ********** **********

                                                                                                                   600
Gauteng     CGAAAAATAA TAGCTTTTTC ATCAATTGCT CACATAGGAT GAATAATAGC TATTATTTGC
FS          CGAAAAATAA TAGCTTTTTC ATCAATTGCT CACATAGGAT GAATAATAGC TATTATTTGC
            ********** ********** ********** ********** ********** **********

                                                                                                                   660
Gauteng     TATAACCCTA ATATTATAAT TCTAAACCTC TTTATTTATA TAAGCATAAC CATTTCATTA
FS          TATAACCCTA ATATTATAAT TCTAAACCTC TTTATTTATA TAAGCATAAC CATTTCATTA
            ********** ********** ********** ********** ********** **********

                                                                                                                   720
Gauteng     TTTATTATCT TTAAAAATAA TAATTCCACT AATATTACAG GCTTATCCTT AATTTATAAT
FS          TTTATTATCT TTAAAAATAA TAATTCCACT AATATTACAG GCTTATCCTT AATTTATAAT
            ********** ********** ********** ********** ********** **********

                                                                                                                   780
Gauteng     AAATCCCCTG TTATAGCCTC ATTATTAGCA CTATTACTTC TATCTTTAGG AGGCTTACCA
FS          AAATCCCCTG TTATAGCCTC ATTATTAGCA CTATTACTTC TATCTTTAGG AGGCTTACCA
            ********** ********** ********** ********** ********** **********

                                                                                                                   840
Gauteng     CCACTTACAG GATTTATACC TAAATGAGCA GTAGTTCAAG AACTAATTAA AAATAATAAT
FS          CCACTTACAG GATTTATACC TAAATGAGCA GTAGTTCAAG AACTAATTAA AAATAATAAT
            ********** ********** ********** ********** ********** **********

                                                                                                                   900
Gauteng     ACAAGTATGG CACTAATTAT ACTAATACTA GCCCTAATTA GCTTATTCTT CTACATACGA
FS          ACAAGTATGG CACTAATTAT ACTAATACTA GCCCTAATTA GCTTATTCTT CTACATACGA
            ********** ********** ********** ********** ********** **********

                                                                                                                   960
Gauteng     CTAATTTACT CAACATCACT AACTATATTC CCATCAATAA ATAATATAAA ATTACACTGA
FS          CTAATTTACT CAACATCACT AACTATATTC CCATCAATAA ATAATATAAA ATTACACTGA
            ********** ********** ********** ********** ********** **********

                                                                                                                  1020
Gauteng     AAATATACAA AAATAAATAG TTATTATCTA ACTTTAACCA CCCTATCTAT TATCTCCATC
FS          AAATATACAA AAATAAATAG TTATTATCTA ACTTTAACCA CCCTATCTAT TATCTCCATC
            ********** ********** ********** ********** ********** **********

Gauteng     TTTATACTT CCACTTTTCC CTATATTAAT AAATTTTACT AA
FS          TTTATACTT CCACTTTTCC CTATATTAAT AAATTTTACT AA
            ********* ********** ********** ********** **
```

Appendix V

Control region alignment of the southern African hedgehog, *Atelerix frontalis* created in MEGA version 3. Dots indicate 100 % base pair match at that site. MID, LYD, FS indicate South African samples, ANG indicates the Angolan sample, TAN the sample collected from Tanzania. The European hedgehogs, *Erinaceus europaeus* and *E. concolor*, the long-eared hedgehog, *Hemichinus auritus*, and the moonrat, *Echinosorex gymnura*, were also included.

```
MID       ATAATCAACA TTAT-TTAAT TACCACATAA TGATATGCAC TTAAATATTA AATAATACAA
LYD       .......... ....-..... .......... .......... .......... ..........
FS        .......... ....-..... .......... .......... .......... ..........
ANG       .......... ....-..... .......... .......... .......... ..........
TAN       .......... .C..-..... .....T.... .......... .......C.C ....G..T.T
CONCOLOR  .CT.CT.... ...AA....C ...AC..... .......... .........T ....G.CT..
EUROPAEU  .CT.CT.... ...AA..... ...AC..... .......... .........T ....G.CT..
Aur       C.T..T..A. C.TATG..CA ACTAG....T AT.C...T.. A.T..AC.A. ....T....T

MID       AG-ACATTAA ATTA-ATATT TACTATAAAT TTATGTAAAA CTAGCATATA AGCATGTACA
LYD       ..-....... ....-..... .......... .......... .......... ..........
FS        ..-....... ....-..... .......... .......... .......... ..........
ANG       ..-....... ....-..... .......... .......... .......... ..........
TAN       ..-....... ....-..TC. .......T.. .......... .......... ..........
CONCOLOR  ..-....... ....T..TC. .......T.. .........TT .......... ..........
EUROPAEU  ..-....... ....T..TC. .......T.. .........TT .......... ..........
Aur       .AT....... ....T..TC. ..T..-...C .......C.. .......... TA........

MID       TTAAATCTTA ATTATTACAT AATACATTAA ATTATCTCAC AACTTTAAAA TAAATAACAA
LYD       .......... .......... .......... .......... .......... ..........
FS        .......... .......... .......... .......... .......... ..........
ANG       .......... .......... ..C....... .......... ........G. ..........
TAN       .......... .......... .......... .C...T.A.. ......C.T. .TT.......
CONCOLOR  .....CTC.. -..A..... ..A....... .C...TAA.. ...AA.C.T. .T..ATT...
EUROPAEU  .....CTC.. -..A..... ..A....... .C...TAAT. ...AA.C-T. .T..ATT...
Aur       .....CTAA. ..-....... .......... ....CAAACT ...A-..... .TT..T.A..

MID       TACGAATATC TAAATCAATT ATAATTTATT AATATTACAT AGTACATATT AATATTAATC
LYD       .......... .......... .......... .......... .......... ..........
FS        .......... .......... .......... .......... .......... ..........
ANG       .......... .......... .......... .......... .......... ..........
TAN       ....G....T C......... .......... .......... .......... ......G...
CONCOLOR  .........T C......... ...GG..... .......... .......... ......G...
EUROPAEU  .........T C......... ...GG..... .......... .......... ......G...
Aur       C.T.G....T ....C..... ...G...... .......... .........- ...G.AT...

MID       GTACATAGCG CATTCTATTA ATAAATT-TT CTCTACCACC CGCATATCAC CTCCATTAGG
LYD       .......... .......... .......-.. .......... .......... ..........
FS        .......... .......... .......-.. .......... .......... ..........
ANG       .......... .......... .......-.. .......... .......... ..........
TAN       T........A ...CTC.... ....T.CA.. .......... .........T ..........
CONCOLOR  .......... ...C...... .....C.-.. A......... .......... ..........
EUROPAEU  .......... .......... .....C.-.. A......... .......... ..........
Aur       ........TA ...C...... ......A-.. ..T.T..... .......... ..........

MID       TTATTTCTTA ATCTACCAAC TCACGTGAAA CCAACAACCC TTGTGAACAG TATCCCTCTC
LYD       .......... .......... .......... .......... .......... ..........
FS        .......... .......... .......... .......... .......... ..........
ANG       .......... .......... .......... .......... .......... ..........
TAN       .......... .......... .......... ......-... .......... ..........
CONCOLOR  .......... .......... .......... .......... .......... ..........
EUROPAEU  .......... .......... .......... .......... .......... ..........
Aur       .......... .......... .......... .......... ....A..A.. ..........

MID       CTCGCCCCGG GCCCAT
LYD       .......... ......
FS        .......... ......
ANG       .......... ......
TAN       .......-.. ......
CONCOLOR  .......... ......
EUROPAEU  .......... ......
Aur       .....T.... ......
```

Appendix VI

A table illustrating the p-distance values for the western European hedgehog, *Erinaceus europaeus*, and the long-eared hedgehog, *Hemichinus auritus*, to illustrate that the p-distance for the entire Cyt-*b* gene is larger than the p-distance for 377 bp region sequenced in the present study and therefore the molecular clock imposed in the present study is probably an underestimation of the divergence times.

Gene (length)	European hedgehog *vs* Long-eared hedgehog	Clock (MYA)
Cyt-*b* (full length)	0.192	9.6
Cyt-*b* (377 bp)	0.143	7.15
D-loop (377 bp)	0.203	10.15

Appendix VII

The p-distance table illustrating that the western European hedgehog, *Erinaceus europaeus*, is identical to the eastern European, *Erinaceus concolor* C1-13 isolate.

```
[ 1] #EUROPAEU
[ 2] #E._concolor_C1-12
[ 3] #E._concolor_C2-03
[ 4] #E._concolor_concolor4
[ 5] #E._concolor_C1-11
[ 6] #E._concolor_C1-10
[ 7] #E._concolor_C1-09
[ 8] #E._concolor_C1-07
[ 9] #E._concolor_C1-05
[10] #E._concolor_C1-13
[11] #E._concolor_C1-08
[12] #E._concolor_C1-06
[13] #E._concolor_C1-04
[14] #E._concolor_C1-03
[15] #E._concolor_C1-02
[16] #E._concolor_C1-01
[17] #E._concolor_C2-04
[18] #E._concolor_C2-01
[19] #E._concolor_C2-02
```

```
          1     2     3     4     5     6     7     8     9    10    11    12    13    14    15    16    17    18
[ 1]
[ 2]  0.011
[ 3]  0.040 0.046
[ 4]  0.005 0.005 0.040
[ 5]  0.005 0.005 0.040 0.000
[ 6]  0.005 0.005 0.040 0.000 0.000
[ 7]  0.011 0.005 0.046 0.005 0.005 0.005
[ 8]  0.008 0.008 0.043 0.003 0.003 0.003 0.003
[ 9]  0.008 0.008 0.043 0.003 0.003 0.003 0.003 0.000
[10]  0.000 0.011 0.040 0.005 0.005 0.005 0.011 0.008 0.008
[11]  0.008 0.008 0.043 0.003 0.003 0.003 0.003 0.000 0.000 0.008
[12]  0.008 0.008 0.043 0.003 0.003 0.003 0.003 0.000 0.000 0.008 0.000
[13]  0.008 0.013 0.043 0.008 0.008 0.008 0.008 0.005 0.005 0.008 0.005 0.005
[14]  0.011 0.013 0.046 0.011 0.011 0.011 0.008 0.008 0.008 0.011 0.008 0.008 0.003
[15]  0.008 0.013 0.043 0.008 0.008 0.008 0.008 0.005 0.005 0.008 0.005 0.005 0.000 0.003
[16]  0.008 0.013 0.043 0.008 0.008 0.008 0.008 0.005 0.005 0.008 0.005 0.005 0.000 0.003 0.000
[17]  0.040 0.040 0.011 0.040 0.040 0.040 0.040 0.043 0.043 0.040 0.043 0.043 0.043 0.043 0.043 0.043
[18]  0.035 0.040 0.005 0.035 0.035 0.035 0.040 0.038 0.038 0.035 0.038 0.038 0.038 0.040 0.038 0.038 0.005
[19]  0.038 0.043 0.003 0.038 0.038 0.038 0.043 0.040 0.040 0.038 0.040 0.040 0.040 0.043 0.040 0.040 0.008 0.003
```

The southern African hedgehog, *Atelerix frontalis* (A. Smith, 1831) is listed as near-threatened in the *Red Data Book of South African Mammals* (Friedman & Daly 2004) and has a disjunct distribution of two allopatric populations (Skinner & Smithers 1990; Mills & Hes 1997; Skinner & Chimimba 2005) that led to the recognition of two subspecies (Rautenbach 1978). These include *A. f. frontalis* (A. Smith, 1831) that is confined to the eastern parts of southern Africa, and *A. f. angolae* (Thomas, 1918) that is restricted to the western parts of the subregion mostly in Namibia and extralimitally to south-western Angola (Skinner & Smithers 1990; Mills & Hes 1997; Skinner & Chimimba 2005). However, the recognition of these two subspecies is despite reservations expressed on the limited knowledge of both the nature and extent of geographic variation within *A. frontalis* (Corbet 1974; Gillies 1989; Skinner & Smithers 1990).

It is for this reason that the present study was initiated to assess geographic variation within *A. frontalis* in order to test the validity of the subspecies designations. It represents the first analysis of geographic variation in the southern African hedgehog, and includes the largest sample and widest geographical coverage than has hitherto been considered for the species, and is based on a multidisciplinary approach involving traditional and two-dimensional geometric morphometric analysis of the cranium and mandible, and molecular data.

Of significance in the present study is that the analyses on the multidisciplinary characterization of the southern African hedgehog were largely based on museums specimens for the morphometric as well as the molecular analyses but also included opportunistically-obtained fresh material that augmented the molecular part of the study in order to avoid sampling live individuals. Given the near-threatened listing of the southern African hedgehog in the *Red Data Book for South African Mammals* (Friedman & Daly 2004) and its currently decreasing suitable habitat (Friedman & Daly 2004), study material of the southern African hedgehog is generally limited. The

general paucity of study mater : by the generally secretive nature of hedgehogs (Morris 19

However, prior to the main analyses to assess the nature and extent of geographic variation within the southern African hedgehogs a number of preliminary considerations were investigated. The first included the selection of meaningful taxonomic characters for use in assessing the nature and extent of variation within the southern African hedgehog using traditional and geometric morphometric analyses of the cranium and mandible. These measurements were selected to adequately represent cranial and mandibular phenotypes in the southern African hedgehog.

Although neglected, such a taxonomic measurement selection procedure is highly recommended in systematics since it attempts to fulfill two important requirements, namely, 1) "comprehensiveness" through the consideration of adequate coverage of the phenotype, and 2) "economy" through the removal of redundant measurements. Chimimba & Dippenaar (1995) reported that the use of unevaluated taxonomic measurements may affect subsequent morphometric analyses. These include the distortions of inter-OTU relationships to an increase in analysis time when processing large data matrices (Chimimba and Dippenaar 1995). Previous studies reported that after the assessment of redundancy (or linear dependency) and co-linearity, large quantitative measurement sets can be reduced to a few and still contain equivalent information. More importantly, the measurement selection procedure adopted in the present study which reduced an initial set of 70 measurements to 30, have been applied in a range of vertebrate and invertebrate taxa that include small carnivores (Taylor & Meester 1993), murid rodents (Chimimba and Dippenaar), and weevils (Janse van Rensburg *et al.* 2003). The present analysis represents the first study in which such a procedure has been applied to the southern Africa hedgehog and the measurements selected could also be applied in future studies of other species of hedgehogs.

The second preliminary consideration prior to the main morphometric analyses was to assess the nature and extent of geographic variation in the southern African hedgehog. This included the analysis of non-geographic variation at the level of sexual dimorphism and age variation using traditional and geometric morphometric

analyses of the cranium and m sexual dimorphism and age variation was undertaken with establishing whether sexes should be treated separately or together, and which specimens have reached adult dimensions and were, therefore, suitable for measurement recording and analysis in the subsequent assessment of the nature and extent of variation in the southern African hedgehog.

The results obtained from the homogeneous sample showed a lack of sexual dimorphism but remarkable variation between juveniles of age classes I and II and adults of age classes III and IV. These results justified the pooling of sexes as well as individuals of age classes III and IV for subsequent measurement recording and analysis directed at samples obtained from across the distributional range of this species. The procedure adopted in the analysis of sexual dimorphism and age variation in the southern African hedgehog has previously been applied in other small mammals. For example, the procedure has been used to demonstrate the general lack of sexual dimorphism and the presence of marked age variation in bathyergid rodents such as the social mole-rats, *Cryptomys hottentottus hottentotus* and *C. damarensis* (Bennett *et al.* 1990) and in murid rodents of the genus *Aethomys* (Chimimba & Dippenaar 1994). The present study represents the first known analysis of non-geographic variation in the southern African hedgehog, and the procedure followed can also be applied to other taxa in general and other species of hedgehogs in particular.

The preliminary analyses of measurement selection, sexual dimorphism, and age variation were followed by the main series of analyses to assess the nature and extent of geographic variation in the southern African hedgehog using a multidisciplinary approach involving traditional and two-dimensional geometric morphometric analysis of the cranium and mandible, and molecular data. Of particular significance is that the results of both univariate and multivariate analyses of the traditional morphometric data that are generally considered to be inferior to geometric morphometric data (Marcus & Corti 1996) were congruent.

All these morphometric results suggest a north-westerly–south-easterly clinal pattern of variation with cranial configuration being positively correlated with both

latitude and longitude. No pronal pattern of variation were evident suggesting a clinal north-western populations (representing the currently recognized *A. f. angolae*) being narrower and the south-eastern populations (representing the currently recognized *A. f. frontalis*) being broader in cranial and mandibular configuration.

The results of the morphometric analyses with respect to the validity of the current sub-specific status being questionable were supported by Neighbour-joining, Maximum Likelihood, and Maximum Parsimony analyses of Cyt-*b*, ND2 and control region data of the mitochondrial genome. Data from the first two gene regions revealed no variation across a 377 bp and 1034 bp region sequenced for each gene, respectively, whilst analysis of the 377 bp of the control region revealed low levels of variation between representatives of the two recognized subspecies (0.54 % pairwise sequence divergence). These results together with the lack of pronounced steps in the clinal pattern of variation suggest that the recognition of subspecies within *A. frontalis* may be untenable.

Taken together these results indicate that the disjunct distribution of *A. frontalis* may represent a recent divergence event and may have implications in the conservation management strategies for *A. frontalis* since it could be argued that one disjunct population could act as a source population for the other. Nevertheless, it is highly recommended that prior to the implementation of conservation management plans for the species, further studies involving a wide range of alternative systematic techniques should first be undertaken in order to gain a better understanding of the nature and extent of geographic variation within the southern African hedgehog.

Interestingly, clinal patterns of variation have also been reported in other southern African small mammals such as the murid rodents *Aethomys granti* (Chimimba *et al.* 1998) and *A. ineptus* (Chimimba 2001). Although in homeotherms, such clines have often been interpreted in terms of Bergmann's (1874) rule, others have argued that they may be a function of a complex combination of interdependent climatic factors (Sokal and Rinkel 1963; Rising 1970; Gould and Johnston 1972; Endler 1977; Ellison *et al*. 1993). It is therefore, suggested that there is a critical need for future studies on the southern African hedgehog, as well as other small mammal in the southern

African subregion. Such suggestions should be based on comprehensive sampling as well as analyses involving additional genetic parameters and/or climatic variables that may assist in identifying factors that may explain both the disjunct distributions and clinal pattern of variation in the subregion. For example, it has been reported that forced movements due to climatic fluctuations result in differences in dispersal conditions, which may influence the consequent genetic diversity (Seddon *et al.* 2001). It is possible that this may explain the slight separation of the Angolan sample from the South African samples in some of the phylogenetic analyses in the present study, and may require further investigation.

Of additional relevance in the present study is marked molecular differences shown between hedgehog samples from Tanzania and the southern African subregion. The results confirm the presence of a distinct species of hedgehog in East Africa and provides the first divergence estimate (approximately 4 MYA) for these con-generics, While these results are based on small sample sizes, there is a critical need for additional studies based on comprehensive sampling in both East and southern Africa. Such a study should perhaps also focus on morphometrics as well as on developing *Atelerix*-specific primers that may allow the generation of complete gene sequences and a more accurate estimation of the time of divergence between the southern and East African hedgehogs.

The problem of reduced sample size in the present study was particularly pronounced in the molecular part of the study as this necessitated the use of museum-preserved material, due to limited fresh material. Museum specimens normally contain highly degraded DNA and the presence of chemical inhibitors leading to a general reduction in the efficiency of PCR amplification (Yang *et al.* 1997) which is further exacerbated by the low copy number of target DNA. In the present study, however, the procedures followed in extracting DNA from museum samples permitted a preliminary, but critically required first assessment of genetic variation and phylogenetic relationships in the southern African hedgehog. The present study may, therefore, serve as a model for other similar studies involving non-invasive sampling, particularly with reference to threatened taxa.

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