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SYSTEMATICS OF THE SOUTH AFRICAN LEPORIDAE

by

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requirements for the degree of

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ABSTRACT

SYSTEMATICS OF THE SOUTH AFRICAN LEPORIDAE

by

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Co-supervisor: Professor J.M.P. Geerthsen
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New and traditional systematic parameters were used to examine the taxonomic relationships of the South African Leporidae. Conclusions on taxonomic status were based on univariate and multivariate morphometric analyses, comparative karyology, interspecific variation in cross sections through the principal upper incisors, sperm morphology, electrophoretic analyses of serum proteins and red blood cell enzymes and qualitative pelage characteristics.

Multivariate analyses of the genus *Pronolagus* clearly indicate the cranial distinctiveness of the species *P. rupestris, P. crassicaudatus* and *P. randensis.* This treatment was supported *a posteriori* by univariate discriminant function analyses, and also by the unique pelage characteristics of the three species. Principal upper incisor width and the morphology of these teeth in cross section can be used to distinguish *P. rupestris* from *P. crassicaudatus* and *P. randensis.* No interspecific variation of systematic value was evident from detailed chromosome comparisons, the electrophoretic examination of blood samples, or the silver staining patterns of the spermatozoa. The diploid number of the genus was established as 2n = 42.

Univariate and multivariate morphometric analyses of the South African *Lepus* provided evidence of two phenetically distinct species, *L. capensis* and *L. saxatilis.* The delimitation of these taxa was largely dependent on overall size, *L. saxatilis* being, on

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average, larger than *L. capensis*. Analysis of geographic variation showed *L. saxatilis* to vary clinally, with a marked trend towards decreasing size along a SW – NE axis. Although the smaller northern forms of this genus in South Africa have been referred to as *L. crawshayi*, this was not substantiated by the present investigation. The recognition of only two South African species was similarly indicated by almost all other test criteria. *L. capensis* and *L. saxatilis* were clearly separable on incisor tooth morphology, quantitative and qualitative differences in sperm morphology, the presence or absence of erythrocytic carbonic anhydrase and pelage characteristics. The only criterion which failed to provide a means of distinction between these well deliniated taxa was comparative karyology, both species possessing very similar, if not identical G- and C-banded karyotypes. The diploid number for the South African species was 2n = 48.

The bulk of evidence from this study supports the recognition of the monotypic *Bunolagus* as a valid genus. Although the results of multivariate analyses indicate that the taxon is cranially very similar to *Lepus*, the genus to which it has been assigned in the past, exceptionally strong evidence for separate generic status is indicated by its unique karyotype. The presence of 44 chromosomes, many of which are of characteristic morphology, provide unequivocal separation from all *Lepus* species. Marked differences in incisor width, the pattern of the tooth in cross section, sperm morphology, especially with regard to the shape of the acrosome, and pelage characteristics, are reliable criteria for delimitation of this taxon from the South African *Lepus*.

Systematic characters of value in the identification of the various species are presented in the form of keys. The results of this investigation support the recognition of *Pronolagus*, *Lepus* and *Bunolagus* as valid genera for the South African Leporidae, while the retention of three *Pronolagus* species, *P. rupestris*, *P. crassicaudatus* and *P. randensis*, and two *Lepus* species, *L. capensis* and *L. saxatilis*, appears justified.

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the collection of specimens in areas under their jurisdiction. Similarly, access to the lagomorph collections of the British Museum (Natural History) and the Kaffrarian, Natal and Transvaal Museums is acknowledged.

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CHAPTER I

GENERAL INTRODUCTION

TAXONOMIC ACCOUNT

Considerable disagreement prevails with regard to both the generic and specific status of certain of the South African and, on a wider basis, African Leporidae. The three genera recognised by Angermann (1966) as occurring naturally in South Africa were retained by Petter (1972). These are: *Bunolagus, Pronolagus* and *Lepus*. A fourth genus *Oryctolagus*, the Old World rabbit, is an introduced species which, although feral on several South African offshore islands (Skead, 1980), is otherwise primarily represented by laboratory stocks and is consequently excluded from this survey.

At generic level only the status of *Bunolagus* can be regarded as controversial. Originally described as *Lepus monticularis* (Thomas, 1903) the species was separated generically as *Bunolagus* some years later by Thomas (1929). This usage was retained by Allen (1939) and Roberts (1951). However Ellerman and Morrison-Scott (1951) regarded *Bunolagus* as a synonym of *Lepus* and reverted to the use of the original name combination. This was accepted by Ellerman, Morrison-Scott and Hayman (1953) and Meester, Davis and Coetzee (1964). Angermann (1966) argued that the combination of unique features such as the large well defined interparietal bone, a P_3 tooth pattern reminiscent of *Alilepus*$^+$, the *Lepus*-like skull conformation and uniformly coloured tail, strongly supported the recognition of the species as a monotypic genus. This situation was retained by Petter (1972) although with reservation: "the presence of a suture separating this bone (interparietal) from the parietals and supraoccipital in adults has been considered a generic character, but the variability of the character in *L. habessinicus* shows clearly that it is not adequate for generic separation." The taxonomic problems associated with the two remaining genera, *Pronolagus* and *Lepus*, centre on the validity of the various species.

The genus *Pronolagus* Lyon, 1904 is restricted to Africa south of the Sahara. Roberts (1951) recognised six distinct species as occurring in South Africa: *P. barretti* Roberts 1948; *P. crassicaudatus* (I. Geoffroy, 1832); *P. curryi* (Thomas, 1902); *P. melanurus* (Rüppell, 1842); *P. randensis* Jameson, 1907 and *P. rupestris* (A. Smith, 1834). However, Ellerman *et al.* (1953) recognised the validity of only three, *P. crassicaudatus, P. randensis* and *P. rupestris*, this treatment of the species being based primarily on occipitonasal and bulla lengths.

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A further attempt to reduce the number of species soon followed. Lundholm (1955) considered two of the taxa recognised by Ellerman *et al.* (1953), *P. crassicaudatus* and *P. randensis*, as inseparable, with the latter name as a junior synonym. His grounds for adopting this synonomy were based on what could be considered tenuous evidence of clinal variation within separating characters (bulla length, occipitionasal length), since the diagnosis was made on single specimens (except in two cases of three and five) from each locality.

Further pertinent contributions of a taxonomic nature are those of Petter (1972) and Peddie (1975). Petter (1972), while stating that the genus was in need of revision, reverted to the use of the three species originally proposed by Ellerman *et al.* (1953). However Peddie (1975) using measurements of the bulla, skull length and standard external body measurements on material from South Africa and Zimbabwe concluded that "no reason could be found to suggest the existence of more than one species in southern Africa...."

Distributional data relating to the genus and the species considered by the various authors to constitute it, both within the study area and in other regions of southern Africa, are described by Shortridge (1934), Roberts (1951), Ellerman *et al.* (1953), Cabral (1966), Smithers (1971), Pringle (1974), Kingdon (1974), Lynch (1975), Smithers and Lobâo Tello (1976), Ansell (1978), Rautenbach (1978a) and Stuart and Lloyd (1979).

The taxonomy of African members of the genus *Lepus* Linnaeus, 1758 is highly complex. In one of the earliest listings of African mammals (Allen, 1939) no less than 30 different species of *Lepus* were recorded for Africa, although only two were regarded as occurring in South Africa. Subsequent attempts at revising the African *Lepus* resulted in many of the species being synonomised and reduced to subspecies status (Ellerman and Morrison-Scott, 1951; Petter, 1959, 1961, 1963; Petter and Genest, 1965). At present only five African species are regarded as valid, *L. capensis* Linnaeus, 1758, *L. saxatilis*, F. Cuvier, 1823, *L. habessinicus* Hemprich and Ehrenberg, 1832, *L. whytei* Thomas, 1894, and *L. crawshayi* de Winton, 1899 (See Petter, 1972). Angermann (in Petter, 1972), is however of the opinion that *L. crawshayi* is a junior synonym of *L. whytei*. This suggested synonomy has been provisionally adopted by Corbet and Hill (1980), although no taxonomic investigation has been made to test the validity of Angermann's hypothesis. Consequently, for the purpose of the present study it has been decided to follow Petter (1972) and consider *L. crawshayi* as a valid species pending further investigation.

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The two species recorded by Allen (1939) as occurring in South Africa were *L. capensis* and *L. saxatilis*. This was accepted by Roberts (1951), but Petter (1959, 1972) using the variation found in the principal upper incisors as a diagnostic character in conjunction with the width of the mesopterygoid space and cranial length, recognised the presence of a third species, *L. crawshayi*, whose distribution is reported to extend into parts of South Africa, notably northern Natal and the Transvaal.

In his key to the African *Lepus*, Petter (1972) separated *L. saxatilis* which Ellerman *et al.* (1953) considered a synonym of *L. europaeus* (Pallas 1778), entirely on size: "Head and body length more than 500 mm, ear more than 130 mm, length of cranium more than 92 mm". This is complicated by the fact that Smithers (1971), using data from various localities in southern Africa, found some evidence of clinal variation in both ear length and total length of the skull.

More recently, Rautenbach (1978a) criticised the diagnostic characters proposed by Petter (1972) for the delimitation of *L. crawshayi*. He was unable to distinguish the latter species using the existing keys and treated it as a junior synonym of *L. saxatilis*.

SYSTEMATIC METHODS

Most previous studies involving the taxonomy of the South African Leporidae have been based on qualitative pelage characteristics, standard body measurements and relatively few cranial characters. The only exception to the more classical approach to the taxonomic complexities of the South African lagomorphs was the work of Petter (1972), who utilised the variation found in the principal upper incisor tooth pattern of *Lepus* as an additional character in the delimitation of various species of this genus.

It was felt that a complete re-examination of the Leporidae was necessary, using additional and diverse techniques, in the hope that new parameters or combinations thereof, would facilitate a more realistic identification of the various taxa. Systematic parameters incorporated in this study were therefore:

(a) analyses of skull and standard body measurements using univariate and multivariate techniques;
(b) karyotypic comparisons between taxa based on G- and C-banding techniques;
(c) an evaluation of incisor tooth pattern variation as an aid in the separation of the Leporidae at both generic and species level;

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(d) comparative sperm morphology and the associated silver staining properties of the spermatozoa;
(e) electrophoretic studies of serum proteins and red blood cell enzymes;
(f) species specific pelage characteristics.

Each of these parameters and the degree of congruence between them is presented independently in subsequent chapters.

STUDY AREA AND COLLECTION LOCALITIES

Collection localities were chosen so as to afford good coverage of each species' range, although care was also taken to sample material from representative areas in the biotic zones where the Leporidae have been reported to occur (Rautenbach, 1978b). The location of the sample areas and their proximity to each other are shown in Fig. 1, while map references for each site are appendixed in the gazetteer (Appendix I).

MATERIAL

It is of fundamental importance in a systematic investigation of this nature, that data pertaining to each of the investigatory parameters should, as far as possible, be available for each specimen utilised. This necessitated the collection of material specifically for this study and the use of museum specimens was restricted to those taxa where the incorporation of additional material was unavoidable for certain of the analyses.

Specimens were collected at night using a spotlight and ,410 guage shotgun. Where possible the following material and measurements were taken from each individual:

(a) Material: Ear biopsies were taken in the field and stored in Eagles MEM with Hank's salts supplemented with 5% bovine serum, and transported to the laboratory for subsequent cytogenetic analyses. Blood samples were collected from almost all specimens utilised. A sperm smear was routinely made from an epididymus of each adult male. The skull, skin and post-cranial skeleton excluding the foot and hand bones were retained; skull and study skins of all specimens collected, have been deposited in the mammal collection of the Transvaal Museum.

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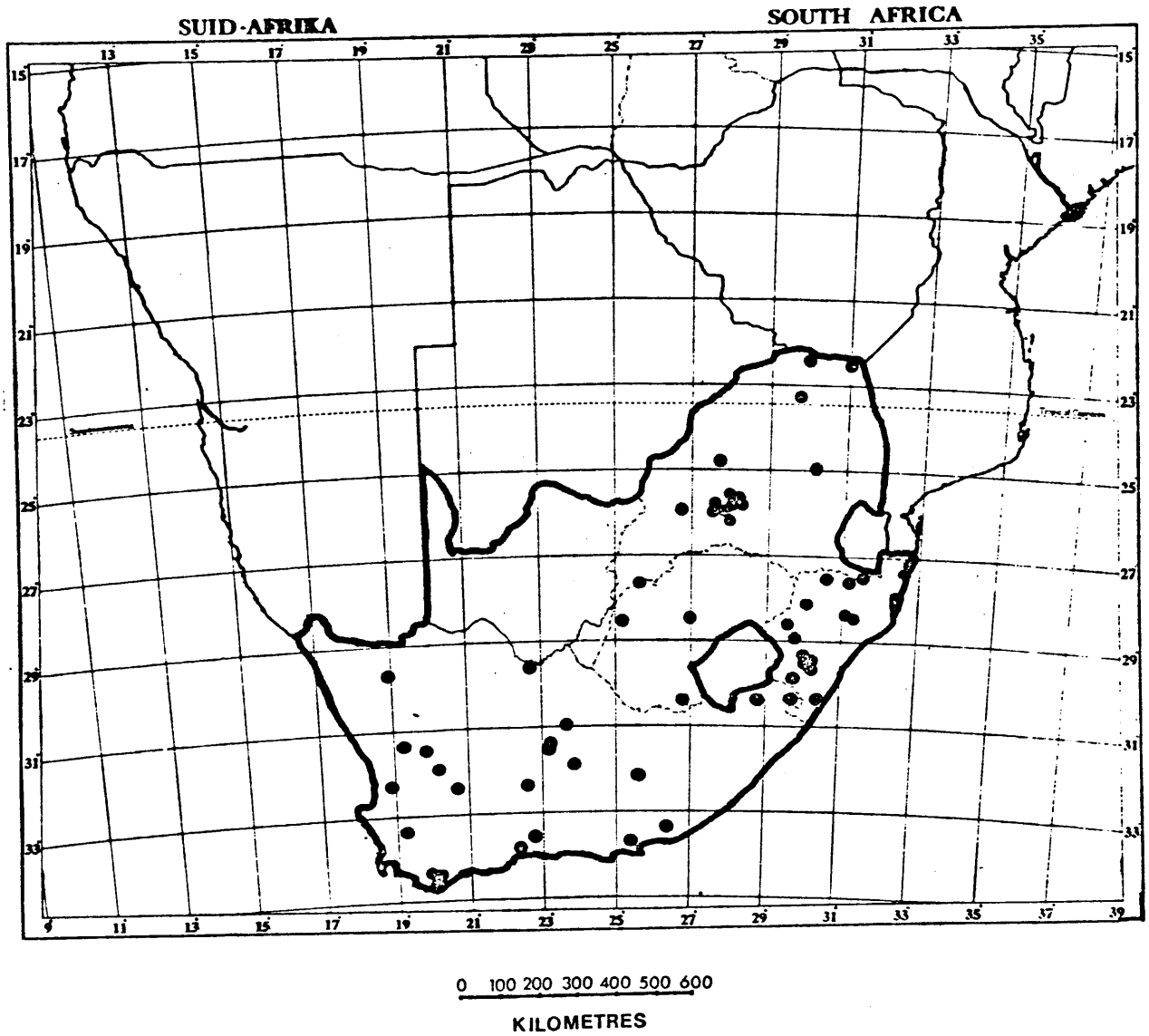

Fig. 1: *Collection localities from which specimens utilised in the present investigation were sampled. The map co-ordinates of the various localities are presented in gazetteer (Appendix I).*

(b) Measurements: Standard body measurements and mass were routinely recorded. The reproductive status and habitat associated with each specimen was noted.

Detailed descriptions of the methods used for examining the various parameters studied, are presented in their respective chapters.

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OBJECTIVES

The aims of this study are twofold. First, an effort has been made to examine the taxonomic status of the genera and species of the South African Leporidae using a wide variety of techniques, most of which have hitherto not been applied to the group, in an attempt to clarify the taxonomic confusion which prevails at present. Secondly, to provide an identification scheme for use by systematists and so create a reliable framework for further taxonomic research, especially at infraspecific level.

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CHAPTER 2

UNIVARIATE AND MULTIVARIATE STATISTICAL ANALYSES

INTRODUCTION

Univariate and multivariate morphometrics are finding increasing use in taxonomic studies. Recent examples of investigations employing these techniques to assess inter-specific and intraspecific relationships of certain leporid genera are those of Taylor, Freedman, Olivier and McCluskey (1977), Baker, Peterson, Eger and Manning (1978), Yates, Genoways and Jones (1979) and more recently Diersing and Wilson (1980). To date however, no attempt has been made to evaluate the status of the South African Leporidae using modern methods of data analysis.

MATERIAL AND METHODS

Material:

Specimens utilised in the morphometric analyses were derived from two sources.

Field specimens:

A total of 206 specimens representative of the three genera and seven species listed by Petter (1972), were collected throughout South Africa. The need to collect a large sample of leporids specifically for this study was dictated by the type of parameters on which this investigation is based, most of which (e.g. karyology, electrophoretic analyses, sperm morphology) are not available from conventional museum preparations.

A further advantage associated with the use of field specimens was that it was possible to reliably age the material used in the morphometric analyses, thus largely removing any bias which may have been inadvertantly introduced through the inclusion of juvenile and subadult material. The criterion used was that of skeletal maturity as indicated by the closure of the proximal epiphysis of the humerus. This method of age determination has

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been used widely in the Leporidae (Thomsen and Mortensen, 1946, Hale, 1949, Petrides, 1951, Lechleitner, 1959, Walhovd 1965, Walhovd, 1966, Bothma, Teer and Gates, 1972).

Since specimens were collected at night it was to be expected that the sample would comprise a percentage of juvenile and subadult individuals. Of the 206 specimens collected 24% were found on later examination to be skeletally immature and were consequently excluded from the morphometric analyses.

Museum material:

The use of standard museum preparations was restricted, since data pertaining to most of the other systematic criteria were not available. The absence of postcranial material from museum specimens also preclude the use of the epiphysial closure of the humerus for ageing and other methods had to be employed. Consequently the use of museum material was limited to those taxa where, due largely to small sample sizes, their inclusion was unavoidable.

These were:

B. monticularis which, with the exception of a single specimen collected for this study in 1979 (Robinson, 1981), is known entirely from museum material. Extensive use was made of existing collections in the British Museum (Natural History, London), and Kaffrarian (King William's Town) and Transvaal (Pretoria) Museums.

P. crassicaudatus and *P. randensis* were particularly poorly represented in field collections due in part to their relatively limited distributions. The collection of larger samples for any of the *Pronolagus* species was hampered by the rugged terrain in which the species occur (rocky situations on hills, mountain slopes and summits), and their nocturnal and shy habits. Additional *P. crassicaudatus* and *P. randensis* specimens were included from the Natal (Pietermaritzburg) and Transvaal Museum collections.

Preliminary investigations of skull and in particular the incisor tooth pattern data from *L. saxatilis*, resulted in the need to include supplementary information on these two aspects from localities in the central and southern Karoo and the mountainous regions of the Cape Province. Additional data were obtained from specimens housed in the Transvaal Museum which originated from suitable localities in these areas.

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Measurements:

Four external measurements, head and body length, tail length, hind foot length (cu) and ear length were recorded for all field specimens. Body mass measured on a spring balance to the nearest 50 g, was also recorded. External measurements for museum material were obtained directly from specimen labels.

Twenty eight skull and toothrow measurements were recorded, whenever possible, from each specimen used in the analyses (Fig. 2). All cranial measurements were taken to the nearest 0,1 mm with a dial caliper. The bulla length measurement, which has been used extensively by other workers (Ellerman *et al.*, 1953, Lundholm, 1955; Petter 1972; Pringle, 1974), was found to have low repeatability. Consequently bulla breadth, which proved more reliable, was substituted as a measure of this structure. Most other cranial measurements are the same as those used by Taylor *et al.* (1977).

Cleaning and ageing of cranial and skeletal material:

As much as possible of the soft tissue was removed prior to boiling the carcasses in a 15% sodium hydroxide solution for approximately 15 min. The material was finally cleaned by manual scraping off any of the remaining soft tissue, the bones allowed to dry and then boxed.

The epiphyseal groove can be readily seen in bones from young specimens prepared in this way, since the soft tissue shrinks on drying and is no longer flush with the surface. Based on the degree of closure of the proximal epiphyseal cartilage, material could be classified into the three categories proposed by Walhovd (1966):

(a) specimens showing epiphyseal cartilage, detected by the presence of a definite groove;

(b) specimens with the epiphyseal plate nearly closed, as revealed by a light line on the surface of the proximal humerus;

(c) specimens with no indication of an epiphyseal line in the humerus.

Only those field specimens classified as falling within category c were utilised in the morphometric analyses.

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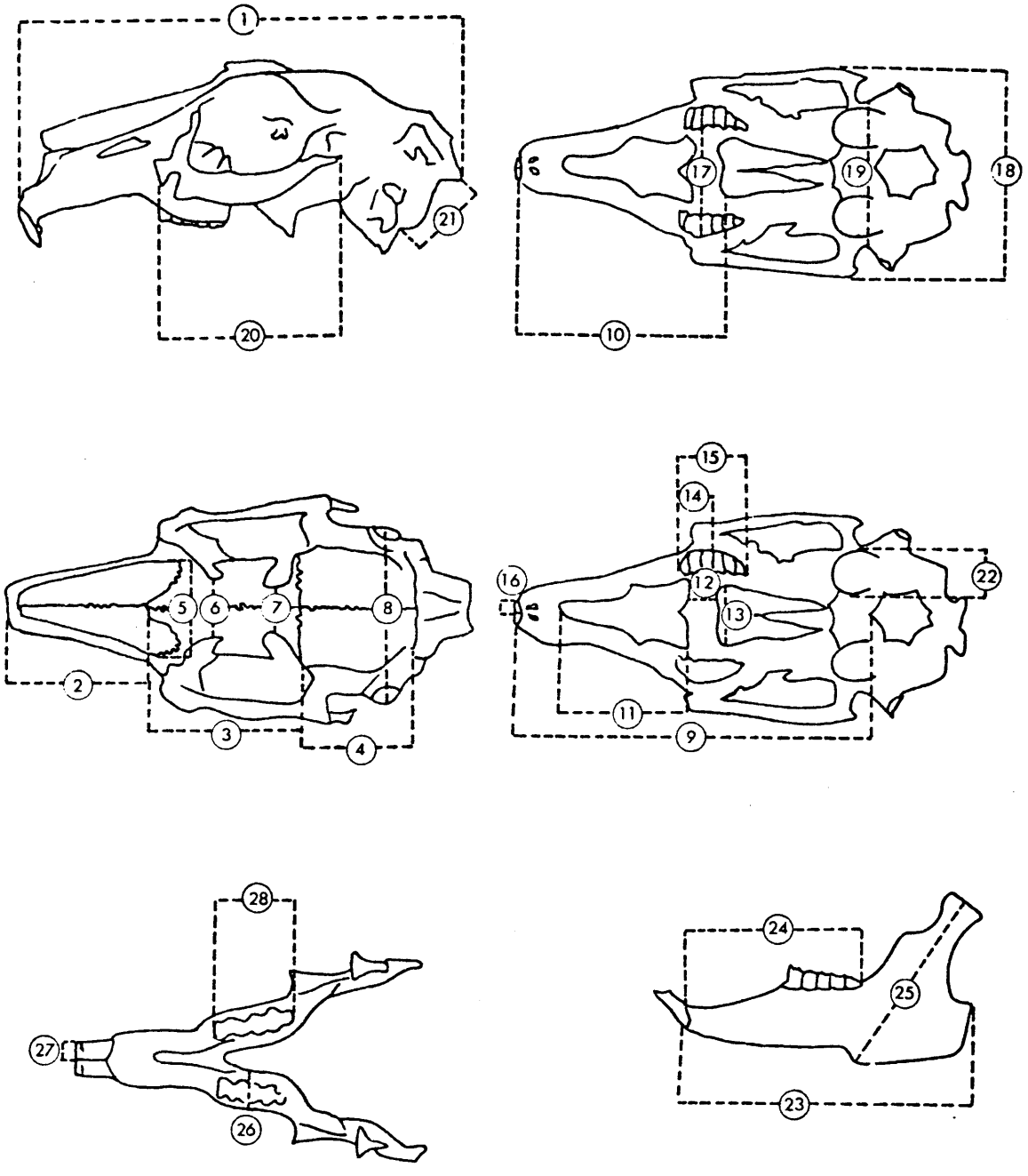

Fig. 2.: *Diagrammatic representation of the 28 cranial measurements utilised in the present study.*
1 = total length of skull; 2 = muzzle length; 3. = frontal length; 4 = parietal length; 5 = posterior muzzle breadth; 6 = anterior frontal breadth; 7 = posterior frontal breadth; 8 = interauditory breadth; 9 = basal cranial length; 10 = palate incisor length; 11 = palate vacuity length; 12 = hard palate length; 13 = mesopterygoid space; 14 = maxillary premolar length; 15 = maxillary premolar-molar length; 16 = principal I^1 breadth; 17 = palatal breadth between P^2 and P^3; 18 = bizygomatic breadth; 19 = maximum cranial breadth; 20 = zygomatic arch length; 21 = posterior cranial height; 22 = bulla breadth; 23 = maximum mandibular length; 24 = mandibular toothrow length; 25 = maximum mandibular height; 26 = mandibular body breadth; 27 = I_1 breadth; 28 = mandibular premolar length.

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The absence of postcranial material for museum specimen necessitated the use of alternate ageing criteria. Cranial methods, used successfully in *Sylvilagus*, based on the fusion between the exoccipital and supraoccipital bones (Hoffmeister and Zimmerman, 1967) or alternatively, between the basisphenoid and basioccipital bones (Yates *et al.*, 1979) tended to be variable, particularly the latter, when tests were conducted on field material already segregated using the closure of the epiphyseal line as criterion. In view of the far higher degree of concordance between the method involving the fusion of the exoccipital and supraoccipital bones and the presence or absence of epiphyseal cartilage in the humerus, it was considered justified, in the absence of postcranial material, to class as adult:

(a) those specimens showing visible evidence of fusion along the entire length of the suture between the exoccipital and supraoccipital bones;

(b) those specimens showing no evidence of a suture line between the bones, the line having been entirely obliterated.

Computations:

Univariate analyses yielding standard statistics including the arithmetic mean, range, standard error, standard deviation and coefficient of variation, among others, were computed using the CONDESCRIPTIVE subprogramme of the statistical package for the Social Sciences (SPSS; Nie, Hull, Jenkins, Steinbrenner and Dent, 1975).

By using the ONEWAY subprogramme of the SPSS package, a single classification analysis of variance (ANOVA) was used to test each of the external and cranial characters (excluding mass) for significant differences between males and females from selected test localities. Mass was excluded, since these data were usually absent for museum specimens.

Geographic variation was analysed using the UNIVAR programme of Power (1970). Variation in characters was assessed across samples using single classification analyses of variance. When sample means were found to be significantly different, the Sum of Squares Simultaneous Test Procedure (SS–STP), an *a posteriori* multiple comparison test, was used to determine maximally nonsignificant subsets.

Multivariate statistical analyses were performed using selected subroutines of the Numerical Taxonomy System of Multivariate Statistical Programmes (NT–SYS) developed by Rohlf, Kispaugh and Kirk (1974). In these analyses the OTU's were either

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individual specimens or samples. Where OTU's were sample localities, arithmetic means were used for each cranial character. In these cases mandibular height was excluded from the data set, but in all other analyses involving individual specimens all 28 cranial characters were utilised.

Although standard external body measurements were available for all field and most museum specimens utilised, these were not routinely included in the multivariate analyses in view of the inherent inaccuracies associated with these measurements, which are often compounded by slight differences in the methodology followed by various workers. This was particularly evident in the body length measurement, where it was not always clear from the museum material whether this parameter referred to the head and body length or the total length of the specimen. However, in the case of multivariate studies involving the South African *Lepus* it was decided to include two external body measurements, hind foot and ear length, in view of the greater accuracy for which these parameters can be recorded and because of the previous implication of ear length in the taxonomy of *L. saxatilis*.

The NT-SYS programme package produces correlation matrices and average taxonomic distance coefficients from standardised character values. Cluster analyses by the un-weighted pair-group method using arithmetic average (UPGMA) were performed on the correlation and distance matrices. Phenograms were generated from each matrix; those based on distance coefficients are referred to as "distance phenograms" in the text and those derived from correlation matrices as "correlation phenograms". These phenograms provide a two dimensional view of the data using unweighted character values. The cophenetic correlation coefficients are presented, providing an indication of the goodness of fit between the phenograms and original matrices. However, in view of the inherent distortion associated with phenograms (Sneath and Sokal, 1973; Moss, Peterson and Atyeo, 1977) they are used largely in this study to summarize the phenetic relationships. Taxonomic conclusions are based primarily on the results of the principal component analyses and the minimum spanning tree.

The principal component analysis (PCA) allows an objective assessment of data without prior grouping of material. The PCA used in the present study was based on correlation coefficients among characters. Pairwise comparisons of the first three components generated by the programme were invariably analysed and two dimensional projections of the samples were made. Furthermore, factor matrices indicating the character loadings in these components and the percentage of variation accounted for by them were computed.

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A minimum spanning tree (=Prim Network; Prim, 1957) was superimposed on the two dimensional projections of the PCA. The minimum spanning tree (MST) shows the affinities of OTU's to each other and indicates the degree of distortion created by representing the OTU's in a two dimensional projection.

Statistical techniques used in this study are largely similar to those of Genoways (1973), Rautenbach and Schlitter (1978), Swanepoel and Genoways (1978), Swanepoel and Schlitter (1978) and in particular Dippenaar (1980).

Computer analyses using the NT-SYS and SPSS packages were conducted on the University of Pretoria IBM 370 computer. The UNIVAR programme for analysis of geographic variation was available on a CDC computer at the Centre for Computing Services, C.S.I.R., Pretoria.

Approach:

The sequence in which the analyses were performed are presented below:

(1) univariate studies of sexual dimorphism were conducted to establish whether subsequent computations could be based on pooled sexes, or alternatively, if the separate treatment of each was warranted;

(2) multivariate morphometric comparisons of genera based predominantly on sample mean values. For certain localities only single specimens were available, consequently their measurements were used as representative of these areas;

(3) the delimitation of species within each genus using the multivariate techniques and univariate calculations of the mean, standard error of the mean, standard deviation and coefficient of variation for both external and cranial measurements;

(4) an analysis of geographic variation in *L. saxatilis*.

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RESULTS AND DISCUSSION

SECONDARY SEXUAL VARIATION:

Although Hall (1951) reported that females are on average larger than males in the Leporidae, recent studies spanning several genera indicate that almost all disparities in measurements between the sexes are slight and not statistically significant (Chapman, 1971; Chapman and Morgan, 1973; Taylor *et al.*, 1977; Baker *et al.*, 1978; Yates *et al.*, 1979; Diersing and Wilson, 1980).

Tests for secondary sexual dimorphism in the South African taxa were hampered to some extent by small sample sizes from individual localities. Consequently it was necessary to pool data from geographically contiguous sample areas for the analyses. The results of a single classification ANOVA on a geographic sample of *L. saxatilis* from (a) Hilton and Winterton in Natal (8♂ : 13♀) and (b) Derdepoort and Kaalplaats near Pretoria (7♂ : 8♀) are presented in Table 1. The results of a pooled sample of *P. crassicaudatus* (5♂ : 10♀) are similarly presented. The *P. crassicaudatus* material, although based largely on field specimens collected at Hilton, was supplemented by the inclusion of museum material from Hilton (1), Howick (1) and Estcourt (2).

Unfortunately, no suitably large samples from single or contiguous localities were available for analysis of the other taxa.

In the pooled Hilton, Winterton *L. saxatilis* sample, females proved to be significantly ($P \leqslant 0,05$) larger than males in the total length of the skull, zygomatic arch length and mandibular height, while conversely males were significantly larger than females in the measurement of the mesopterygoid space. The results obtained for the Derdepoort, Kaalplaats collection, which has a more balanced sex ratio but smaller sample size, indicate that females were significantly larger in only one measurement, that of mandibular height. Significant sexual differences in the *P. crassicaudatus* specimens were shown for principal upper incisor breadth, where females were larger than males, and cranial breadth, where the converse was true.

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Table 1 :

Results of an analysis of variance between males and females of a grouped sample of (a) *L. saxatilis* from Hilton and Winterton (b) *L. saxatilis* from Derdepoort and Kaalplaats and (c) *P. crassicaudatus* from Estcourt, Hilton and Howick. $\bar{X}$ = Arithmetic mean; n = Sample size; P = Level of significance (*P≤ 0,05); tSE = 95% Confidence limit; CV = Coefficient of variation.

Measurement and sex		$\bar{X}$(mm)	n	P	tSE	CV(%)	Range (mm)
		(a) *L. saxatilis* (Hilton and Winterton)					
Head and body length							
	Male	517	8	0,64	16,11	3,7	490–550
	Female	521	13		12,59	4,0	485–555
Tail length							
	Male	114	8	0,46	7,52	7,9	100–125
	Female	119	13		9,46	13,2	90–140
Hind foot length							
	Male	129	8	0,85	2,68	2,5	125–135
	Female	130	13		3,16	4,0	120–140
Ear length							
	Male	106	8	0,21	5,21	6,0	100–115
	Female	108	13		2,27	3,5	100–115
Total length of skull							
	Male	92,0	8	0,04*	1,79	2,3	88,4–95,2
	Female	94,4	12		1,53	2,6	90,2–99,8
Muzzle length							
	Male	41,2	8	0,83	1,21	3,5	38,6–43,0
	Female	41,1	12		0,86	3,3	38,6–43,2
Frontal length							
	Male	37,4	8	0,36	2,29	7,3	32,6–40,5
	Female	38,4	13		1,28	5,5	34,6–41,9
Parietal length							
	Male	20,4	8	0,95	1,37	8,0	18,1–22,8
	Female	20,3	12		0,75	5,8	18,3–21,7
Posterior muzzle breadth							
	Male	21,1	8	0,84	0,86	4,9	19,8–22,5
	Female	21,2	12		0,84	6,2	19,0–22,9
Anterior frontal breadth							
	Male	20,5	8	0,21	1,12	6,5	19,0–22,5
	Female	19,5	13		1,22	10,3	16,0–22,4
Posterior frontal breadth							
	Male	13,2	8	0,21	1,76	16,0	11,4–17,7
	Female	12,3	13		0,63	8,5	10,7–14,0
Interauditory breadth							
	Male	27,6	8	0,48	1,11	4,8	25,0–29,2
	Female	28,0	12		0,55	3,1	26,0–29,8
Basal cranial length							
	Male	74,3	8	0,08	1,39	2,2	71,7–77,0
	Female	76,1	12		1,53	3,2	72,6–81,1

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Table 1 (continued)

Measurement and sex	$\bar{X}$(mm)	n	P	tSE	CV(%)	Range (mm)
Palate incisor length						
Male	37,4	8	0,06	1,00	3,2	35,6–39,1
Female	38,5	12		0,67	2,8	37,0–40,2
Palate vacuity length						
Male	23,9	8	0,36	0,70	3,5	22,8–25,4
Female	24,3	12		0,54	3,5	22,4–25,3
Hard palate length						
Male	6,7	8	0,11	0,49	8,8	5,8– 7,5
Female	7,1	13		0,37	8,6	6,2– 8,0
Mesopterygoid space						
Male	8,9	8	0,01*	0,36	4,8	8,2– 9,4
Female	8,3	13		0,28	5,5	7,7– 9,1
Maxillary premolar length						
Male	8,6	8	0,51	0,25	3,5	8,0– 8,9
Female	8,8	13		0,32	6,1	7,9– 9,9
Maxillary premolar-molar length						
Male	15,8	8	0,52	0,24	1,8	15,4–16,1
Female	16,0	13		0,59	6,0	13,8–17,6
Principal I^1breadth						
Male	3,2	8	0,61	0,09	3,3	3,1– 3,4
Female	3,1	12		0,13	6,7	2,8– 3,4
Palatal breadth						
Male	26,4	8	0,41	0,62	2,8	25,5–27,5
Female	26,7	13		0,63	3,9	25,3–28,4
Bizygomatic breadth						
Male	43,5	8	0,77	1,05	2,9	41,5–45,6
Female	43,7	12		0,93	3,4	41,8–46,3
Maximum cranial breadth						
Male	31,6	8	0,41	0,82	3,1	29,6–32,6
Female	31,2	10		0,72	3,2	29,6–33,2
Zygomatic arch length						
Male	37,8	7	0,04*	1,40	4,0	34,6–39,2
Female	39,4	12		0,92	3,7	37,9–42,7
Posterior cranial height						
Male	18,2	8	0,58	0,78	5,1	17,0–19,4
Female	18,0	13		0,41	3,8	16,6–18,8
Bulla breadth						
Male	8,2	8	0,59	0,44	6,5	7,4– 8,8
Female	8,3	12		0,35	6,6	7,4– 9,0
Maximum mandibular length						
Male	65,9	8	0,16	1,18	2,1	63,6–68,4
Female	67,1	13		1,20	3,0	63,1–71,2
Mandibular toothrow length						
Male	39,2	8	0,29	0,57	1,8	37,8–39,9
Female	39,8	13		0,91	3,8	36,5–41,6
Maximum mandibular height						
Male	43,8	8	0,003*	1,92	5,2	41,1–47,8
Female	46,7	12		0,98	3,3	44,6–49,6
Mandibular body breadth						
Male	6,2	8	0,12	0,41	8,0	5,3– 6,8
Female	6,6	13		0,31	7,8	6,0– 7,9

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Table 1 (continued)

Measurement and sex		$\bar{X}$(mm)	n	P	tSE	CV(%)	Range (mm)
I_1 breadth							
	Male	3,1	8	0,41	0,12	4,8	3,0– 3,4
	Female	3,1	13		0,12	6,3	2,8– 3,4
Mandibular premolar-molar length							
	Male	17,4	8	0,63	0,38	2,6	16,8–18,2
	Female	17,6	13		0,55	5,2	15,8–18,8
(b) *L. saxatilis* (Derdepoort and Kaalplaats)							
Head and body length							
	Male	481	7	0,67	19,30	4,4	440–500
	Female	486	7		20,40	4,5	460–520
Tail length							
	Male	121	7	0,30	9,14	8,1	105–135
	Female	114	8		12,12	12,7	90–130
Hind foot length							
	Male	124	7	0,84	4,94	4,3	120–130
	Female	124	8		3,71	3,6	120–130
Ear length							
	Male	103	7	0,69	2,47	2,6	100–105
	Female	104	7		3,50	3,7	100–110
Total length of skull							
	Male	88,8	6	0,71	1,92	2,1	87,1–91,9
	Female	89,2	6		1,35	1,4	87,0–90,6
Muzzle length							
	Male	39,1	7	0,58	1,42	3,9	36,3–40,6
	Female	39,6	8		1,55	4,7	37,6–43,1
Frontal length							
	Male	36,0	7	0,93	1,05	3,8	33,8–37,6
	Female	36,1	7		2,05	6,2	33,2–38,5
Parietal length							
	Male	19,3	7	0,30	0,63	3,5	18,5–20,4
	Female	19,0	6		0,40	2,0	18,5–19,6
Posterior muzzle breadth							
	Male	20,3	7	0,67	0,66	3,6	19,1–21,2
	Female	20,5	8		1,02	5,9	18,8–22,5
Anterior frontal breadth							
	Male	18,9	7	0,73	1,09	6,3	17,0–20,5
	Female	19,1	8		0,71	4,4	17,9–20,4
Posterior frontal breadth							
	Male	12,3	7	0,80	0,73	6,4	11,5–13,9
	Female	12,2	8		0,69	6,8	10,5–13,0
Interauditory breadth							
	Male	26,2	5	0,17	0,78	2,4	25,5–27,2
	Female	26,9	5		0,87	2,6	26,0–27,8
Basal cranial length							
	Male	71,9	5	0,65	0,32	0,4	71,5–72,2
	Female	71,6	6		1,41	1,9	70,1–73,4
Palate incisor length							
	Male	35,6	7	0,17	0,79	2,4	34,0–36,5
	Female	36,3	8		0,72	2,4	35,1–37,7

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Table 1 (continued)

Measurement and sex		$\bar{X}$(mm)	n	P	tSE	CV (%)	Range (mm)
Palate vacuity length							
	Male	22,4	7	0,77	1,02	4,9	20,9–24,1
	Female	22,5	8		0,50	2,7	21,4–23,1
Hard palate length							
	Male	6,8	7	0,89	0,62	9,9	6,0– 7,7
	Female	6,7	8		0,49	8,8	5,9– 7,5
Mesopterygoid space							
	Male	8,2	7	0,50	0,41	5,4	7,7– 8,8
	Female	8,4	8		0,32	4,6	8,0– 9,0
Maxillary premolar length							
	Male	9,3	7	0,25	0,45	5,2	8,6– 9,9
	Female	9,0	8		0,49	6,6	8,2–10,0
Maxillary premolar-molar length							
	Male	16,7	7	0,41	0,67	4,4	16,0–17,7
	Female	16,4	8		0,61	4,5	15,5–17,8
Principal I^1 breadth							
	Male	3,0	6	0,56	0,04	1,4	2,9– 3,0
	Female	3,0	7		0,11	4,0	2,8– 3,2
Palatal breadth							
	Male	25,7	7	0,94	0,77	3,2	24,1–26,7
	Female	25,7	7		0,54	2,3	25,0–26,7
Bizygomatic breadth							
	Male	42,0	7	0,72	1,04	2,7	40,2–43,4
	Female	42,1	7		0,50	1,3	41,4–42,8
Maximum cranial breadth							
	Male	29,5	6	0,83	1,36	4,4	28,1–31,7
	Female	29,7	5		0,19	0,5	29,5–29,9
Zygomatic arch length							
	Male	36,6	7	0,88	1,30	3,8	35,1–39,1
	Female	36,5	8		1,14	3,7	33,9–37,8
Posterior cranial height							
	Male	18,0	5	0,25	1,40	6,3	17,0–19,8
	Female	17,3	7		0,68	4,2	16,2–18,4
Bulla breadth							
	Male	8,5	7	0,58	0,31	3,9	8,1– 9,0
	Female	8,3	7		0,67	8,7	7,5– 9,2
Maximum mandibular length							
	Male	63,0	7	0,38	1,55	2,7	61,1–65,8
	Female	63,7	7		1,10	1,9	62,1–65,0
Mandibular toothrow length							
	Male	37,4	7	0,29	1,13	3,3	36,0–38,8
	Female	39,3	7		3,92	10,8	36,6–48,8
Maximum mandibular height							
	Male	42,7	7	0,03*	0,93	2,4	41,8–44,7
	Female	43,9	7		0,94	2,3	42,2–45,1
Mandibular body breadth							
	Male	6,2	7	0,89	0,47	8,3	5,6– 6,7
	Female	6,2	7		0,15	2,6	6,0– 6,5
I_1 breadth							
	Male	3,0	6	0,68	0,04	1,3	3,0– 3,1
	Female	3,1	8		0,27	10,7	2,7– 3,6
Mandibular premolar-molar length							
	Male	17,3	7	0,95	0,90	5,6	16,2–18,5
	Female	17,3	7		0,52	3,3	16,6–18,3

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Table 1 (continued)

Measurement and sex		$\bar{X}$(mm)	n	P	tSE	CV (%)	Range (mm)
(c) *P. crassicaudatus* (Estcourt, Hilton and Howick)							
Head and body length							
	Male	518	2	0,71	222,36	4,8	500–535
	Female	522	8		10,23	2,3	510–540
Tail length							
	Male	78	2	0,60	95,30	13,7	70– 85
	Female	73	8		9,74	16,1	50– 80
Hind foot length							
	Male	113	2	0,60	31,77	3,1	110–115
	Female	115	8		5,00	5,2	110–125
Ear length							
	Male	77	2	0,55	44,47	6,5	73– 80
	Female	75	8		2,23	3,6	70– 80
Total length of skull							
	Male	92,1	5	0,61	1,76	1,5	90,6–93,8
	Female	92,5	9		1,08	1,5	90,7–94,8
Muzzle length							
	Male	43,8	5	0,06	1,33	2,5	42,4–45,0
	Female	41,5	9		1,79	5,6	38,3–44,1
Frontal length							
	Male	35,4	5	0,32	3,06	7,0	32,4–39,0
	Female	36,8	10		1,80	6,9	33,1–41,4
Parietal length							
	Male	21,2	5	0,08	0,85	3,3	20,0–21,8
	Female	20,5	10		0,43	2,9	19,4–21,4
Posterior muzzle breadth							
	Male	20,4	5	0,24	1,90	7,5	18,0–22,0
	Female	19,6	10		0,74	5,3	17,5–20,9
Anterior frontal breadth							
	Male	17,5	5	0,53	1,35	6,2	15,9–18,7
	Female	17,0	10		1,08	8,8	14,7–19,5
Posterior frontal breadth							
	Male	13,8	5	0,62	1,04	6,1	12,5–14,6
	Female	13,6	10		0,62	6,3	12,1–15,1
Interauditory breadth							
	Male	28,4	4	0,52	2,59	5,8	26,7–30,6
	Female	27,9	9		0,48	2,2	27,0–28,8
Basal cranial length							
	Male	74,9	5	0,87	1,22	1,3	73,3–75,9
	Female	75,0	9		1,07	1,9	72,3–76,6
Palate incisor length							
	Male	41,9	5	0,41	1,02	2,0	40,8–43,1
	Female	42,8	9		1,85	5,6	40,6–48,4
Palate vacuity length							
	Male	26,3	5	0,75	0,90	2,8	25,6–27,5
	Female	26,5	9		0,60	3,0	25,1–27,6
Hard palate length							
	Male	9,7	5	0,87	0,82	6,8	8,5–10,1
	Female	9,6	10		0,60	8,8	8,8–11,6
Mesopterygoid space							
	Male	5,7	5	0,41	0,30	12,7	4,6– 6,5
	Female	6,0	10		0,38	8,9	5,2– 6,8

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Table 1 (continued)

Measurement and sex	$\bar{X}$(mm)	n	P	tSE	CV(%)	Range (mm)
Maxillary premolar length						
Male	9,6	5	0,19	1,54	13,0	8,3–11,2
Female	9,0	10		0,34	5,3	8,2– 9,8
Maxillary premolar-molar length						
Male	16,5	5	0,17	0,32	1,6	16,2–16,9
Female	16,8	10		0,32	2,6	16,0–17,5
Principal I^1 breadth						
Male	3,1	5	0,02*	0,22	5,7	3,0– 3,4
Female	3,4	7		0,15	4,8	3,2– 3,6
Palatal breadth						
Male	26,4	5	0,53	1,06	3,2	25,7–27,8
Female	26,1	10		0,49	2,6	24,8–27,3
Bizygomatic breadth						
Male	39,3	5	0,32	2,67	5,5	36,0–41,8
Female	40,1	10		0,52	1,8	38,7–41,1
Maximum cranial breadth						
Male	30,5	4	0,02*	1,34	2,8	29,5–31,2
Female	29,6	9		0,30	1,3	29,1–30,4
Zygomatic arch length						
Male	36,7	5	0,24	1,43	3,1	35,2–38,3
Female	37,5	10		0,77	2,9	35,9–38,9
Posterior cranial height						
Male	15,0	5	0,10	0,87	4,7	14,5–16,2
Female	15,7	10		0,51	4,5	14,6–16,8
Bulla breadth						
Male	5,9	5	0,47	0,90	12,4	5,1– 7,0
Female	6,1	9		0,26	5,5	5,5– 6,6
Maximum mandibular length						
Male	68,1	5	0,12	1,16	1,4	67,0–68,9
Female	69,3	10		1,11	2,2	66,5–71,4
Mandibular toothrow length						
Male	41,9	3	0,63	1,97	1,9	41,0–42,5
Female	42,1	10		0,48	1,6	41,1–42,9
Maximum mandibular height						
Male	44,4	5	0,22	1,83	3,3	42,6–46,7
Female	45,3	10		0,80	2,5	42,4–46,2
Mandibular body breadth						
Male	5,8	5	0,82	0,36	5,0	5,5– 6,2
Female	5,8	10		0,23	5,6	5,2– 6,2
I_1 breadth						
Male	3,2	5	0,39	0,32	8,0	2,9– 3,5
Female	3,3	9		0,16	6,1	3,0– 3,6
Mandibular premolar-molar length						
Male	16,4	5	0,77	0,41	2,0	16,2–17,0
Female	16,5	10		0,45	3,8	15,6–17,5

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Conclusions:

It is evident from the results in both *Lepus* and *Pronolagus* that marked differences between the sexes do not exist. Furthermore, there is some evidence from the *Lepus* samples that the irregular nature of characters showing sexual dimorphism in the three groups may be attributable to small sample sizes. Of the 32 variables tested, only mandibular height proved to be significantly dimorphic in more than one sample. Consequently, it was considered expedient to exclude this character from those multivariate analyses based largely on sample mean values (see below), so as to ensure that when the multivariate techniques were used, possible summated sexual differences in this measurement would not effect the results.

DELIMITATION OF GENERA:

In order to provide greater resolution of the phenetic relationships between genera, mean values were calculated from adult specimens of the representative species from each sample locality. For certain localities only single specimens were available, consequently their measurements were used as representative of these areas.

Data from 65 OTU's, based on 27 cranial measurements excluding mandibular height, were analysed using multivariate techniques.

Phenograms illustrating the phenetic relationships among the OTU's were computed from both the distance and correlation matrices. The distance phenogram with a cophenetic correlation coefficient of 0,738 most clearly illustrated the separation of the taxa (Fig. 3). Two major clusters, A and B, are evident. Major cluster A can be subdivided into subclusters C and D, both of which show further subdivisions. Subcluster C comprises all the *L. saxatilis* OTU's, while more specifically subdivisions E and F consist of samples with greatest skull length measurements in excess of 101,9 mm (observed range 101,9–107,7) and less than 100,2 mm (observed range 88,6–100,2) respectively. Subcluster D, which includes all *P. crassicaudatus* and *P. randensis* specimens, compromises two subgroups, G and H. Cluster G consists of *P. crassicaudatus* OTU's, while H with a single misplaced *P. crassicaudatus* OTU from Magudu (48), consists only of *P. randensis.*

The second major cluster, B, can be subdivided into subclusters I and J, with the former containing two further subdivisions K and L; K includes all *L. capensis* and L all

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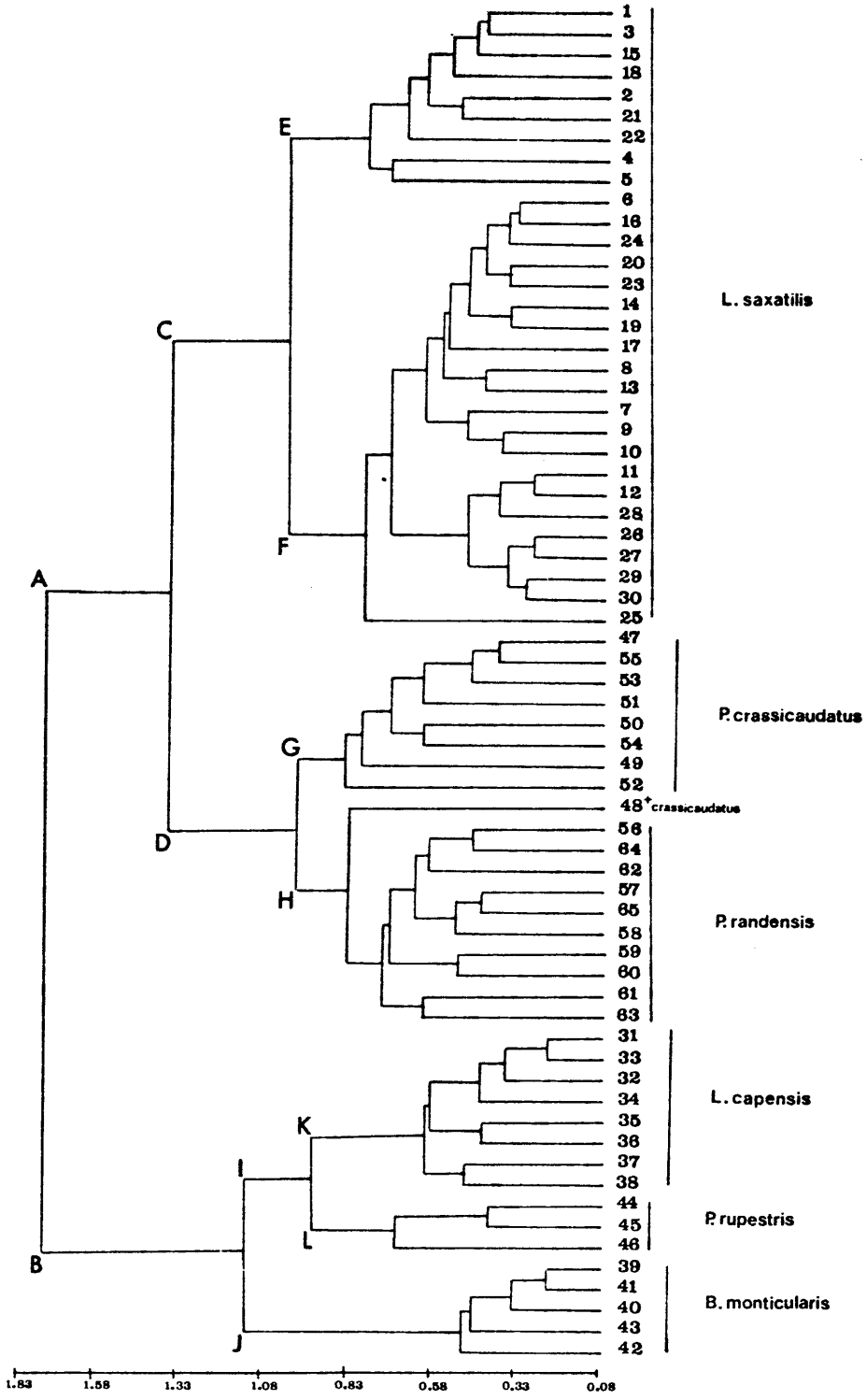

Fig. 3: *Distance phenogram of the South African Leporidae clustered by the unweighted pair-group method using arithmetic averages based largely on sample means. OTU's 1–30* L. saxatilis; *31–38* L. capensis; *39–43* B. monticularis; *44–46* P. rupestris; *47–55* P. crassicaudatus; *56–65* P. randensis. *Cophenetic correlation coefficient = 0,738.*

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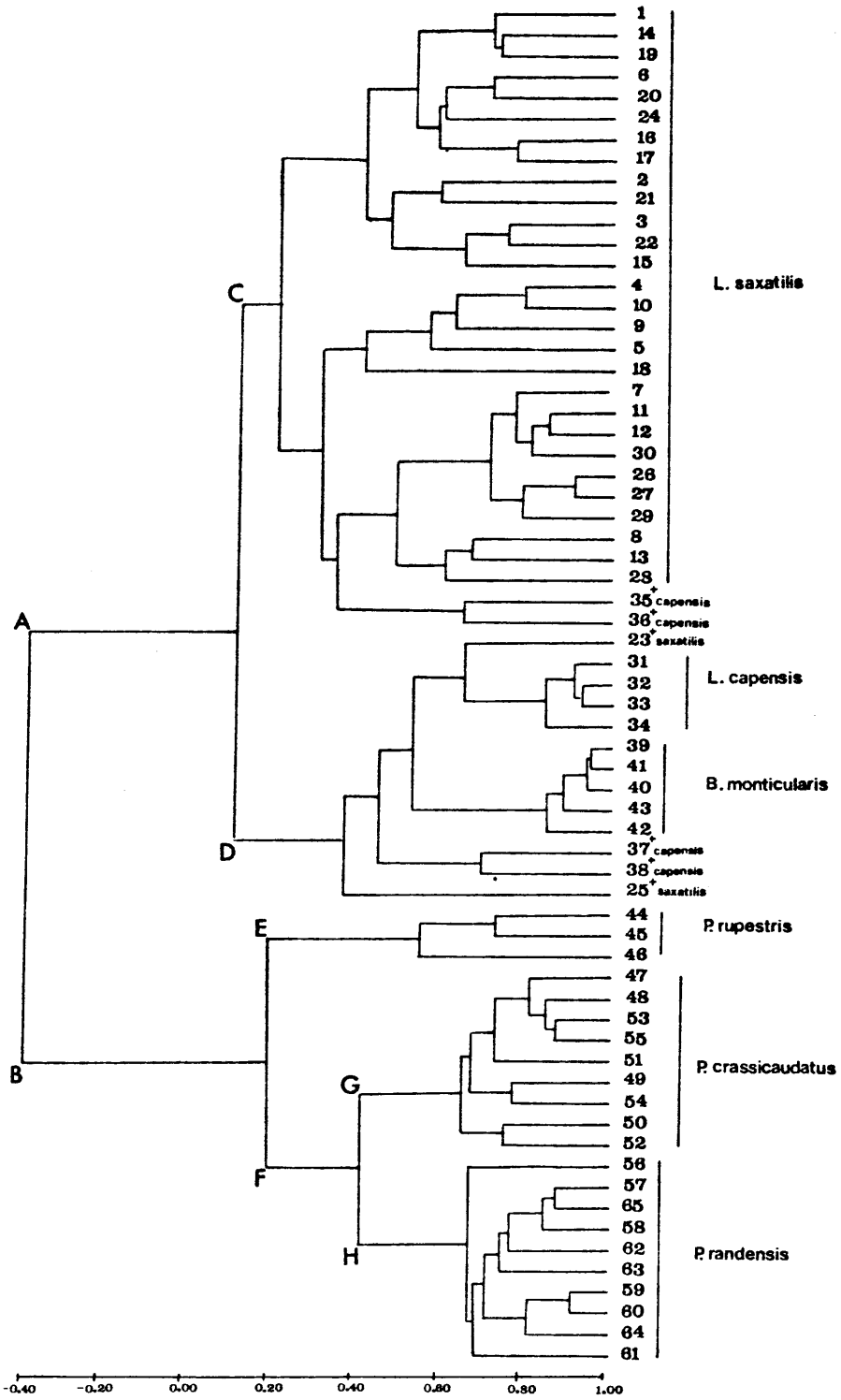

Fig. 4: *Correlation phenogram of the South African Leporidae clustered by the unweighted pair-group method using arithmetic averages based largely on sample means. OTU's 1–30* L. saxatilis; *31–38* L. capensis; *39–43* B. monticularis; *44–46* P. rupestris; *47–55* P. crassicaudatus; *56–65* P. randensis. *Cophenetic correlation coefficient* = 0,876.

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P. rupestris material. Subcluster J is comprised of a tight grouping of five OTU's, representative of all the *B. monticularis* specimens utilised in this study.

The correlation phenogram which had a cophenetic correlation coefficient of 0,876 is illustrated in Fig. 4. Although the clustering of the taxa is largely similar to that illustrated in the distance phenogram, their accentuation is not as clear. In several cases, displacement of OTU's from nearest relatives has occurred.

Two major clusters A and B are evident in the correlation phenogram. Major cluster A can be further subdivided into subclusters C and D, with the former comprised of *L. saxatilis* as well as two misplaced *L. capensis* OTU's (35, 36), while the latter consists primarily of most of the remaining *L. capensis* and all *B. monticularis* samples. However in addition subcluster D also contains two misplaced *L. saxatilis* (23, 25) and *L. capensis* (37, 38) OTU's, which do not cluster with the respective species.

Major cluster B which contains all the *Pronolagus* specimens, shows two distinct subclusters E and F. The former incorporates all the *P. rupestris* material, while F with two further subdivisions, G and H, comprises the *P. crassicaudatus* (including the previously misplaced Magudu OTU, 48) and *P. randensis* samples respectively.

The results of a principal component analysis of these OTU's are given in Table 2, as well as Figs. 5 and 6. The first component accounts for 63,71% of the total variation and is an overall size component as suggested by the usually large and invariably positive coefficients for all measurements (Table 2). The second component, which accounts for an addidional 17,08% of the trace, is a shape component and as is evident from the character loadings, is influenced mostly by palate incisor length, hard palate length, mesopterygoid space and bulla breadth. Component three which contributes an additional 4,43% to the total phenetic variation (Total = 85,22%), is influenced primarily by posterior muzzle breadth, posterior frontal breadth and maxillary premolar length.

The ordination diagram illustrating the pairwise comparison of component one and two (Fig. 5) reflects quite closely the relationships indicated in the phenograms. Those OTU's representing *L. saxatilis* are clustered predominantly in the right half of the first component scale, while tight clusters of the smaller *L. capensis* and *B. monticularis* fall within the left half of the component scale. Separation along the second component was particularly striking. All *Pronolagus* OTU's regardless of species are separated from the other taxa along the second or shape component, with the smaller *P. rupestris* specimens

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Table 2 :

Factor matrix from a 27 variable principal component analysis based largely on sample mean values for the South African Leporidae showing the character loadings on the first three components.

Measurement	Factor I	Factor II	Factor III
Total length of skull	0,969	–0,142	0,135
Muzzle length	0,833	–0,057	0,006
Frontal length	0,723	0,399	0,204
Parietal length	0,590	–0,327	–0,051
Posterior muzzle breadth	0,845	–0,142	–0,324
Anterior frontal breadth	0,858	–0,333	0,085
Posterior frontal breadth	0,204	0,099	0,494
Interauditory breadth	0,897	–0,218	0,197
Basal cranial length	0,964	–0,136	0,111
Palate incisor length	0,740	0,619	0,212
Palate vacuity length	0,666	0,589	0,275
Hard palate length	0,309	0,906	–0,005
Mesopterygoid space	0,484	–0,845	–0,014
Maxillary premolar length	0,742	0,283	–0,468
Maxillary premolar-molar length	0,917	0,163	–0,222
Principal I^1 breadth	0,860	–0,387	–0,185
Palatal breadth between P^2 and P^3	0,954	–0,104	–0,098
Bizygomatic breadth	0,920	–0,247	0,069
Maximum cranial breadth	0,853	–0,351	0,234
Zygomatic arch length	0,948	–0,052	–0,054
Posterior cranial height	0,807	–0,376	0,181
Bulla breadth	0,217	–0,919	0,059
Maximum mandibular length	0,949	0,178	0,077
Mandibular toothrow length	0,867	0,414	0,164
Mandibular body breadth	0,824	–0,207	–0,297
I_1 breadth	0,798	0,391	–0,241
Mandibular premolar-molar length	0,939	–0,106	–0,121

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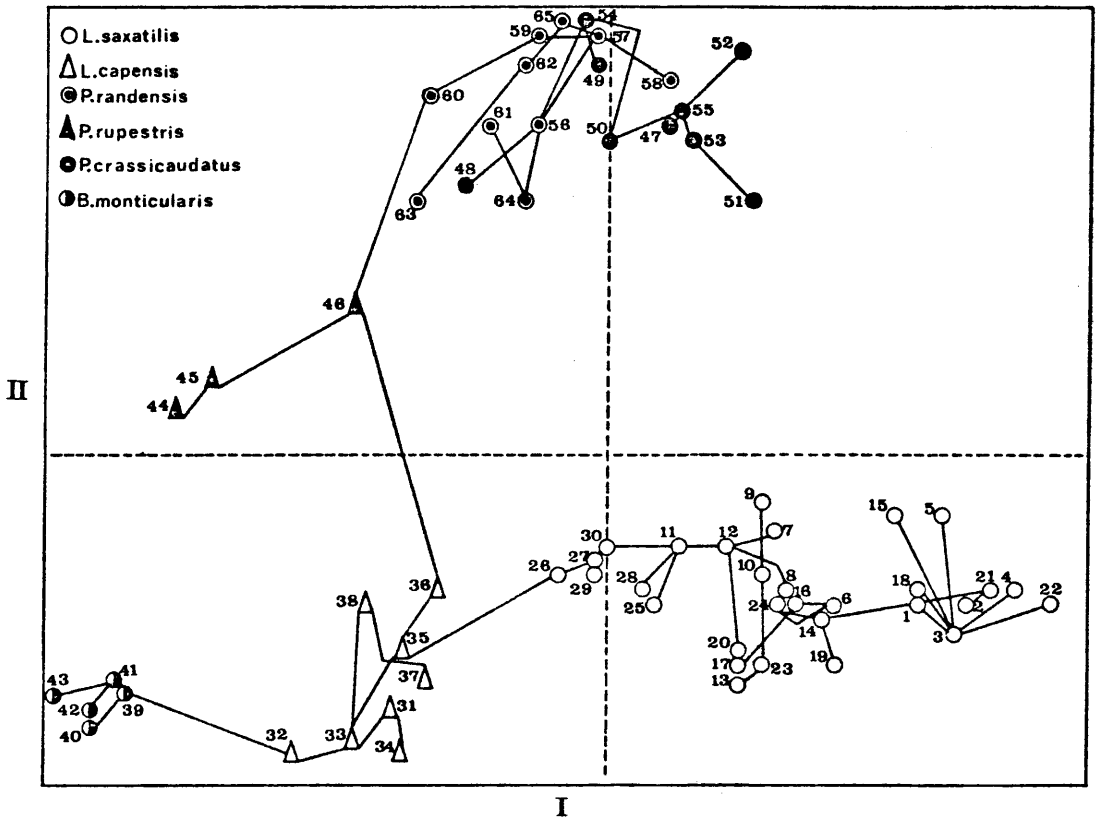

Fig. 5: *Pairwise comparison of components I and II from a principal component analysis of the South African Leporidae based largely on sample means. OTU's 1–30* L. saxatilis; *31–38* L. capensis; *39–43* B. monticularis; *44–46* P. rupestris; *47–55* P. crassicaudatus; *56–65* P. randensis. *The minimum spanning tree (MST) is superimposed.*

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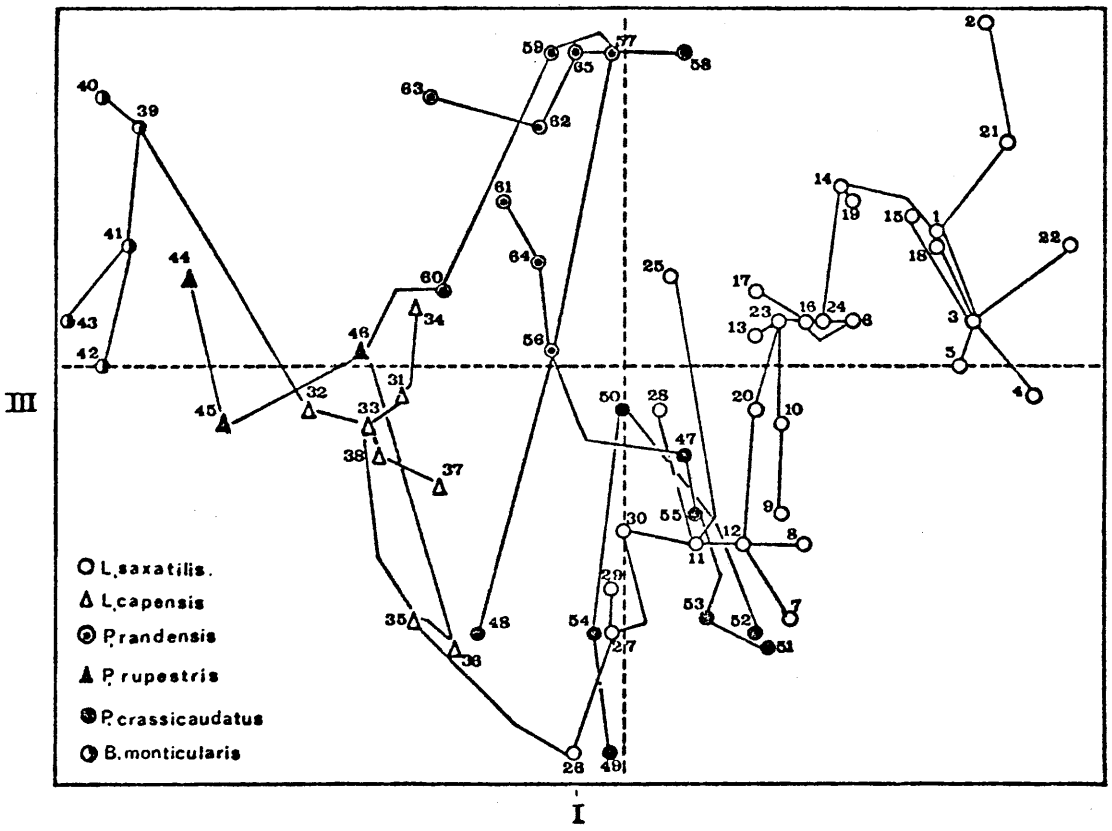

Fig. 6: *Pairwise comparison of component I and III from a principal component analysis of the South African Leporidae based largely on sample means. OTU's 1–30* L. saxatilis; *31–38* L. capensis; *39–43* B. monticularis; *44–46* P. rupestris; *47–55* P. crassicaudatus; *56–65* P. randensis. *The minimum spanning tree (MST) is superimposed.*

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clustering separately from the composite grouping of *P. crassicaudatus* and *P. randensis* OTU's.

Ratios calculated by contrasting the highest positive and negative loadings in components two and three of the factor matrix are presented in Table 3. The results for component two clearly indicate that *Pronolagus* differs markedly from the other genera by having on average a relatively narrow mesopterygoid space in proportion to both palate incisor and hard palate lengths. Furthermore, *Pronolagus* with its relatively small bulla to palate ratio differs from the other taxa which are larger in this measurement.

No clear separation between genera resulted from contrasting components one and three (Fig. 6). However, at species level the segregation of *P. crassicaudatus* (OTU's 47–55) from *P. randensis* (OTU.s 56–65) is worth mention. All *P. randensis* OTU's cluster in the upper half of component three, with the *P. crassicaudatus* OTU's grouping in the lower portion of the scale. The *P. rupestris* specimens occupy a roughly intermediate portion between the two former species. Similar trends were noted in detailed subsequent analyses based on individual specimens in each taxon, and are fully discussed elsewhere.

The distinctiveness of the various taxa and the OTU's comprising them are greatly enhanced by the addition of the MST connections. Furthermore, those OTU's which were misplaced in the distance and correlation phenograms have nearest relative connections in the MST. The only exception is the *P. crassicaudatus* OTU from Magudu (48) which is connected via a *P. randensis* sample (56) to the other *P. crassicaudatus* material. However, subsequent analyses, which deal with the delimitation of the species in *Pronolagus* using individual specimens, all *P. crassicaudatus* specimens, including those from Magudu, are interconnected.

Taxonomic conclusions:

The results of a principal component analysis conducted on OTU's representative of the three genera recognized by Petter (1972), clearly illustrate the cranial uniqueness of *Pronolagus* as distinct from *Bunolagus* and *Lepus*. The separation of *Bunolagus* from *Lepus* (the genus with which it has the greatest cranial affinity) depends largely on size. Based solely on the craniometric data, the recognition of *Bunolagus* as a genus distinct from *Lepus* is not justified. However, substantial support for the recognition of the generic status of the former taxon is provided by karyotype and sperm morphology data, as well

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Table 3 :

Variation in ratios calculated by contrasting cranial measurements which have the highest positive and negative loadings in factor II and III of the factor matrix. $\bar{X}$ = Arithmetic mean; SD = Standard deviation; Observed ranges in parentheses.

Ratio	*L. saxatilis*	*L. capensis*	*B. monticularis*	*P. crassicaudatus*	*P. rupestris*	*P. randensis*
			FACTOR II			
Mesopterygoid space x 100/ palate incisor length	$\bar{X}$ = 23,8 % SD = 1,57 (19,4% –27,1%)	$\bar{X}$ = 24,6 % SD = 1,41 (21,9% –25 ,8 %)	$\bar{X}$ = 24,1 % SD = 0,62 (23,4% –25,1 %)	$\bar{X}$ = 13,7 % SD = 0,93 (12,5% –15,0%)	$\bar{X}$ = 15,4% SD = 0,24 (15,1% –15,5%)	$\bar{X}$ = 11,5% SD = 1,34 (9,0 % –13,4%)
Bulla breadth x 100/ palate incisor length	$\bar{X}$ = 22,7% SD = 1,16 (20,7% –25,4%)	$\bar{X}$ = 25,8% SD = 2,36 (22,0% –29,1%)	$\bar{X}$ = 27,6% SD = 0,72 (27,0% –28,8%)	$\bar{X}$ = 14,8% SD = 1,22 (13,3 % –16,6 %)	$\bar{X}$ = 19,7% SD = 2,55 (17,2 % –22,3%)	$\bar{X}$ = 14,8% SD = 1,44 (12,6 % –17,2%)
Mesopterygoid space x 100/ hard palate length	$\bar{X}$ = 135,9 % SD = 17,72 (90,4 % –175,0 %)	$\bar{X}$ = 148,8 % SD = 19,00 (113,8% –170,5%)	$\bar{X}$ = 153,8 % SD= 5,87 (146,4 % –160,0%)	$\bar{X}$ = 60,2% SD = 5,42 (53,0% –68,9 %)	$\bar{X}$ = 79,8% SD = 4,33 (76,3% –84,6%)	$\bar{X}$ = 53,2% SD = 8,74 (39,0% –64,2%)
Bulla breadth x 100/ hard palate length	$\bar{X}$ = 129,4% SD = 10,76 (114,5 % –153,1%)	$\bar{X}$ = 156,4 % SD = 24,50 (114,6 % –184,8%)	$\bar{X}$ = 176,5 % SD = 9,95 (165,2 % –191,5%)	$\bar{X}$ = 64,9% SD = 5,27 (57,5 % –72,9%)	$\bar{X}$ = 102,3% SD = 13,57 (86,8% –112,3%)	$\bar{X}$ = 68,6% SD = 9,34 (55,4 % –85,4%)
			FACTOR III			
Posterior muzzle breadth x 100/ posterior frontal breadth	$\bar{X}$ = 163,1% SD = 12,42 (131,9 % –194,8%)	$\bar{X}$ = 158,8 % SD = 15,43 (141,1 % –192,7 %)	$\bar{X}$ = 113,1% SD = 9,59 (102,0 % –126,7 %)	$\bar{X}$ = 159,4% SD = 10,30 (144,3 % –171,8%)	$\bar{X}$ = 140,8% SD = 5,61 (135,2 % –146,4%)	$\bar{X}$ = 129,0 % SD= 7,86 (118,9 % –139,2%)
Maxillary premolar length x 100/ posterior frontal breadth	$\bar{X}$ = 68,6 % SD =6,27 (56,9% –85,3 %)	$\bar{X}$ = 64,7% SD =4,72 (60,9% –74,3%)	$\bar{X}$ = 45,6% SD = 1,72 (42,9% –47,5%)	$\bar{X}$ = 75,2% SD = 8,90 (64,0 % –92,5%)	$\bar{X}$ = 66,8% SD = 5,92 (60,2 % –71,6%)	$\bar{X}$ = 59,0% SD = 5,22 (51,7 % –64,8%)

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as the species unique external phenotypic characteristics (Chapters 3, 5 and 7).

DELIMITATION OF SPECIES WITHIN *PRONOLAGUS:*

Petter's key for the delimitation of the three species of *Pronolagus* is based on measurements of the length of the tympanic bulla and skull length (Petter, 1972). However, the low repeatability of the bulla length measurement as reported by Peddie (1975) and experienced in preliminary measurement trials for this study, renders the usefulness of this criterion in the key questionable. Nevertheless, it became increasingly apparent during field work that through the combined use of certain pelage characteristics and the incisor tooth pattern variation (described elsewhere), all *Pronolagus* specimens could be provisionally assigned to one of the three taxa retained by Petter (1972).

In order to assess the interspecific variation of *Pronolagus,* individual specimens were utilised. Unfortunately, specimens deficient in one or more cranial character could not be used in the multivariate analyses, resulting in a reduction of the total sample size. Twenty eight cranial measurements for each of the remaining OTU's were analysed using the multivariate procedures.

Phenograms illustrating the phenetic relationships, were computed from both the distance and correlation matrices. The distance phenogram (Fig. 7) with a cophenetic correlation of 0,803 shows the OTU's falling into two major groups A and B. Major cluster A is further divided into subgroups C and D. Subgroup C comprises all specimens identified as *P. randensis,* but includes two misplaced *P. crassicaudatus* OTU's from Magudu (18 and 24). It is noteworthy that although the two Magudu OTU's are segregated from the other *P. crassicaudatus* specimens, they join the *P. randensis* specimen at an average taxonomic distance only marginally smaller than that of the *P. crassicaudatus* subcluster D (1,15 *cf.* 1,17). Major cluster B provides a well defined grouping of *P. rupestris* specimens. The latter taxon joins the two other samples at an average taxonomic distance of 1,79.

The correlation phenogram which had a cophenetic correlation coefficient of 0,794 is presented in Fig. 8. The principal differences between this and the distance phenogram (Fig. 7) lie in the placement of the *P. crassicaudatus* OTU's from Magudu, which are now correctly grouped with the other specimens of the species, and two *P. randensis* OTU's (9 and 12), which have moved to positions which give no indication of their relationship to the remainder of the *P. randensis* material.

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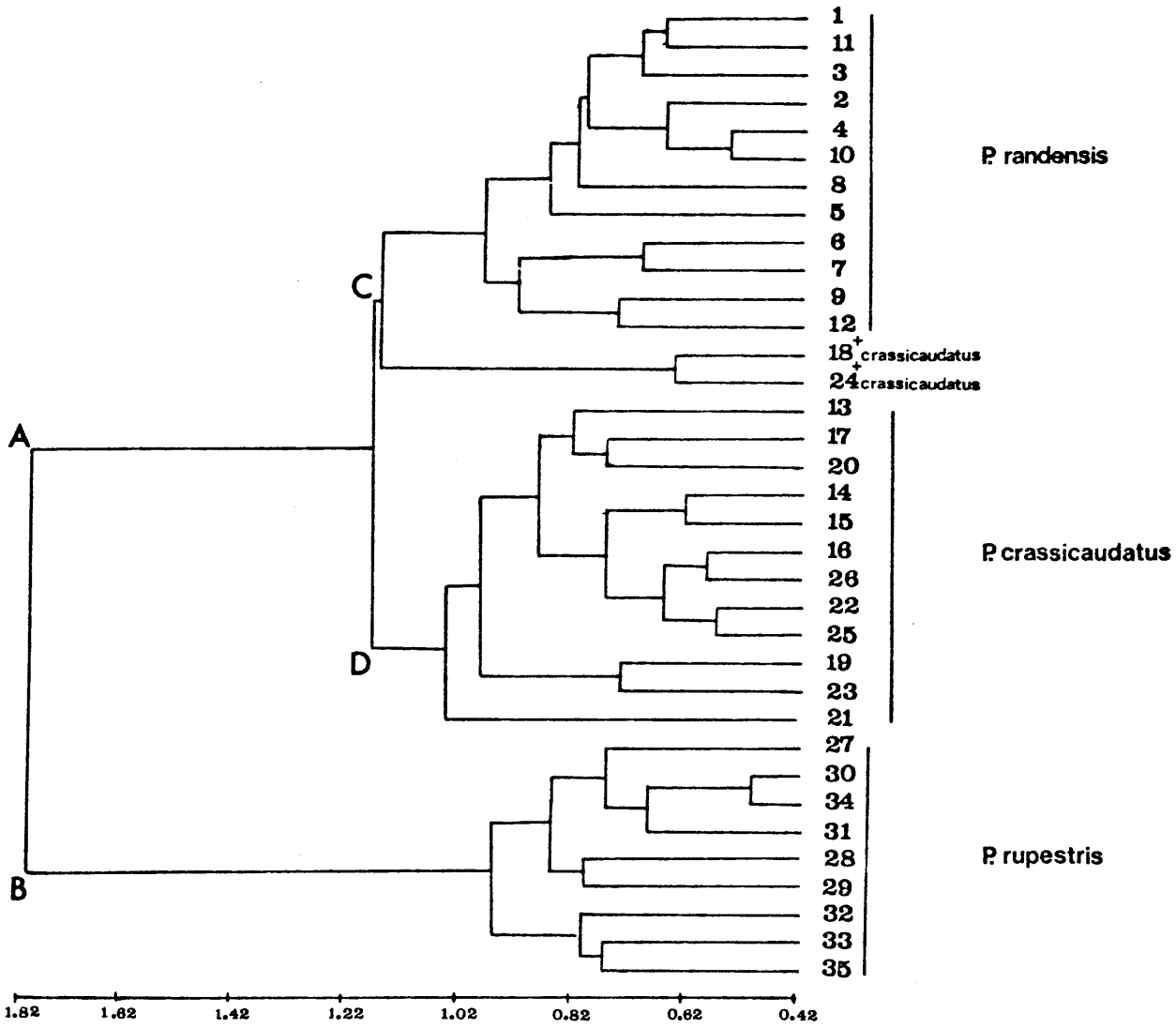

Fig. 7: *Distance phenogram of specimens of* Pronolagus *clustered by the unweighted pair-group method using arithmetic averages. OTU's 1–12* P. randensis; *13–26* P. crassicaudatus; *27–35* P. rupestris. *Cophenetic correlation coefficient = 0,803.*

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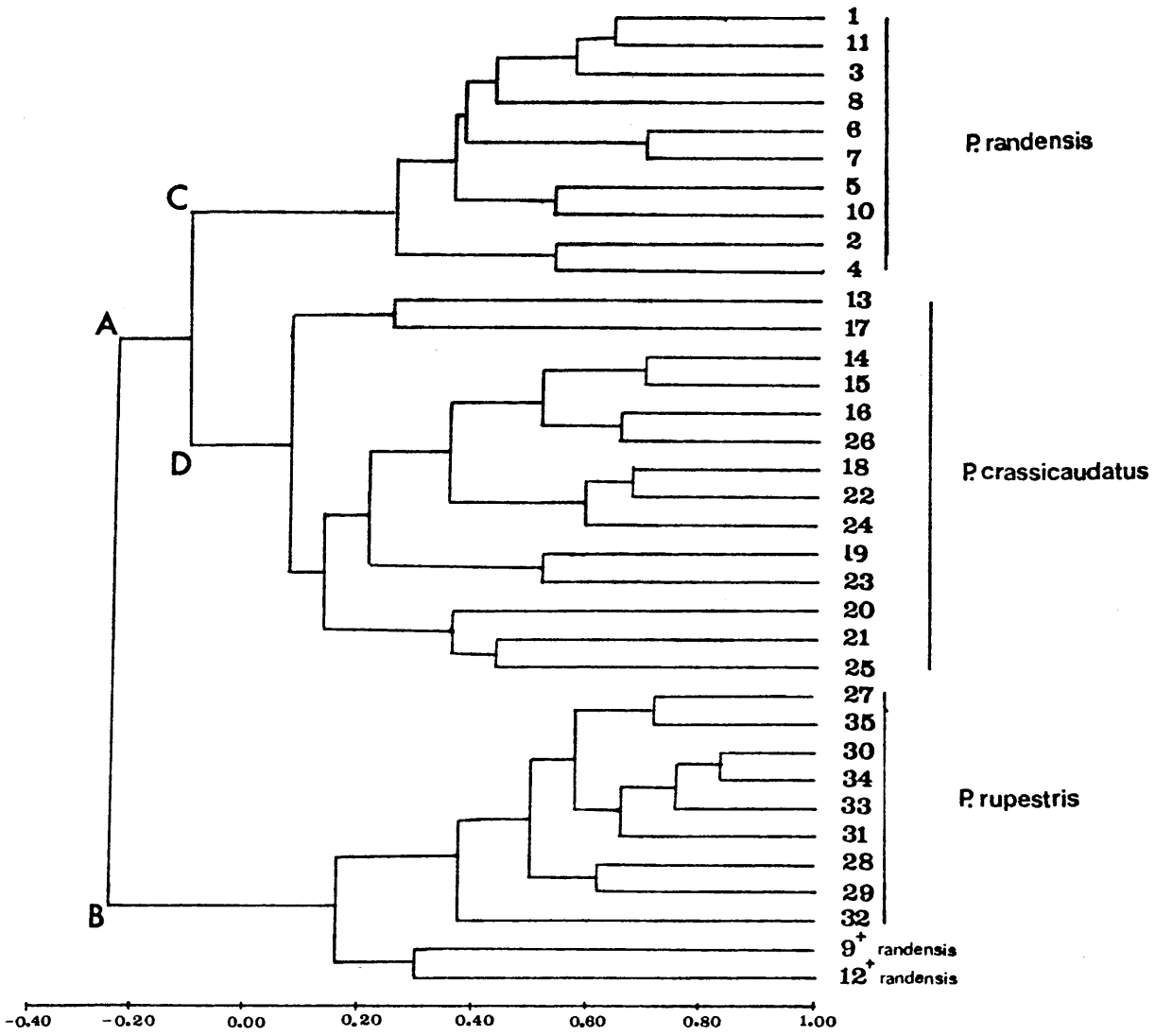

Fig. 8: *Correlation phenogram of specimens of* Pronolagus *clustered by the unweighted pair-group method using arithmetic averages. OTU's 1–12* P. randensis; *13–26* P. crassicaudatus; *27–35* P. rupestris. *Cophenetic correlation coefficient = 0,794.*

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Table 4:

Factor matrix from a 28 variable principal component analysis of specimens of *P. crassicaudatus, P. rupestris* and *P. randensis* showing the character loadings on the first three components.

Measurement	Factor I	Factor II	Factor III
Total length of skull	0,943	− 0,061	0,132
Muzzle length	0,765	0,139	0,070
Frontal length	0,462	− 0,647	− 0,106
Parietal length	0,539	0,458	0,377
Posterior muzzle breadth	0,747	0,311	− 0,378
Anterior frontal breadth	0,792	− 0,197	− 0,008
Posterior frontal breadth	0,772	− 0,308	− 0,194
Interauditory breadth	0,759	− 0,072	0,102
Basal cranial length	0,925	− 0,010	0,139
Palate incisor length	0,913	− 0,276	− 0,004
Palate vacuity length	0,809	− 0,262	0,121
Hard palate length	0,830	− 0,079	− 0,295
Mesopterygoid space	− 0,041	0,673	0,600
Maxillary premolar length	0,558	0,516	− 0,202
Maxillary premolar-molar length	0,854	0,317	− 0,070
Principal I^1 breadth	0,903	0,225	− 0,111
Palatal breadth between P^2 and P^3	0,919	0,279	− 0,062
Bizygomatic breadth	0,780	− 0,399	0,203
Maximum cranial breadth	0,664	− 0,261	0,432
Zygomatic arch length	0,856	0,011	0,004
Posterior cranial height	0,281	− 0,643	0,164
Bulla breadth	− 0,789	0,130	0,120
Maximum mandibular length	0,914	− 0,046	0,193
Mandibular toothrow length	0,914	− 0,168	0,116
Mandibular height	0,941	0,003	− 0,037
Mandibular body breadth	0,762	0,331	− 0,188
I_1 breadth	0,835	0,357	− 0,061
Mandibular premolar-molar length	0,907	0,188	− 0,054

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The results of a principal component analysis of these OTU's are given in Table 4 and Figs. 9 and 10. The amount of phenetic variation represented in the first three principal components was 61,48% for component one, 10,25% for component two and 4,55% for component three (Total = 76,28%). The factor matrix (Table 4) indicates that the first component is heavily influenced by overall size, with measurements such as total length of the skull, basal cranial length and mandibular height, among others, showing the highest loadings. Only two measurements, mesopterygoid space and bulla breadth have negative loadings. Separation along the second component is influenced primarily by frontal length, width of the mesopterygoid space, maxillary premolar length and posterior cranial height. The third component appears to be influenced predominantly by breadth measurements such as posterior muzzle breadth, mesopterygoid space and maximum cranial breadth. One length measurement, parietal length, also has a relatively high loading in this component.

Examination of the two-dimensional plot I:II (Fig. 9) reveals a pattern similar to that shown in the distance and correlation phenograms. Three distinct clusters corresponding to the species *P. crassicaudatus, P. rupestris* and *P. randensis* are evident. *P. crassicaudatus* and *P. randensis* specimens are roughly of equivalent overall size (See Appendix II, Table 3), with both species falling predominantly in the right half of the first component scale. *P. rupestris* which is smaller than other taxa on absolute values (Appendix II, Table 3), is separated along the first component. Cranial ratios (Table 5) calculated by contrasting bulla breadth (high negative) with total length of skull, basal cranial length and mandible height (all high positive), indicate that *P. rupestris* has, on average, broader bullae in relation to the contrasted characters than either *P. crassicaudatus* or *P. randensis.* Both *P. crassicaudatus* and *P. randensis* are very similar in respect of these ratios.

However, good distinction is particularly evident between *P. crassicaudatus* and *P. randensis* as evidenced by the spread of specimens along component two. All *P. crassicaudatus* specimens cluster in the upper half of the second component scale, with *P. randensis* grouped in the lower half of the scale. Cranial ratios (Table 5) indicate that *P. crassicaudatus* usually has a porportionately longer maxillary premolar toothrow in relation to frontal length and posterior cranial height than *P. randensis* and *P. rupestris,* although greater individual overlap is evident in comparison with the latter species. In addition, both *P. crassicaudatus* and *P. rupestris* have on average, a proportionately wider mesopterygoid space in relation to frontal length and posterior cranial height than *P. randensis.*

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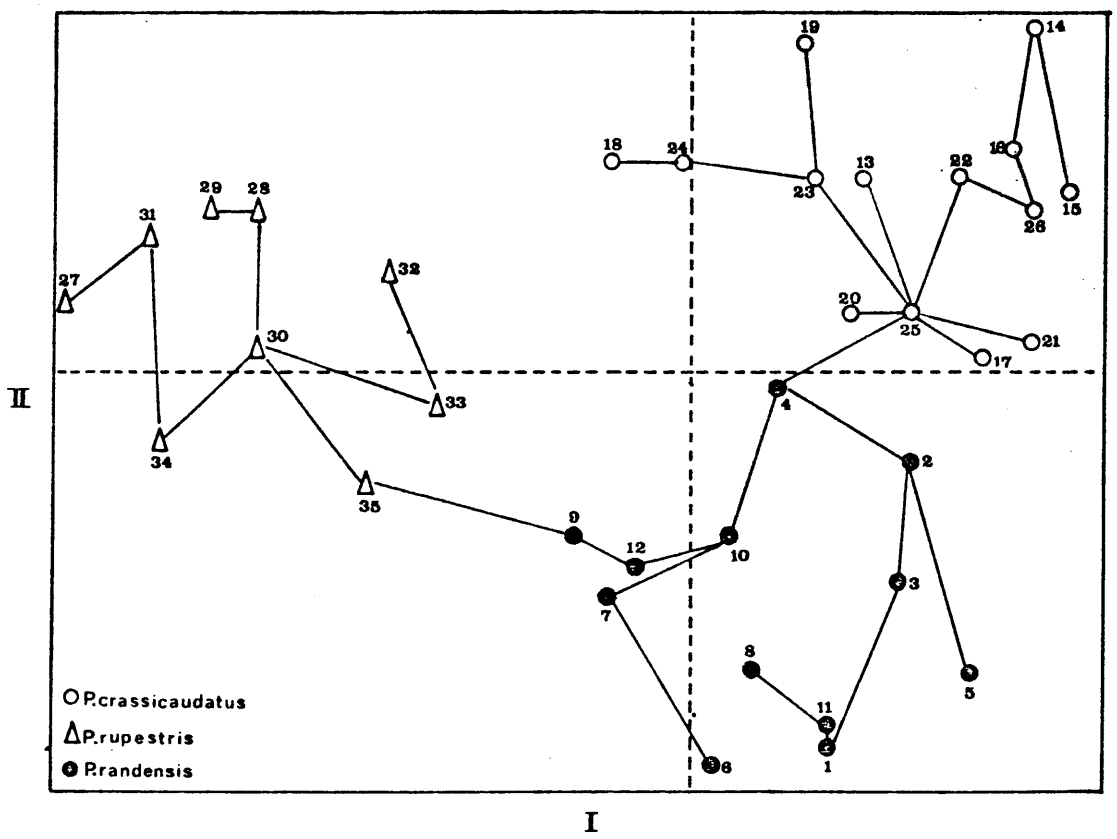

Fig. 9: *Pairwise comparison of components I and II from a principal component analysis of* Pronolagus *specimens. OTU's 1–12* P. randensis; *13–26* P. crassicaudatus; *27–35* P. rupestris. *The minimum spanning tree (MST) is superimposed.*

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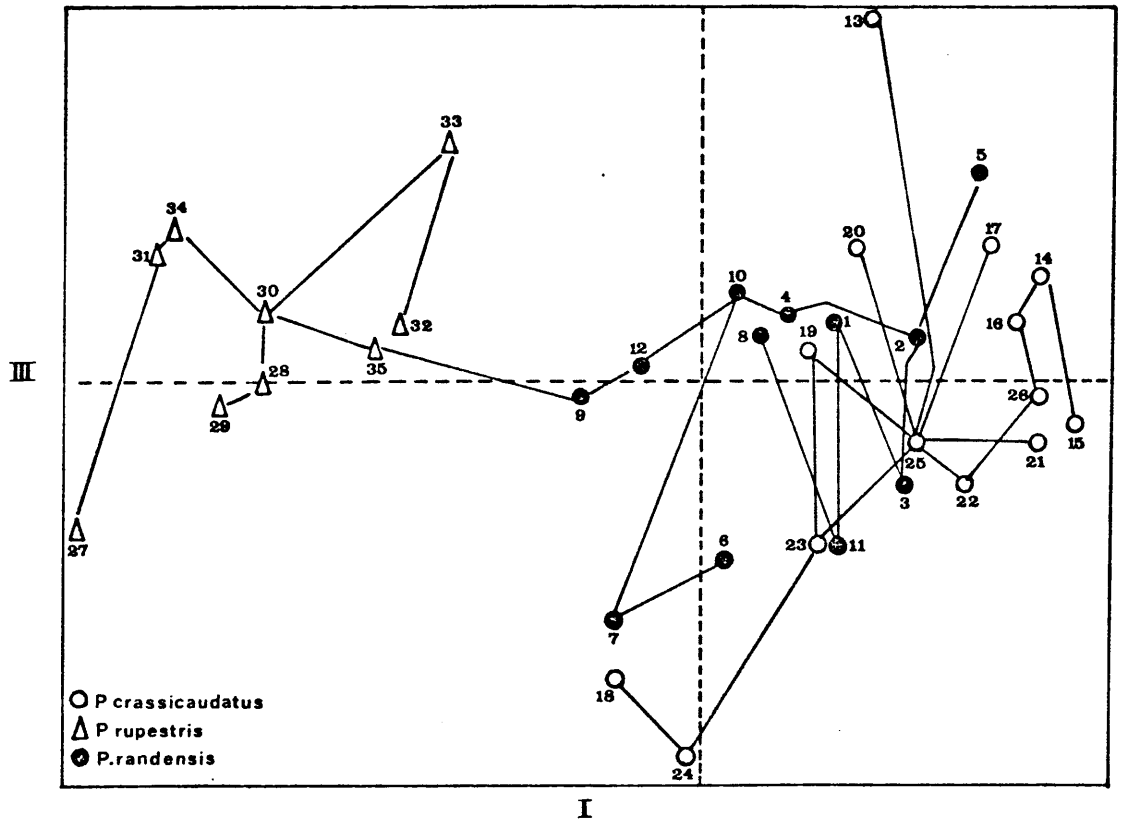

Fig. 10: *Pairwise comparison of components I and III from a principal component analysis of* Pronolagus *specimens. OTU's 1–12* P. randensis; *13–26* P. crassicaudatus; *27–35* P. rupestris. *The minimum spanning tree (MST) is superimposed.*

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Table 5:

Variation in ratios calculated by contrasting cranial measurements which have the highest positive and negative loadings in factor I and II of the factor matrix. $\bar{X}$ = Arithmetic mean; SD = Standard deviation; Observed ranges in parentheses.

Ratio	*P. crassicaudatus*	*P. rupestris*	*P. randensis*
		FACTOR I	
Bulla breadth x 100/total length of skull	$\bar{X}$=6,6% SD=0,58 (5,5% – 7,8%)	$\bar{X}$= 9,0% SD=0,80 (7,7% – 10,7%)	$\bar{X}$= 6,9% SD=0,56 (6,0% – 7,9%)
Bulla breadth x 100/ basal cranial length	$\bar{X}$= 8,2% SD=0,70 (6,8% – 9,5%)	$\bar{X}$= 11,2% SD=0,87 (9,8% – 13,0%)	$\bar{X}$= 8,6% SD=0,71 (7,3% – 9,7%)
Bulla breadth x 100/ mandibular height	$\bar{X}$= 13,6% SD=1,18 (11,5% – 16,1%)	$\bar{X}$= 19,9% SD=1,65 (17,1% – 22,7%)	$\bar{X}$= 14,4% SD=1,17 (12,6% – 17,0%)
		FACTOR II	
Maxillary premolar length x 100/frontal length	$\bar{X}$= 26,1% SD=2,65 (21,9% – 32,7%)	$\bar{X}$= 22,2% SD=2,63 (18,6% – 28,1%)	$\bar{X}$= 19,7% SD=1,53 (17,7% – 21,7%)
Maxillary premolar length x 100/post.cranial height	$\bar{X}$= 60,8% SD=6,55 (52,1% – 77,1%)	$\bar{X}$= 48,2% SD=5,21 (40,4% – 55,9%)	$\bar{X}$= 47,1% SD=3,48 (41,0% – 53,3%)
Mesopterygoid space x 100/ frontal length	$\bar{X}$= 15,3 % SD=1,90 (12,0% – 19,1%)	$\bar{X}$= 16,3 % SD=0,77 (15,4% – 17,5%)	$\bar{X}$= 11,9% SD=1,39 (9,4% – 14,0%)
Mesopterygoid space x 100/ post. cranial height	$\bar{X}$= 35,5% SD=4,51 (29,3% – 43,0%)	$\bar{X}$= 35,4% SD=2,46 (32,1% – 40,6%)	$\bar{X}$= 28,5 % SD=2,91 (22,2% – 32,9%)

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Examination of the ordination diagram for component I and III (Fig. 10) provides no evidence of species separation along the third axis. However, the *P. crassicaudatus* specimens 13 (Hilton), and 18 and 24 (Magudu), cluster respectively above and below the other representatives of this species. The anomalous placement of specimen 13 in respect to the other *P. crassicaudatus* material appears in part, to be attributable to a relatively small posterior muzzle breadth to cranial breadth ratio (57,7% *cf.* 70,4%, the average for the species) and a larger mesopterygoid space to posterior muzzle breadth ratio (36,1% *cf.* 27,4%). Conversely, specimens 18 and 24 are characterised by a marginally larger posterior muzzle breadth ratio (72,9% and 83,6% respectively) but smaller mesopterygoid space ratios (23,0% ; 21,4%) than is average for the species.

A minimum spanning tree (MST) superimposed on the ordination diagrams (Figs 9 and 10) clearly shows the three species to be discretely clustered, the representatives of which all have closest relative connections. Consequently it would seem that the displacement of OTU's in the phenograms is attributable to distortions.

Taxonomic conclusions:

Based on the results of the morphometric data analyses using the multivariate procedures, the recognition of three species, *P. crassicaudatus, P. randensis* and *P. rupestris* in South Africa appears justified. These results confirm the earlier treatment of the genus by Ellerman *et al.* (1953) and the subsequent retention of these taxa by Petter (1972). No evidence could be found to support Lundholm's (1955) argument that *P. crassicaudatus* and *P. randensis* are synonyms, or alternatively, that *Pronolagus* is a monotypic genus (Peddie, 1975).

DELIMITATION OF SPECIES WITHIN *LEPUS*

Multivariate analyses of the South African *Lepus* were conducted on data sets comprising two external body measurements, ear length and hind foot length, and 28 cranial measurements. Phenograms diagramming the phenetic relationships were computed from both the distance and correlation matrices. The phenogram based on the distance matrix (Fig. 11) had a correlation coefficient of 0,722 and clearly shows the OTU's falling into two major clusters A and B.

Major cluster A is a complex entity comprising several subclusters which consist entirely of specimens regarded as being distinct from *L. capensis* and which were provisionally

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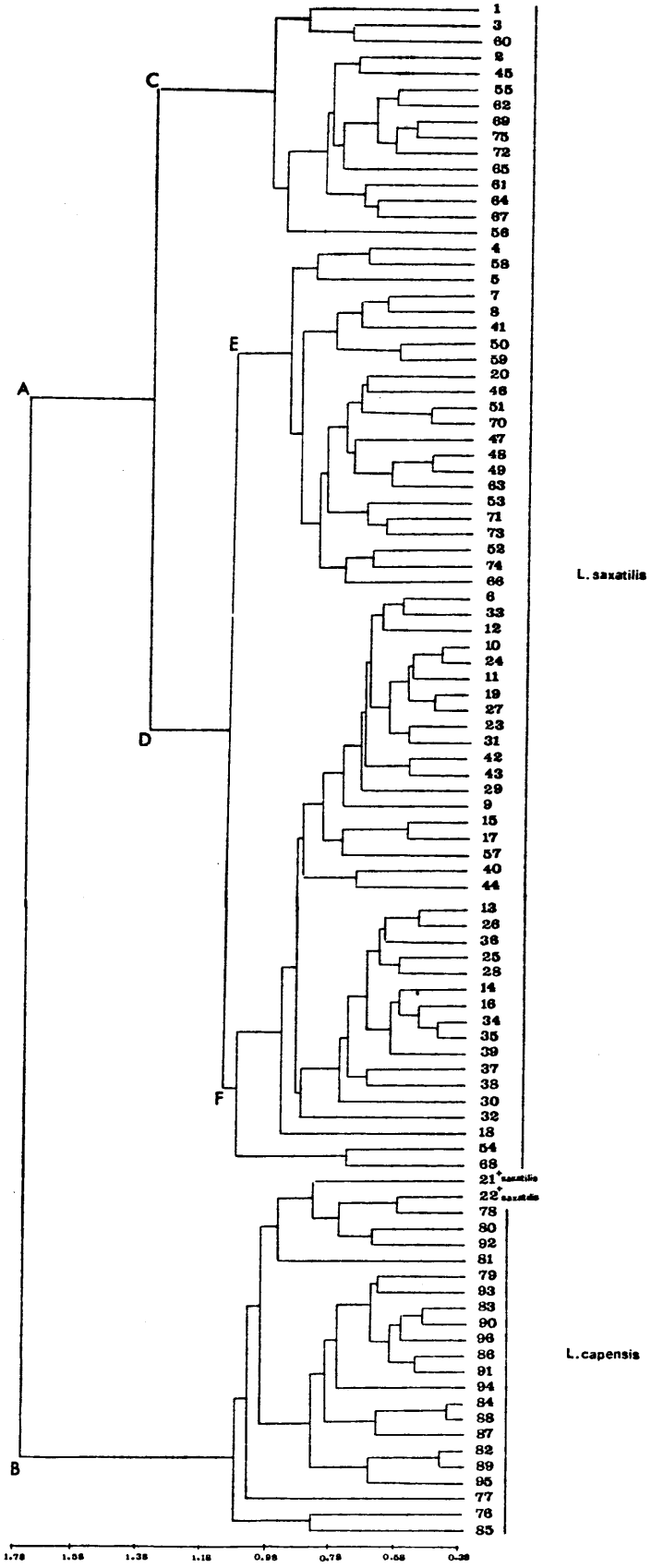

Fig. 11: *Distance phenogram of South African* Lepus *specimens clustered by the unweighted pair-group method using arithmetic averages. OTU's 1–75* L. saxatilis; *76–96* L. capensis. *Cophenetic correlation coefficient = 0,722.*

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classified as *L. saxatilis.* Major cluster B which represents a well deliniated group, includes all the *L. capensis* specimens used in the present study, as well as two misplaced *L. saxatilis* OTU's from Winterton (OTU's 21 and 22). This cluster(B), is well set off from the bulk of the *L. saxatilis* specimens, joining the latter at the relatively high average taxonomic distance of 1,74.

As can clearly be seen from Fig. 11, major cluster A can be split into subclusters C and D with the latter showing two further divisions E and F. The grouping of OTU's into the constituent clusters seems to depend largely on overall size as reflected by skull length. Those OTU's comprising clusters C, E and F were found to have mean greatest skull length measurements of 104,2 mm (observed range 101,7–107,7); 98,0 mm (observed range 94,3–102,0) and 92,3 mm (observed range 87,0–97,2) respectively.

The correlation phenogram with a cophenetic correlation coefficient of 0,676 is presented in Fig. 12. Two major clusters A and B are apparent. However, in this case the cluster composition bears scant resemblance to that illustrated in the distance phenogram. Particularly striking is the high number of *L. saxatilis* OTU's which are misplaced from their nearest relatives and grouped within the *L. capensis* cluster.

Uncertainties regarding the misplaced OTU's in both the distance and correlation phenogram were resolved by the principal component analysis. As will be seen, all the constituent OTU's of both *L. capensis* and *L. saxatilis* have nearest relative connections in the minimum spanning tree.

The first three principal components were extracted from the matrix of correlation among the 30 characters used and, which combined, expressed 76,00% of the total phenetic variation. Table 6 shows the weighting of each character on the first three components. The amount of phenetic variation presented in component one was 62,00% and appears to be heavily influenced by overall size as indicated by the invariably positive and high loadings of almost all characters. Examination of the two dimensional plot of component one and two (Fig. 13) clearly reflects the importance of overall size, with the larger *L. saxatilis* clustering predominantly in the right half of this component scale and the smaller *L. capensis* in the left.

The two species do not separate along the second (10,16% of the total phenetic variation) or the third component (3,84% of the total phenetic variation, Fig. 14) and the spread of OTU's along them reflects intrapopulation and interpopulation differences rather than species differences.

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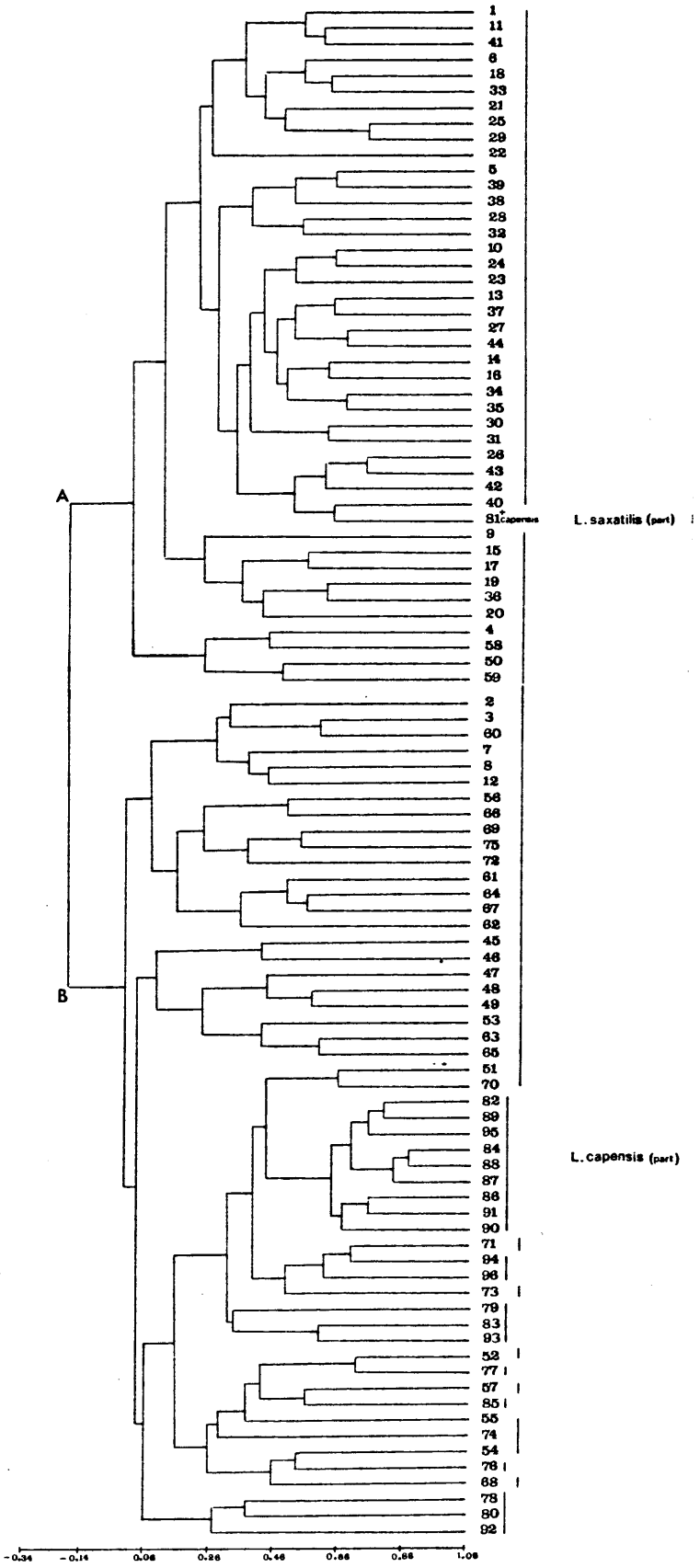

Fig. 12: *Correlation phenogram of South African* Lepus *specimens clustered by the unweighted pair-group method using arithmetic averages. OTU's 1–75* L. saxatilis*; 76–96* L. capensis. *Cophenetic correlation coefficient = 0,676.*

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Table 6:

Factor matrix from a 30 variable principal component analysis of specimens of *L. capensis* and *L. saxatilis* showing the character loadings on the first three components.

Measurement	Factor I	Factor II	Factor III
Hind foot length	0,777	− 0,133	0,155
Ear length	0,509	− 0,707	− 0,006
Total length of skull	0,963	− 0,178	0,095
Muzzle length	0,785	− 0,038	0,323
Frontal length	0,776	0,193	− 0,205
Parietal length	0,594	− 0,313	− 0,105
Posterior muzzle breadth	0,761	0,040	− 0,186
Anterior frontal breadth	0,761	0,089	− 0,321
Posterior frontal breadth	0,447	− 0,322	− 0,629
Interauditory breadth	0,777	− 0,383	0,080
Basal cranial length	0,950	− 0,175	0,112
Palate incisor length	0,948	− 0,069	0,181
Palate vacuity length	0,867	− 0,269	0,079
Hard palate length	0,638	0,326	0,275
Mesopterygoid space	0,650	− 0,444	− 0,133
Maxillary premolar length	0,800	0,268	− 0,041
Maxillary premolar-molar length	0,838	0,155	− 0,058
Principal I^1 breadth	0,772	0,421	− 0,095
Palatal breadth between P^2 and P^3	0,910	0,172	− 0,024
Bizygomatic breadth	0,929	0,019	− 0,109
Maximum cranial breadth	0,789	− 0,338	− 0,183
Zygomatic arch length	0,895	0,188	0,132
Posterior cranial height	0,711	− 0,263	− 0,026
Bulla breadth	0,147	− 0,702	0,212
Maximum mandibular length	0,947	− 0,030	0,169
Mandibular toothrow length	0,956	− 0,037	0,140
Mandibular height	0,867	0,309	0,186
Mandibular body breadth	0,651	0,578	0,034
I_1 breadth	0,755	0,409	− 0,256
Mandibular premolar-molar length	0,871	0,281	− 0,071

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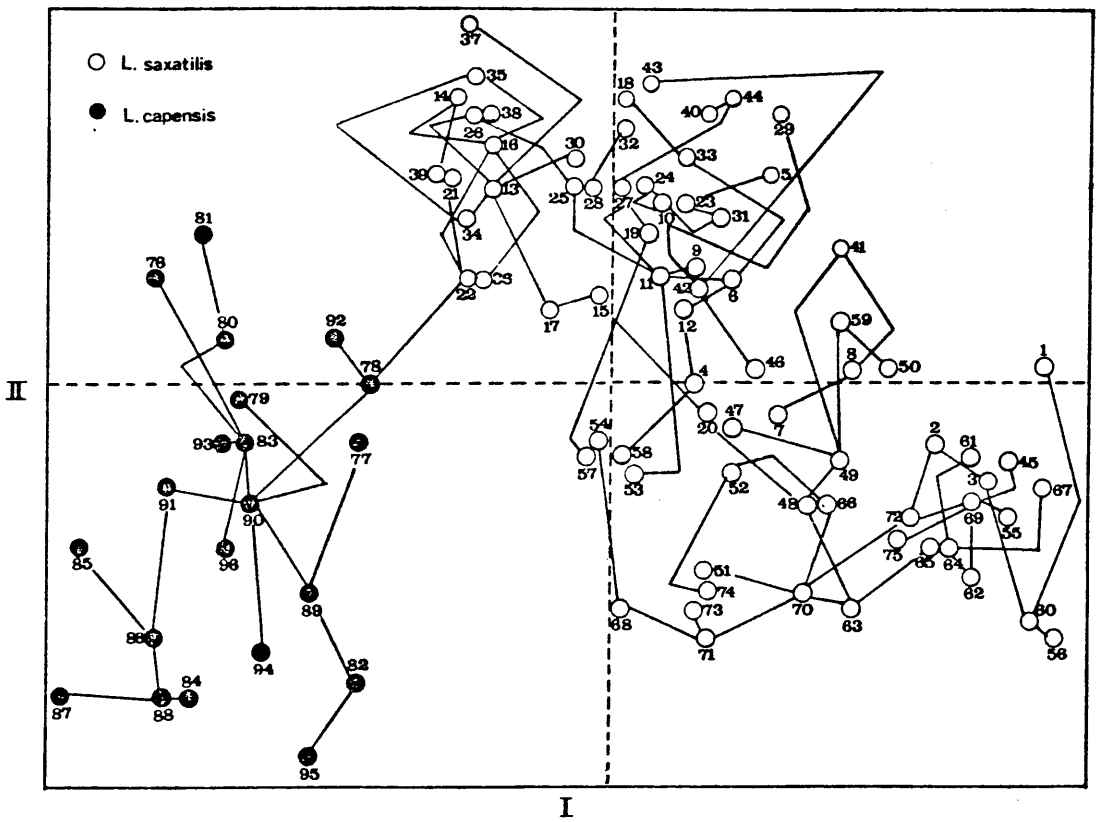

Fig. 13: *Pairwise comparison of components I and II from a principal component analysis of South African* Lepus *specimens. OTU's 1–75* L. saxatilis; *76–96* L. capensis. *The minimum spanning tree (MST) is superimposed.*

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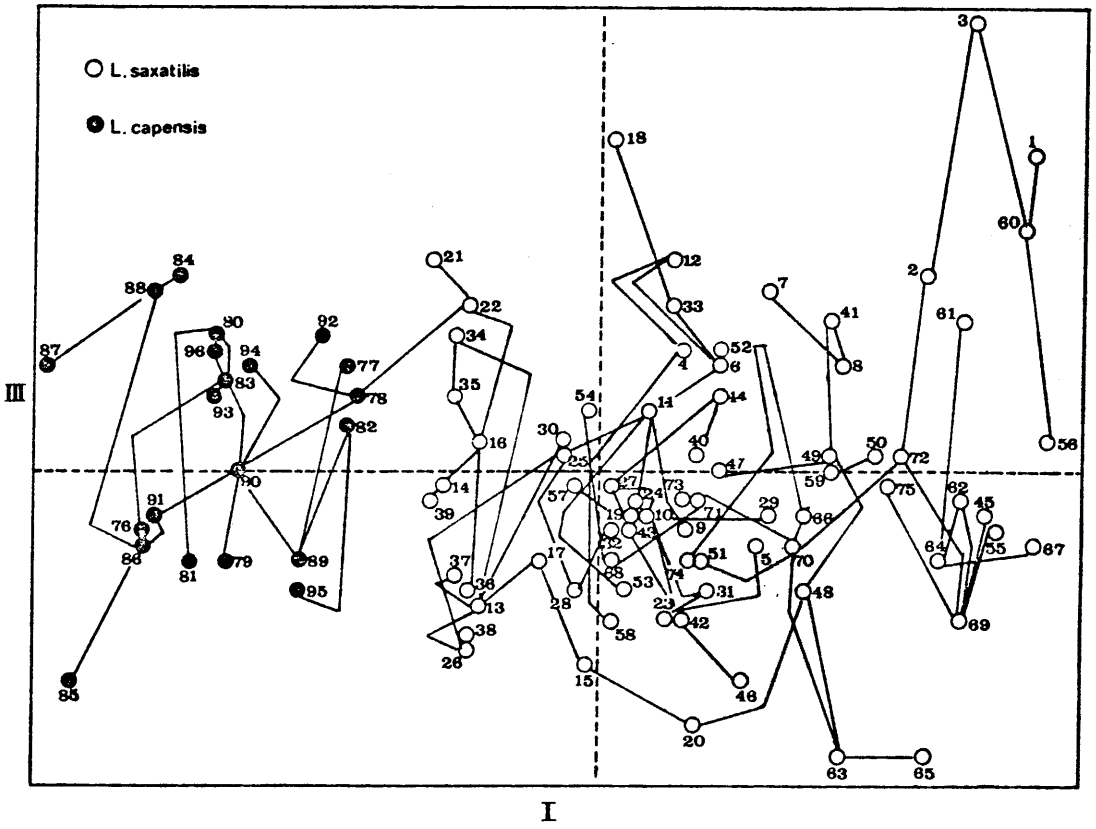

Fig. 14: *Pairwise comparison of components I and III from a principal component analysis of South African* Lepus *specimens. OTU's 1–75* L. saxatilis; 76–96 *L. capensis.* The minimum spanning tree (MST) is superimposed.

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The distinction between *L. capensis* and *L. saxatilis* is considerably enhanced by the addition of the MST which shows the two taxa linked via a single branch of the tree network. Furthermore, the constituent OTU's of each species are evident as well knit clusters showing nearest relative connections.

Good evidence exists in the above analyses for the delimitation of *L. capensis*, a species which is reported to have a prodigious range extending from the Cape Province northwards through Africa, Europe and Asia (Petter, 1972). However, it should be realized that the recognition of the remaining OTU's provisionally identified as *L. saxatilis* in this study includes specimens which, using Petter's key (Petter, 1972), could be asigned to *L. crawshayi*. Consequently it was decided to exclude the *L. capensis* material from the following analysis and to process the remaining OTU's independently. It was hoped that by removing the source of variation provided by *L. capensis*, a clearer indication of the phenetic relationships of the remaining OTU's would result.

The residual 75 OTU subset comprising only *L. saxatilis* specimens was analysed using the same procedures as before. The distance phenogram (Fig. 15) had a cophenetic correlation coefficient of 0,645. Two major clusters A and B can be clearly identified. Major cluster A is split into subclusters C and D which comprise OTU's with mean greatest skull measurements of 106,5 mm (observed range 105,5–107,7) and 102,1 mm (observed range 98,1–106,0) respectively. Major cluster B can be subdivided into subclusters E and F, with the latter showing further groupings of OTU's, G and H. Mean cranial length calculated for the constituent OTU's in E, G and H are 97,0 mm (observed range 94,3–100,5), 94,6 mm (observed range 90,7–97,2) and 90,6 (observed range 87,0–95,0) respectively.

Using Petter's (1972) criteria for the identification of *L. saxatilis*, "... ear more than 130 mm, length of cranium more than 92 mm" (the head body criterion also used by this author is considered too variable for use as a taxanomic character), most of the OTU's comprising subclusters C and D would be classified as *L. saxatilis*. However, the five specimens clustering together at the bottom of subcluster D (7, 8, 41, 50, 59) have an ear length less than 130 mm, the stipulated limit for *L. saxatilis*. These OTU's represent the largest individual specimens from localities (Grahamstown, Matatiele, Rouxville, Winburg) whose remaining constituents fall within subclusters E and G.

All the OTU's comprising subcluster E have skull lengths greater than the prescribed limit for *L. saxatilis*. However, approximately half of these have ear length measurements less than 130 mm. Examination of the OTU's clustering within group G showed that

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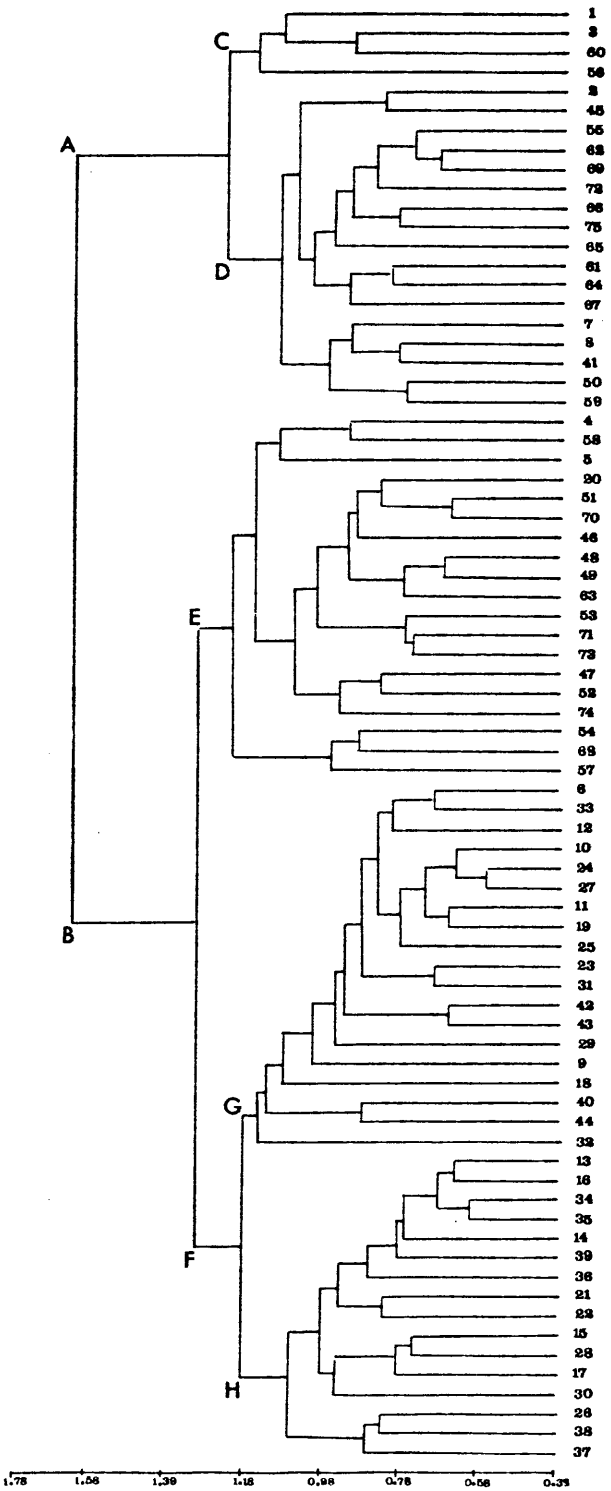

Fig. 15: *Distance phenogram of South African* Lepus *specimens, excluding those OTU's which were classified as* L. capensis, *clustered by the unweighted pair-group method using arithmetic averages. OTU 1 Hutchinson; 2–3 Deelfontein; 4–5 Bloemhof; 6 Boshof; 7–12 Winburg; 13 Babanango; 14–18 Magudu; 19–27 Winterton; 28–33 Hilton; 34–35 Kaalplaats; 36 Lydenburg; 37–39 Derdepoort; 40–44 Matatiele; 45–50 Rouxville; 51–52 Prieska; 53 Uitenhage; 54 Cradock; 55 Murraysburg; 56 Beaufort West; 57–59 Grahamstown; 60 Calvinia; 61–62 Sutherland; 63–67 Nieuwoudtville; 68–69 Clanwilliam; 70 Lemoenshoek; 71 Wolseley; 72 Cape Agulhas; 73 Bredasdorp; 74 Kammanassie; 75 Zoentendalsvlei. Cophenetic correlation coefficient = 0,645.*

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Table 7:

Factor matrix from a 30 variable principal component analysis of South African *Lepus* specimens, excluding those OTU's which were classified as *L. capensis*, showing the character loadings on the first three components.

Measurement	Factor I	Factor II	Factor III
Hind foot length	0,717	– 0,047	0,092
Ear length	0,773	0,339	– 0,081
Total length of skull	0,960	0,119	0,091
Muzzle length	0,669	– 0,026	0,340
Frontal length	0,515	– 0,177	– 0,258
Parietal length	0,498	0,412	– 0,056
Posterior muzzle breadth	0,561	0,102	– 0,214
Anterior frontal breadth	0,418	0,266	– 0,283
Posterior frontal breadth	0,371	0,527	– 0,384
Interauditory breadth	0,820	0,204	0,110
Basal cranial length	0,949	0,045	0,068
Palate incisor length	0,901	0,042	0,282
Palate vacuity length	0,864	0,087	– 0,030
Hard palate length	0,277	– 0,180	0,665
Mesopterygoid space	0,738	0,194	– 0,295
Maxillary premolar length	0,547	– 0,439	– 0,180
Maxillary premolar-molar length	0,673	– 0,297	– 0,218
Principal I^1 breadth	0,361	– 0,591	– 0,276
Palatal breadth between P^2 and P^3	0,781	– 0,290	– 0,062
Bizygomatic breadth	0,826	0,098	– 0,054
Maximum cranial breadth	0,784	0,194	– 0,257
Zygomatic arch length	0,724	– 0,119	0,334
Posterior cranial height	0,742	0,208	0,046
Bulla breadth	0,600	0,299	0,187
Maximum mandibular length	0,916	– 0,031	0,185
Mandibular toothrow length	0,934	– 0,069	0,064
Mandibular height	0,648	– 0,344	0,371
Mandibular body breadth	0,069	– 0,730	0,116
I_1 breadth	0,347	– 0,492	–0,545
Mandibular premolar-molar length	0,653	– 0,433	–0,250

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with one exception in the case of skull length (OTU 19 Magudu, 90,7 mm) and two in ear length (OTU's 40 and 44, Matatiele, 130 mm), all the constituent OTU's had total skull length measurements greater than the limit set for *L. saxatilis*, but ear lengths less than 130 mm.

Group H comprises specimens of which only three OTU's (14 and 15, Magudu; 28, Hilton) have both ear and cranial length measurements that could result in their classification as *L. crawshayi.* However, with the exception of Babanango (n = 2) and Derdepoort (n = 8),all the localities represented in cluster G (Babanango, Derdepoort, Kaalplaats, Lydenburg, Magudu, Winterton) have specimens with skull lengths often exceeding or else marginally less than 92,0 mm, the lower limit for *L. saxatilis.*

Limited additional information resulted from the examination of the correlation phenogram (not presented). It was however, characterised by the fragmentation of those OTU's which had previously been clustered together in group H of the distance phenogram. No clear pattern was discernable in the placement of OTU's in this phenogram (cophenetic correlation coefficent 0,544).

The percentage of phenetic variation represented by the first three principal components for the 75 OTU subset was 47,62% for component one, 9,37% for component two and 6,78% for component three (total = 63,77%). From the factor matrix (Table 7) it can be seen that the first component is heavily influenced by overall size with greatest length of skull, basal cranial length, mandibular length and mandibular toothrow length carrying the heaviest loadings. Examination of the first two dimensional plot (I–II, Fig. 16) reveals a pattern similar to that shown in the distance phenogram.

The larger OTU's with skull and ear length measurements conforming to those stipulated for *L. saxatilis* (Petter, 1972) and which were previously grouped in clusters C and D of the distance phenogram, are clustered in the right half of the first component scale. Conversely, the smallest OTU's (skull and ear lengths almost invariably less than 92 mm and 130 mm respectively; cluster H in the distance phenogram) are grouped on the extreme left of the first component scale. Those specimens with skull and ear length measurements, which on average fall between the two extremes (previously clusters E and G in the distance phenogram), are grouped near the middle of the two dimensional space. In broad geographic terms the segregation of OTU's along the first component, as reflected in Fig. 16 and supported by the distance phenogram, indicates a tendency for the largest specimens to originate from the southwest Cape and adjacent arid Karoo. Extending from these regions in a north-

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Table 8:

Variation in ratios calculated by contrasting cranial measurements which have the highest positive and negative loadings in factor II of the factor matrix. The cluster composition is based on the results of the distance phenogram (p. 46). $\bar{X}$ = Arithmetic mean; SD = Standard deviation; Observed ranges in parentheses.

Ratio	OTU's comprising clusters C + D	OTU's comprising cluster E	OTU's comprising cluster G	OTU's comprising cluster H
Post frontal breadth x 100/ mandibular body breadth	$\bar{X}$=210,2% SD=22,54 (164,7% –240,3%)	$\bar{X}$=229,9% SD=19,44 (190,3% –258,3%)	$\bar{X}$=190,8% SD=18,74% (156,2 % –283,3%)	$\bar{X}$=198,4% SD=17,14 (177,2 % –239,3%)
Post frontal breadth x 100/ Principal I^1. breadth	$\bar{X}$=416,0% SD=43,07 (339,4% –483,3%)	$\bar{X}$= 466.1% SD=39,31 (393,3% –565,4%)	$\bar{X}$=386,9% SD=35,00 (316,7 % –466,7%)	$\bar{X}$= 405,6% SD=30,62 (362,5 % –460,0%)

easterly direction,a trend for smaller overall size is evident, with the smallest specimens being found in the more northeastern parts of South Africa.

Separation along the second axis is more complex and principally affects those OTU's comprising cluster E, which are grouped predominantly in the upper half of this component scale and cluster G which is restricted largely to the lower half of the scale (Fig. 16). Mean ratio values calculated by contrasting posterior frontal breadth (positive) with mandibular body breadth and width of the upper principal incisor (both negative) for OTU clusters C and D, E, G and H are presented in Table 8.

It would appear that on average, the individual specimens falling in the upper portion of the second component scale (cluster E) have proportionately wider posterior frontal breadth measurements in relation to the two contrasted parameters than those grouped in the lower half of this scale (cluster G). However, although considerable overlap in the ratios of individual specimens comprising the various clusters (C – D, E, G and H) is apparent, subtle shape differences are undoubtedly responsible for the segregation of OTU's along the second component, particularly with regard to OTU's comprising clusters E and G. The placement of individual specimens along this axis clearly reflects both intra-population (for example position of OTU's from Clanwilliam 68–69, Grahamstown 57–59, Nieuwoudtville 63–67, Rouxville 45–50, and Winterton 19–27) and interpopulation

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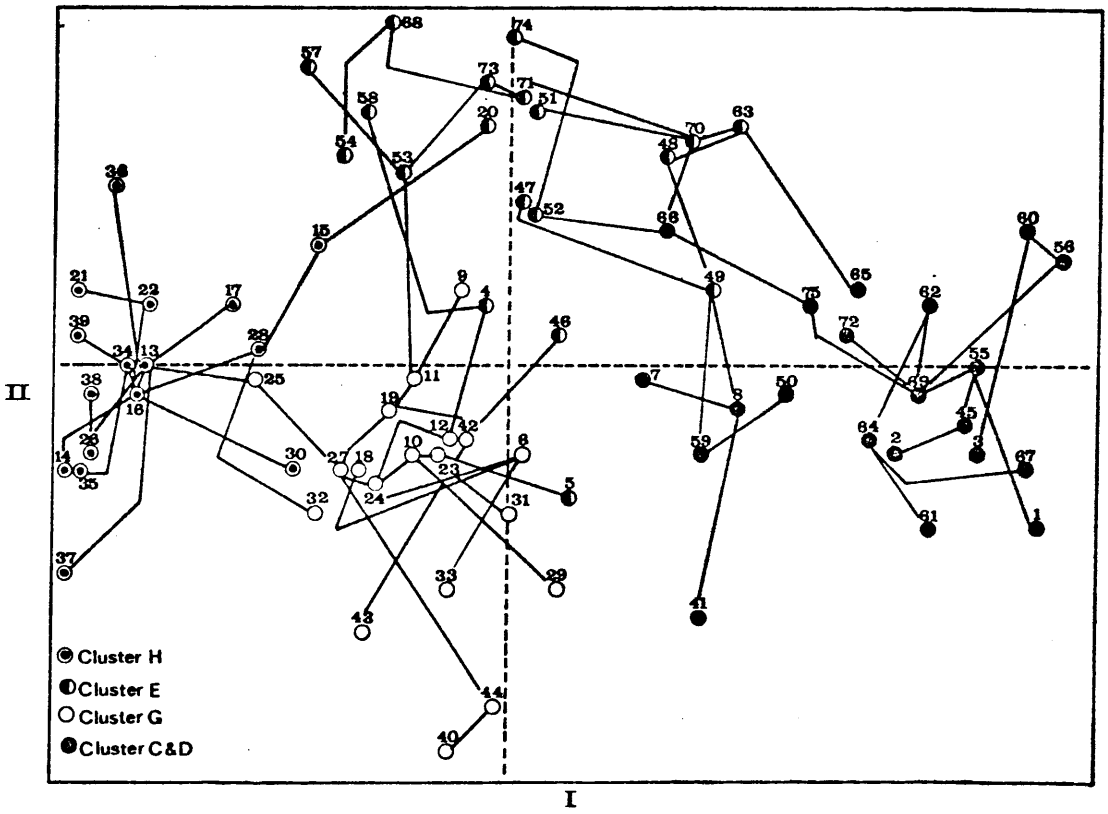

Fig. 16: *Pairwise comparison of components I and II from a principal component analysis of South African* Lepus *specimens excluding those OTU's which were classified as* L. capensis. *See Fig. 15 for localities of OTU's.*

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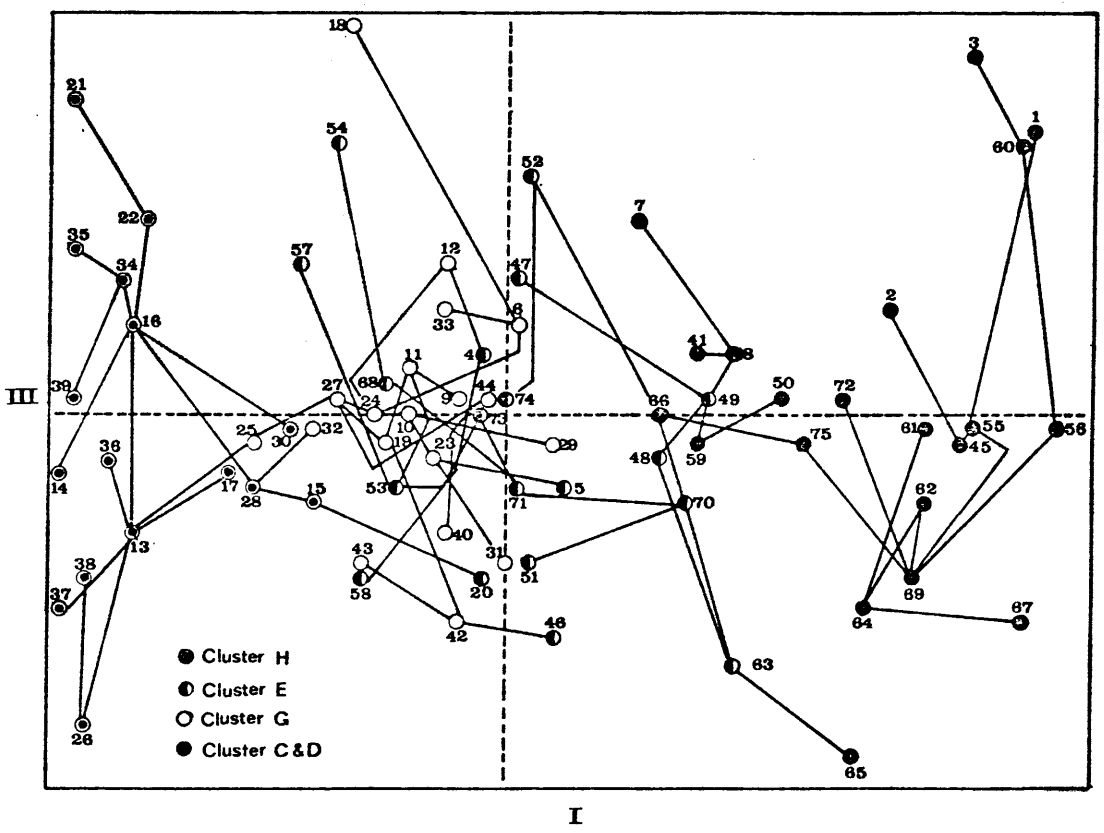

Fig. 17: *Pairwise comparison of components I and III from a principal component analysis of South African* Lepus *specimens excluding those OTU's which were classified as* L. capensis. *See Fig. 15 for localities of OTU's.*

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variation, but not species differences. These observations are further supported by the interconnected nature of the clusters as illustrated by the MST (Fig. 16). Examination of the ordination diagram for components one and three (Fig. 17) reveals no meaningful separation of OTU's along the third axis.

The tendency for OTU's to segregate on size and to assume positions in both the cluster and ordination modes of analysis, which are roughly congruent with their geographic positions along a northeasterly axis, raises the question whether the variation between populations is clinal or marked by definite character discontinuities. This is particularly pertinent with respect to the use of the size criteria proposed by Petter (1972) for the delimitation of *L. crawshayi* from *L. saxatilis*. The absence of marked discontinuities in characters could be interpreted as providing further evidence that the criteria proposed by Petter for the separation of the two species do not hold and that in addition to the well deliniated *L. capensis*, support exists for the recognition of only one species, *L. saxatilis*, in South Africa.

In order to statistically evaluate and illustrate the variation among samples, use was made of the SS–STP analysis of geographic variation. To ensure adequate sample sizes ($\geqslant 4$) for use in this analysis, it was necessary to pool specimens from certain broad geographically contiguous localities. Material from Lydenburg (n = 3), Kammanassie (n = 2), Prieska (n = 1) and Springbok (n = 1), which were considered too distant from other collection sites and were consequently not pooled, had to be excluded from the analysis due to inadequate numbers. Specimens from all other localities throughout South Africa were grouped into 12 areas (Fig. 18).

The single classification analysis of variance revealed statistically significant differences among samples in the two external measurements tested, hind foot length and ear length, as well as in all the cranial characters except hard palate and mandibular body breadth. Results of the SS–STP analysis indicate that the consistent trend in most characters, particularly the length measurements, is for a decrease in size extending from the southern regions of the Cape Province (samples 1, 2 and 3) along a northeasterly axis towards the northern borders of South Africa (samples 9, 10, 11 and 12). Of all the characters tested, only ear length showed evidence of a distinct break in clinal variation along this axis (Table 9). The results indicate that specimens from localities in the central and northern regions of Natal (samples 9–11), as well as the central southern Transvaal (sample 12), have significantly shorter ears than specimens from all other test localities with the exception of Winburg in the Orange Free State (sample 7). The decline in ear length is particularly evident in Natal, with specimens from the southern regions of the

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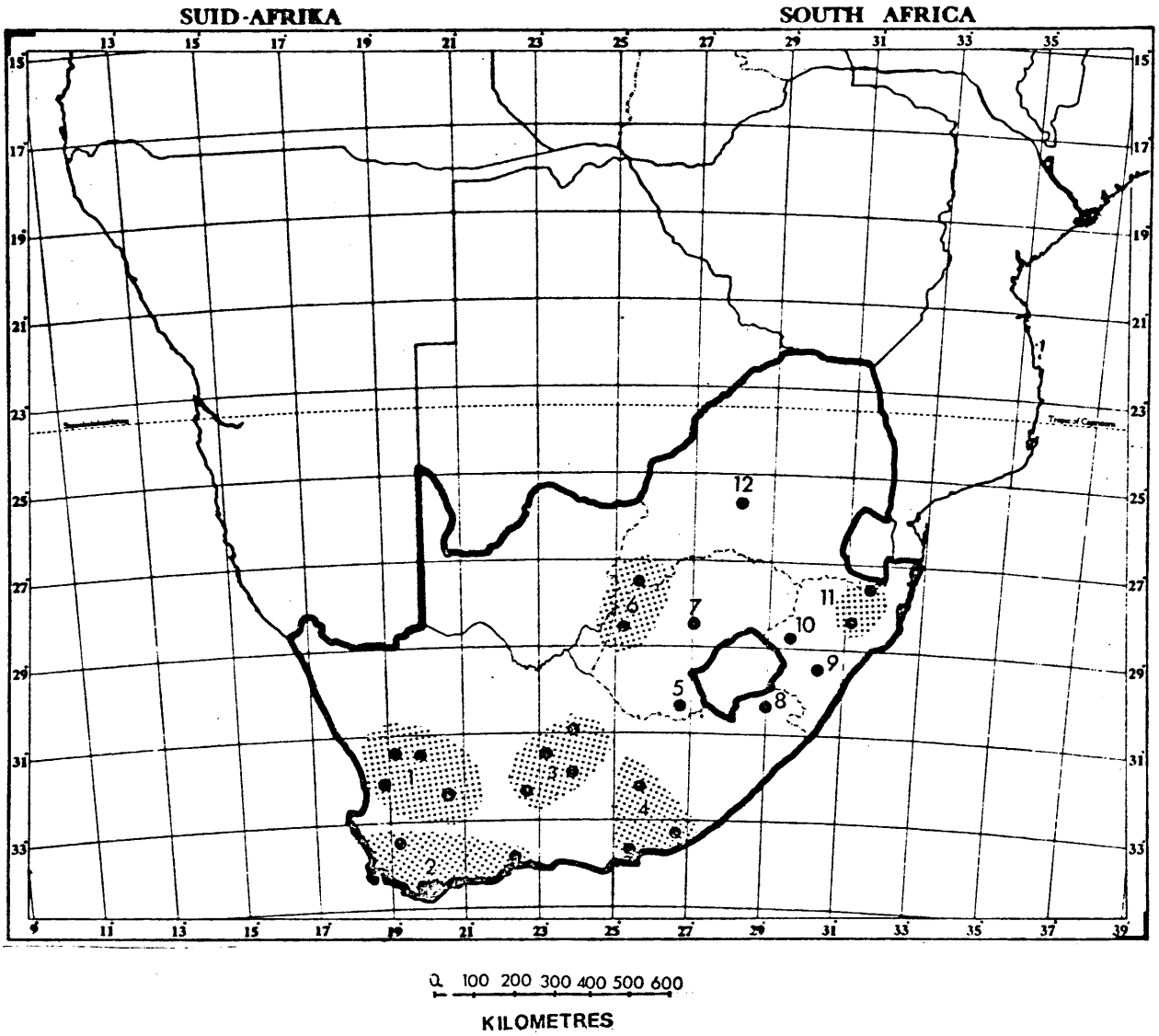

Fig. 18: *Localities of samples included in the analysis of geographic variation of* L. saxatilis: sample 1 = *Calvinia, Clanwilliam, Nieuwoudtville, Sutherland; sample 2 = Bredasdorp, Cape Agulhas, Lemoenshoek, Wolseley, Zoetendalsvlei; sample 3 = Beaufort West, Deelfontein, Hutchinson, Murraysburg; sample 4 = Cradock, Grahamstown, Uitenhage; sample 5 = Rouxville; sample 6 = Bloemhof, Boshof; sample 7 = Winburg; sample 8 = Matatiele; sample 9 = Hilton; sample 10 = Winterton; sample 11 = Babanango and Magudu; sample 12 = Derdepoort and Kaalplaats.*

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Table 9:

Results of the SS-STP analysis of geographic variation in two external and 28 cranial characters in *L. saxatilis*. Nonsignificant subsets are indicated by vertical lines to the right of each array of means. The locality codes correspond to those in Fig. 18.

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
	Hind foot length			**Ear length**	
1	11	145,0	1	11	144,0
3	5	142,4	2	5	144,0
6	5	136,6	3	5	138,0
5	7	135,3	5	7	126,1
7	6	132,5	4	5	124,6
8	7	132,1	6	5	122,0
10	12	130,8	8	7	121,4
9	9	128,0	7	6	118,3
2	5	127,0	10	12	107,5
4	5	124,6	9	9	107,2
12	15	124,0	12	14	103,2
11	10	119,50	11	10	101,3
	Total length of skull			**Muzzle length**	
3	5	105,6	3	5	46,8
1	11	102,3	1	11	44,3
2	5	100,4	5	7	44,0
5	7	98,9	6	5	42,9
4	5	96,9	2	5	42,3
7	6	96,5	7	6	42,1
6	5	95,7	4	5	42,0
8	7	95,7	9	9	41,7
9	9	94,2	8	7	41,7
10	11	92,8	10	11	40,7
11	10	90,7	11	10	40,4
12	12	89,0	12	15	39,4

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Table 9 (continued)

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
	Frontal length			**Parietal length**	
3	5	40,3	1	11	22,0
1	11	40,2	5	7	21,9
8	7	39,9	2	5	21,9
7	6	39,7	4	5	21,8
5	7	38,8	3	5	21,6
10	12	38,3	9	8	20,5
6	5	37,9	10	12	20,3
2	5	37,8	8	9	20,1
4	5	37,8	7	6	19,6
9	9	37,7	6	5	19,4
11	9	37,6	12	13	19,2
12	14	36,1	11	9	19,2
	Posterior muzzle breadth			**Anterior frontal breadth**	
6	5	22,6	6	5	22,0
1	11	22,3	3	5	21,7
3	5	22,1	1	11	21,3
9	9	21,7	5	7	20,9
2	5	21,4	9	9	20,4
7	6	21,6	4	5	20,2
5	7	21,3	7	6	20,2
8	7	20,9	2	5	20,1
10	11	20,7	10	12	19,5
4	5	20,5	11	10	19,1
12	15	20,4	12	15	19,0
11	10	20,1	8	7	19,0

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Table 9 (continued)

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
Posterior frontal breadth			**Interauditory breadth**		
1	11	14,4	3	5	30,9
2	5	13,6	1	11	29,7
4	5	13,5	2	5	29,5
5	7	13,4	7	6	29,5
9	9	13,0	5	7	28,8
6	5	12,8	4	5	28,5
3	5	12,8	10	11	28,1
11	9	12,6	6	5	28,0
10	12	12,4	9	9	27,6
8	7	12,3	8	5	27,6
12	15	12,2	11	7	27,1
7	6	12,2	12	10	26,6
Basal cranial length			**Palate incisor length**		
3	5	85,2	3	5	43,4
1	11	82,4	1	11	40,8
2	5	81,4	2	5	40,7
5	7	78,6	5	7	40,0
7	6	78,0	7	6	39,1
4	5	77,7	6	5	39,0
8	7	77,5	8	7	38,5
6	5	77,4	4	5	38,3
9	9	76,4	9	9	38,1
10	11	74,6	10	11	38,0
11	8	73,2	11	10	37,3
12	11	71,7	12	15	36,0

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Table 9 (continued)

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
	Palate vacuity length			**Hard palate length**	
3	5	27,0	3	5	7,7
1	11	26,9	5	7	7,1
2	5	26,5	7	6	7,0
5	7	25,3	10	12	7,0
6	4	25,1	8	7	7,0
7	6	24,6	4	5	7,0
9	9	24,4	9	9	6,8
8	7	24,3	6	5	6,8
10	11	23,9	12	15	6,7
4	5	23,8	2	5	6,6
11	10	23,4	1	11	6,6
12	15	22,4	11	10	6,6
	Mesopterygoid space			**Maxillary premolar length**	
1	11	10,2	6	5	9,5
3	5	10,0	3	5	9,3
2	5	10,0	5	7	9,3
5	7	9,8	12	15	9,1
4	5	9,7	8	7	9,0
6	5	9,4	2	5	9,0
8	7	9,2	1	11	9,0
7	6	8,8	9	9	8,8
9	9	8,6	10	12	8,7
10	12	8,4	11	10	8,7
12	15	8,3	4	5	8,6
11	10	8,2	7	6	8,6

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Table 9 (continued)

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
Maxillary premolar-molar length			**Principal I^1 breadth**		
3	5	17,3	8	7	3,4
6	5	17,2	3	5	3,3
1	11	17,1	9	9	3,2
5	7	17,0	1	11	3,2
7	6	16,5	7	6	3,1
12	15	16,5	10	11	3,1
8	7	16,5	11	10	3,1
2	5	16,4	2	5	3,0
9	9	16,3	5	7	3,0
4	5	16,2	6	5	3,0
10	12	15,7	12	13	3,0
11	10	15,5	4	5	2,9
Palatal breadth			**Bizygomatic breadth**		
3	5	28,2	3	5	45,8
1	11	28,1	1	10	45,4
2	5	27,2	5	7	45,2
8	7	27,1	2	5	44,5
6	5	27,0	9	9	44,4
9	9	26,8	7	6	44,1
7	6	26,7	4	5	44,1
4	5	26,6	6	5	43,9
5	7	26,5	8	7	43,8
10	12	26,5	10	11	43,0
11	9	25,8	11	8	43,0
12	14	25,7	12	14	42,1

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Table 9 (continued)

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
Maximum cranial breadth			**Zygomatic arch length**		
1	11	33,5	3	5	41,4
5	7	33,3	5	7	39,9
3	5	33,2	7	6	39,9
2	5	33,2	1	10	39,8
8	6	32,0	9	8	39,3
4	5	31,9	6	5	39,3
9	8	31,5	8	7	39,1
6	4	31,5	2	5	39,1
7	6	31,4	4	5	38,8
10	10	31,2	10	11	38,5
11	8	30,6	11	10	38,0
12	11	29,6	12	15	39,6
Posterior cranial height			**Bulla breadth**		
3	5	20,6	3	5	9,7
5	6	20,3	2	5	9,3
1	11	20,1	1	11	9,2
2	5	19,8	4	5	9,0
7	6	19,5	7	6	9,0
6	5	18,8	5	7	8,8
8	7	18,5	6	5	8,7
4	5	18,5	11	7	8,7
10	12	18,1	8	7	8,6
9	9	18,1	12	14	8,4
12	12	17,6	10	11	8,3
11	8	17,5	9	9	8,2

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Table 9 (continued)

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
Maximum mandibular length			**Mandibular toothrow length**		
3	5	74,6	3	5	44,1
1	11	71,6	1	11	42,8
2	5	70,4	2	5	42,4
5	7	69,4	5	7	41,7
7	6	69,2	6	5	40,8
8	7	68,5	7	5	40,7
4	5	68,3	8	7	40,5
6	5	68,0	4	5	39,9
9	9	66,9	9	9	39,9
10	12	66,5	10	12	39,4
11	8	65,0	11	10	38,5
12	14	63,3	12	14	37,7
Maximum mandibular height			**Mandibular body breadth**		
3	5	48,5	8	7	6,7
7	6	46,8	10	12	6,4
8	7	46,5	9	9	6,4
5	7	46,4	6	5	6,4
9	8	46,3	3	5	6,3
6	5	46,2	1	11	6,3
1	11	46,0	4	5	6,2
10	12	45,1	12	14	6,2
11	9	44,9	7	6	6,2
2	5	44,4	11	10	6,2
4	5	43,7	5	7	6,1
12	14	43,3	2	5	6,1

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Table 9 (continued)

Locality code	Sample size	Arithmetic mean	Locality code	Sample size	Arithmetic mean
	I_1 breadth			Mandibular premolar-molar length	
8	7	3,4	3	5	18,2
3	5	3,2	1	11	18,2
1	11	3,2	5	7	18,0
9	9	3,1	6	5	17,9
6	5	3,1	9	9	17,8
5	7	3,1	7	6	17,8
7	6	3,1	2	5	17,7
10	12	3,1	8	7	17,7
11	10	3,0	12	14	17,3
2	5	3,0	10	12	17,3
12	14	3,0	11	10	16,7
4	5	3,0	4	5	16,7

province (sample 8) being characterised by an average ear length measurement of 121 mm, which in central Natal has decreased to approximately 107 mm (samples 9–10).

In contrast to the phenetic discontinuity reflected in ear length, total length of the skull shows an even decline from the southern regions of the Cape Province northeastwards (Table 9). OTU's from sample areas 10, 11 and 12 which are the smallest in this measurement are all north of the discontinuity shown by ear length and differ significantly only from specimens in the south-central and south-western Cape Province (samples 1, 2 and 3), which on average have the longest craniums for the species (100,4 – 105,6 mm). Samples 4 – 10, of which localities 9 and 10 were also north of the discontinuity shown in ear length, are on average intermediate for the taxon (92,8 – 98,9 mm) and fall within the same nonsignificant subset; these areas correspond in very broad geographic terms to the more central regions of South Africa.

Of interest is the tendency for specimens from the south-central Cape Province (sample 3) to average larger in most cranial length measurements than specimens from the more

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southwestern sample areas (1, 2). However, since the differences among character means are small and all three geographic areas are generally grouped together in the same non-significant subset, this may imply some sampling artifact arising from small numbers.

The pattern of geographic variation in most other cranial length characters, particularly muzzle length, basal cranial length, palate incisor length, palate vacuity length, mandibular length and mandibular toothrow length, was similar to that illustrated by greatest skull length.

There seemed to be little geographic variation in frontal length as is illustrated in Table 9. Furthermore, two toothrow measurements, maxillary premolar and maxillary premolar-molar length, as well as zygomatic arch length, parietal length, mandibular premolar-molar length and the remaining external measurement (hind foot length), tended to vary irregularly among samples.

A similar gross trend to that illustrated by greatest skull length and most other length measurements, was found for interauditory breadth, bulla breadth, palatal breadth, bizygomatic breadth, cranial breadth and mesopterygoid space. As was evident in several length measurements, the specific ranking of samples (particularly 4–9) varied slightly. However, the differences in mean values were slight and considerable overlap was evident between subsets.

The clear clinal variation in one of the breadth measurements, width of mesopterygoid space (Table 9), is of particular interest. This criterion expressed as a percentage of the hard palate length is regarded by Petter (1972) as the most important character for the recognition of the northern extralimital *L. whytei*, which is reported to be very narrow in this measurement. Should the same tendencies illustrated in this study for these parameters extend northwards (no significant variation in hard palate length; clinal variation in mesopterygoid space), the validity of *L. whytei* would be in serious doubt. In fact, the choice of this ratio in Petter's (1972) diagnostic key is surprising in view of earlier observations in which he considers this criterion too variable to be of taxonomic significance (Petter, 1959).

Little, if any, indication of geographic variation resulted from the SS–STP analysis of posterior frontal breadth and lower incisor breadth. Furthermore, posterior muzzle breadth, anterior frontal breadth, and principal upper incisor breadth did not follow the predominant trend, varying irregularly among samples. Of the two height measure-

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ments used, maximum mandibular height varied irregularly, while the pattern of geographic variation displayed by posterior cranial height (Table 9) was essentially the same as has been described for most cranial length measurements.

The most noticeable trend to emerge from these results is for a steady decrease in size from the southwestern and south-central Cape Province northeastwards. However, ear length which shows a discontinuity in clinal variation deserves special mention. The discontinuity in this one character coincides largely with the reported distribution of *L. crawshayi* in South Africa (northern Natal and Transvaal; Petter, 1972). The relatively sharp morphological transition in this character, which could in part be due to sampling artifact, or paralleled by some not readily discernable environmental transition, is not considered sufficient justification in view of the bulk of evidence to support the assigning of the more northern forms in South Africa to a separate species.

Taxonomic conclusions:

Multivariate analyses using the NT–SYS procedure clearly illustrate the phenetic distinctness of *L. capensis*, thereby supporting the recognition of this taxon as a separate species.

The result of similar multivariate studies conducted on a residual subset comprising *Lepus* specimens other than *L. capensis*, which originate from localities throughout South Africa, failed to provide evidence of more than one species in the test material, the prior name for which is *L. saxatilis* F. Cuvier, 1823. This conclusion was further supported by the univariate SS–STP analysis of geographic variation. The bulk of evidence clearly illustrates that the major trend is for a decrease in size along a SW–NE axis. This seriously compromises Petter's (1972) recognition of the smaller more northern forms in South Africa as a separate species distinct from *L. saxatilis*.

No conclusions regarding the synonomy of *L. saxatilis* and *L. crawshayi* can be reached at this stage since material from the major portion of the latter's range (Botswana, northern South West Africa and northwards to Kenya, the type locality, and southern Sudan; see Petter 1972) has not been included in this study. However, should the clinal variation illustrated in *L. saxatilis* extend northwards, the validity of *L. crawshayi* as a separate species would have to be critically reassessed.

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CHAPTER 3

CHROMOSOME ANALYSES

INTRODUCTION

Studies of chromosome morphology and number, and more recently of differentially stained chromosomes using banding techniques, are being increasingly employed in mammalian systematic and phylogenetic investigations. The literature pertaining to these aspects is prodigious and does not warrant extensive review here. Some relevant studies, including many of the references contained therein, are those of Árnason (1974a, 1974b, 1977) on pinnipeds and cetaceans, Buckland and Evans (1978) on bovids, Wurster-Hill and Gray (1973) and Wurster-Hill and Bush (1980) on carnivores, Dutrillaux, Couturier and Chauvier (1980b) on primates and numerous workers on bats and rodents (for example Mascarello and Hsu, 1974; Greenbaum, Baker and Bowers, 1978; Baker and Bass, 1979; Haiduk, Robbins, Robbins and Schlitter, 1980; Elder, 1980).

Karyotype studies within the family Leporidae have been limited and at present published information is restricted to five of the ten recognised genera (Table 10). Data from the remaining taxa are still outstanding. Although the diploid number of species examined range from 2n = 38 for *S. aquaticus*, to 2n = 48 for *Lepus, S. bachmani* and *Romerolagus diazi* (Table 10), early banding studies on *O. cuniculus, S. bachmani* and *L. americanus* (Stock, 1976) gave clear indication that the banding patterns of major chromosomes or chromosome arms tend to be strongly conserved in the Leporidae. These data illustrated the usefulness of chromosome investigations for assessing phylogenetic and taxonomic relationships within the family.

In the present study the inclusion of detailed cytogenetic analyses was prompted by several considerations. It was hoped that the inclusion of this parameter would facilitate the delimitation of the various taxa and thereby provide additional information for use in the classification of the South African Leporidae. Furthermore, in so far as the data will allow, I have attempted to identify the mechanisms of chromosomal evolution within these taxa and have speculated on some of the factors which may have influenced chromosomal change within the group.

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MATERIAL AND METHODS

All specimens karyotyped for this investigation were prepared as museum study skins and skulls and deposited in established mammal collections. The only exceptions were the *Oryctolagus* (n = 2) and *L. timidus* (n = 6) material, which was obtained respectively from a commercial breeder, and the Department of Zoology, University of Aberdeen, Scotland.

Specimens of the South African taxa have been incorporated into the Transvaal Museum mammal collection, while those of the extralimital species that were used for comparative cytogenetic purposes are available from the Appalachian Environmental Laboratory, University of Maryland, Frostburg, Maryland, USA (*S. aquaticus, S. floridanus, S. transitionalis*) and the Instituto de Biología, National University of Mexico, Mexico (*R. diazi*). Since accession numbers are not yet available for most of the material, these data could not be included, but will eventually be available on request.

Collection of biopsies:

Tissue cultures were established primarily from skin biopsies (ear clippings) taken in the field. Material to be collected was vigorously cleaned with 70% alcohol in an attempt to reduce contamination. Clippings were usually placed briefly (2 – 3 h) in tissue flasks containing MEM with Hank's salts (Paul, 1975), 5% bovine serum and high concentrations of antibiotics (penicillin 0,24 mg/ml medium; streptomycin 0,4 mg/ml medium; Novo Industries, Johannesburg, South Africa). Following this pretreatment, the biopsies were transferred to a storage medium similar to that indicated above, but with standard antibiotic levels (penicillin 0,12 mg/ml medium; streptomycin 0,2 mg/ml medium) pending culture in the laboratory. Biopsy specimens of the South African *Lepus* species which were kept refrigerated in storage medium were usually viable three months *post mortem*, while those of *Pronolagus* were more susceptible to storage.

Initiation of primary cultures:

Prior to initiation in culture the biopsies were again carefully cleaned with 70% alcohol, this time under sterile conditions, minced, then placed in 30 cm^2 Lux tissue flasks (Lux Scientific Corp. Newbury Park, California, USA).

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Table 10:

Summary of known karyotypic data for the family Leporidae. Data on species composition of *Lepus* largely after Flux (*in litt.*)*[1], *Sylvilagus* (Chapman, *in litt.*)*[2] and *Pronolagus* and *Bunolagus* (present study). Other taxonomic data principally from Corbet and Hill (1980). In the case of the *Oryctolagus* karyotype which has been extensively described, only the most recent authorative reference has been presented.

Genus and Species	2n	Source
Pentalagus furnessi	–	
Pronolagus crassicaudatus	42	Robinson (in press); present study.
Pronolagus randensis	42	Present study.
Pronolagus rupestris	42	Robinson (1980); present study.
Romerolagus diazi	48	Uribe-Alcocer *et al.* (1975); Hsu and Benirschke (1975); Van der Loo *et al.* (1979); Robinson *et al.* (1981).
Caprolagus hispidus	–	
Lepus alleni	48	Hsu and Benirschke (1967);
Lepus americanus	48	Hsu and Benirschke (1970); Stock (1976).
Lepus brachyurus	–	
Lepus californicus	48	Worthington and Sutton (1966); Hsu and Benirschke (1967); Schröder *et al.* 1978 b).

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Table 10 (continued)

Genus and species	2n	Source
Lepus callotis	–	
Lepus capensis	48	Robinson (in press).
Lepus castroviejoi	–	
Lepus crawshayi	–	Robinson *et al.* (1981) described the karyotype of specimens from northeastern South African which, using Petter's (1972) key, were referable to *L. crawshayi.* However name not valid for South African species (present study).
Lepus europaeus	48	Gustavsson (1971); Schröder *et al.* (1978 a); Schröder *et al.* (1978 b).
Lepus flavigularis	–	
Lepus habessinicus	–	
Lepus insularis	–	
Lepus mandshuricus	–	
Lepus nigricollis	48	Sharma *et al.* (1975).
Lepus oiostolus	–	
Lepus peguensis	–	
Lepus ruficaudatus	–	
Lepus saxatilis	48	Robinson (1980), Robinson *et al.* (1981) described as *L. crashayi;* Robinson (in press); present study.
Lepus sinensis	48	Tateishi (1936) described as *L. formosus* (in Dave *et al.*, 1965).

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Table 10 (continued)

Genus and species	2n	Source
Lepus timidus	48	Makino (1944, 1952) described as *L. gichiganus ainu;* Dave *et al.* (1965); Gustavsson (1971); Rausch and Ritter (1974) described as *L. othus;* Schröder *et al.* (1978 a).
Lepus townsendii	48	Jalal *et al.* (1967); Hsu and Benirschke (1971).
Lepus whytei	–	
Lepus yarkandensis	–	
Poelagus marjorita	–	
Sylvilagus aquaticus	38	Unpublished data; previously given as 2n = 42 by Earley and Mouton (1973).
Sylvilagus audubonii	42	Worthington and Sutton (1966).
Sylvilagus bachmani	48	Worthington (1970). Stock (1976).
Sylvilagus brasiliensis	–	
Sylvilagus cunicularius	–	
Sylvilagus floridanus	42	Palmer and Armstrong (1967); Holden (1968); Holden and Eabry (1970); Hsu and Benirschke (1971); Unpublished data.
Sylvilagus graysoni	42	Diersing and Wilson (1980).
Sylvilagus idahoensis	44	Schröder and Van der Loo (1979).
Sylvilagus insonus	–	
Sylvilagus mansuetus	–	
Sylvilagus nuttallii	42	Worthington and Sutton (1966); Schröder and Van der Loo (1979).

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Table 10 (continued)

Genus and species	2n	Source
Sylvilagus palustris	–	
Sylvilagus transitionalis	46	Unpublished data; previously reported as 2n = 52, Holden and Eabry (1970).
Bunolagus monticularis	44	Present study.
Oryctolagus cuniculus	44	Hageltorn and Gustavsson (1979).
Nesolagus netscheri	–	

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NOTE: The status of many species, particularly in *Lepus*, are uncertain and this list cannot be considered definitive.

Culture medium (MEM containing Hanks's salts, 15% foetal or bovine serum, standard antibiotics including Fungizone = 0,005 mg/ml; Squibb Lab. Isando, South Africa), sufficient to cover the tissue pieces, was added and the sealed containers incubated at 37°C. Within 3–4 days to as long one week, many of the fragments put out fibroblast cells which, after they become numerous and form large sheets around the fragments, can be removed with trypsin and subcultured using standard techniques. (Schwarzacher and Wolf, 1974; Paul, 1975). These cells subsequently yield good fibroblast cell lines which can be repeatedly subcultured and harvested, thereby providing a reliable source of good quality mitotic preparations for cytogenetic analyses.

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Mitotic arrest, hypotonic treatment and fixation of cells:

In order to obtain a high mitotic index, cultures must be in a logarithmic growth phase which is usually attained some 24–48 h following each subculture. Colchicine (BDH Chemicals Ltd. Poole, England) a mitotic inhibitor, was added to each flask 4 h prior to harvest (0,4 µg/ml medium). The harvested cells were subsequently treated with a 0,075 M KCl hypotonic solution for 12 min. and fixed using a freshly prepared 1:3 acetic – methanol solution. The precise protocol followed for the harvesting and fixation of cells in this study are similar to those of Schwarzacher and Wolf (1974).

Slide preparation:

Air dried slide preparations were made by releasing 3–4 drops of the acetic-methanol cell suspension onto cold, wet slides. Although excess water must be shaken off the slide prior to the liberation of the cell suspension, care must be taken to ensure that the slide surface is uniformly covered by a thin film of water. The optimum height for dropping the suspension must be tested; for most leporid preparations this distance is about 60 cm. The slides were air dried in a vertical position at ambient temperature, boxed and immediately incubated at 37°C for 24 h. The incubation period appears to "harden" the chromosomes making them more resistant to the trypsin treatment, which appears necessary for good resolution of the G-bands.

Banding techniques:

G- and C-band preparations were made following the methods of Wang and Fedoroff (1972) and Sumner (1972) respectively, following which the slides were stained in a 5% phosphate buffered giemsa solution (T. Gurr Ltd. Essex, England). The phosphate buffer (Sörensen's buffer) is made by combining stock solutions of 0,067 M K_2HPO_4 and 0,067 M Na_2HPO_4 (Schwarzacher and Wolf, 1974).

Using slides prepared as described above, optimum G-banding patterns were obtained on preparations less than one week old. Slides older than 7 days were characterised by poor resolution of the band patterns or their total absence. The duration of the enzyme treatment varied from 20–45 sec., but did not appear to be species specific.

In the case of the C-banding procedure, the age of the preparation appeared to be of little consequence and good C-bands were obtained on slides in excess of 6 months. Treat-

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ment in $Ba(OH)_2$ varied from 30–120 sec., but did not appear to be species specific.

Photomicrography:

All G- and C-banded chromosomes as well as unbanded preparations used in this study were photographed using a Zeiss photomicroscope and Ilford Pan F film. In order to achieve greater resolution and contrast, photomicrographs were taken with a 25 ASA setting and developed in Ilford Perceptol developer. In order to assess the degree of possible intraspecific variation within the South African Leporidae, an attempt was made wherever possible, to obtain good quality G- and C-banded preparations of at least one male and female of each representative taxon per locality.

In determining G-band homologies between species, a minimum of 10 karyotypes of each species were compared with at least 10 karyotypes of the other species, the only exceptions being *R. diazi* (n = 6) and *P. crassicaudatus* (n = 8).

Measurements:

The absence of interspecific variation within the genus *Lepus* and *Pronolagus*, with regard to gross chromosome morphology and G-banding patterns, simplified the presentation of karyotypes for these taxa. This lack of variation made it possible to extend the format for the ranking and presentation of chromosomes, which was obtained from the standardised karyotypes of *L. saxatilis* and *P. rupestris*, to the remaining constituent species of their respective genera. The karyotypes were standardised and the chromosomes numbered based on the chromosome lengths expressed as a percentage of a haploid female karyotype.

All measurements were taken directly from photographs using dial calipers. To ensure the correct identification of individual chromosomes only G-banded preparations were used. Similar methodology was followed for standardising the *Bunolagus* karyotype.

The choice of the two species *L. saxatilis* and *P. rupestris* for use in the determination of the chromosomal format within the respective genera was prompted by their larger sample sizes and wider geographic representations. Other studies employing similar methods for determining the ranking sequence of chromosomes, are those of Zimmerman and Sihvonen (1973), Elder (1978) and Lee and Martin (1980).

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CHROMOSOME ANALYSES OF THE SPECIES

A. *LEPUS:*

L. saxatilis (2n = 48)

The G-banded chromosomes of *L. saxatilis* are shown in Fig. 19A. The chromosomes have been numbered and ranked according to their relative lengths expressed as a percentage of a haploid female karyotype (Table 11). The male chromosomal complement comprises eight pairs of metacentric or submetacentric autosomes (pairs 1–8) and 15 pairs of subtelocentric and acrocentric autosomes (pairs 9–23). The X is a medium sized submetacentric and the Y a small telocentric chromosome.

As is evident from Fig. 19A, there is a gradual decrease in the size of the chromosomes constituting each group. The G-band patterns of individual chromosome pairs are distinctive; however, high resolution preparations are necessary to distinguish between many of the pairs for example 4 and 5, 9 and 10, 14 and 15 and 19 and 20. The centromeric regions of most chromosomes were invariably found to be G-band negative.

Table 11:

Relative chromosome lengths of *L. saxatilis* expressed as a percentage of a haploid female karyotype (n = 14). $\bar{X}$ = Arithmetic mean; tSE = 95% Confidence limit.

Chromosome number	$\bar{X}$ length	tSE	Chromosome number	$\bar{X}$ length	tSE
1	6,4	0,17	14	4,3	0,19
2	5,8	0,17	15	4,2	0,09
3	4,8	0,13	16	4,1	0,11
4	4,7	0,17	17	3,9	0,13
5	4,4	0,17	18	3,7	0,13
6	3,2	0,11	19	3,4	0,11
7	2,6	0,09	20	3,0	0,13
8	2,4	0,06	21	2,8	0,13
9	6,2	0,11	22	2,2	0,11
10	6,1	0,15	23	1,9	0,11
11	5,9	0,19	X	4,8	0,26
12	4,8	0,17	Y	1,8	0,11
13	4,6	0,13			

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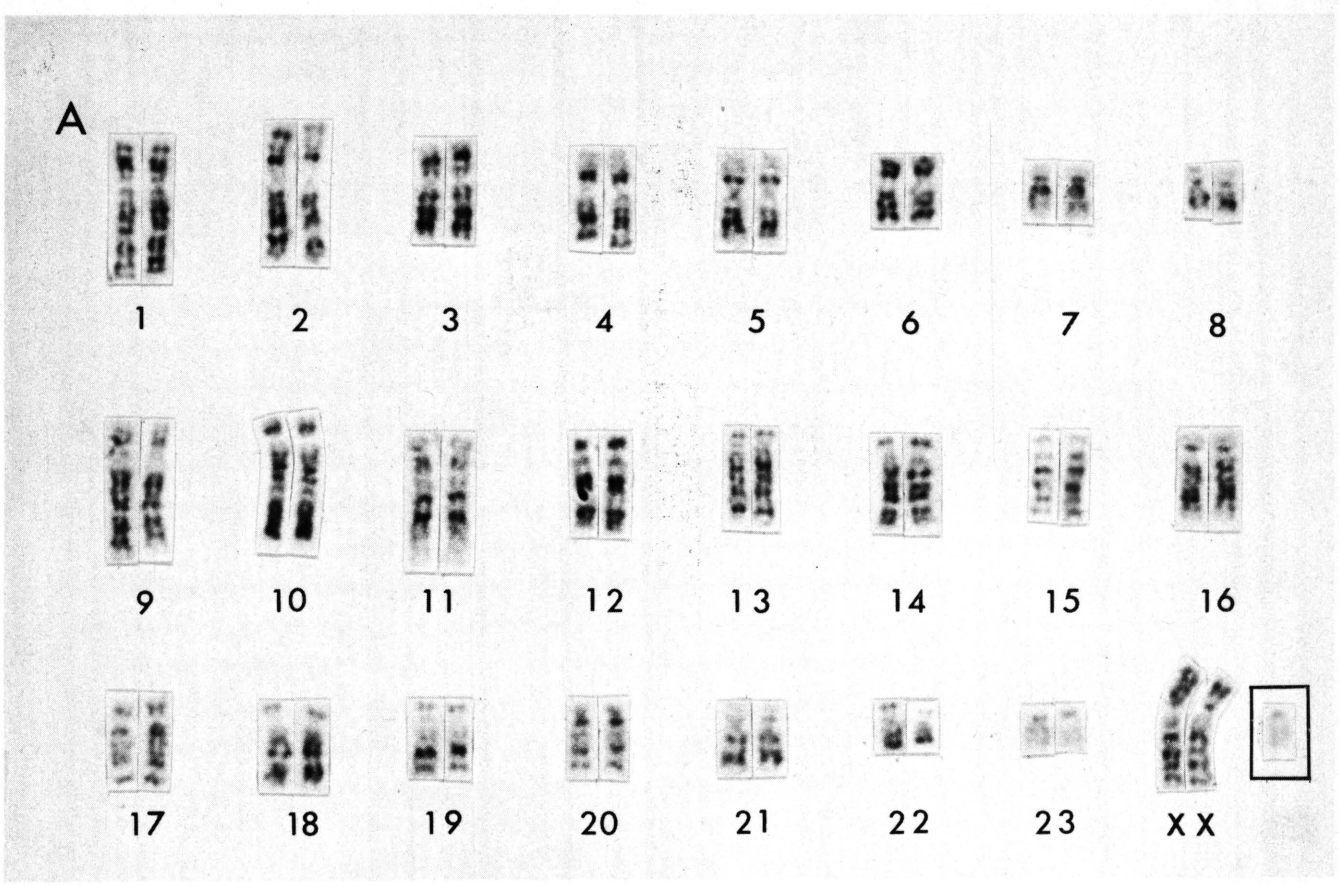

Fig. 19A: *G-banded karyotype of a female* L. saxatilis *(2n = 48). The inset shows the Y chromosome of this species*

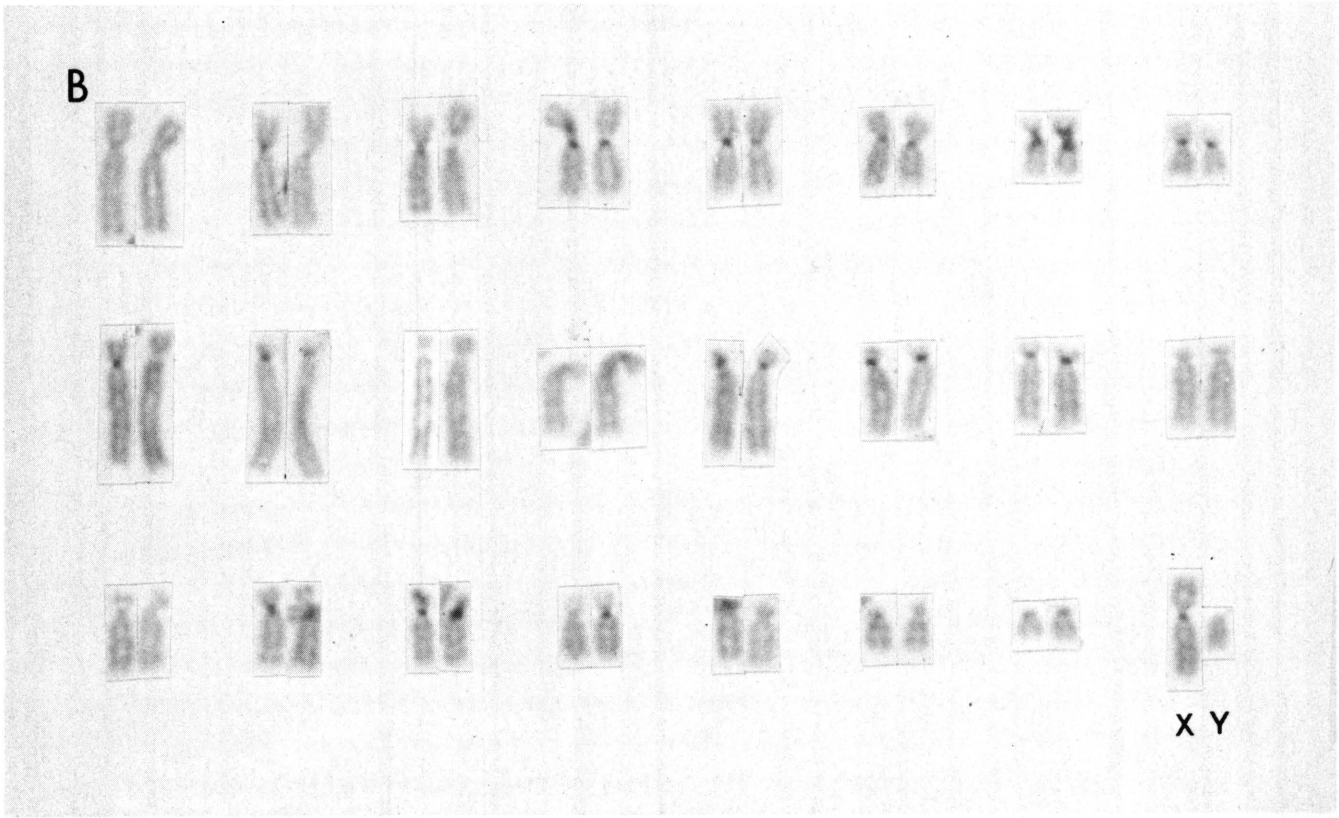

Fig. 19B: *C-banded karyotype of a male* L. saxatilis (2n = 48).

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The C-banded karyotype of *L. saxatilis* is shown in Fig. 19B. The gradation in the length of the chromosomes precludes the identification of each of the chromosomes by the C-banding pattern alone. Only the very best preparations reveal the small amounts of pericentric heterochromatin present in the chromosomes of this species. There was no indication of telomeric or interstitial banding. The C-banding patterns of the X chromosome are similar to those of the submetacentric autosomes of comparable size, while the Y appeared to be largely heterochromatic in most preparations studied although not as intensely staining as the pericentric autosomal heterochromatin.

Despite the large number of specimens examined (n = 73), and their varying geographic origins, no evidence was found of any intraspecific G- or meaningful C-band variation.

L. capensis (2 n = 48)

Since the G- and C-banding patterns as well as the gross morphology of the *L. capensis* chromosomes closely resemble those of the previous species, no attempt has been made to describe this species karyotype in great detail.

The G- and C-banded karyotypes are presented in Figs. 20A and B respectively. The male karyotype compromises eight pairs of metacentric or submetacentric autosomes (pairs 1–8) and 15 pairs of subtelocentric and acrocentric autosomes (pairs 9–23). As in the previous species, the X is a medium sized submetacentric and the Y a small telocentric chromosome. The C-banding patterns were similar to those of the previous species.

Interspecific comparisons:

The G-banded chromosomes of *L. saxatilis* are compared with those of *L. capensis* in Fig. 21. The chromosomes of the two species are indistinguishable both on morphology and their G-banding patterns. No observable differences were apparent in the amount and distribution of constitutive heterochromatin between taxa.

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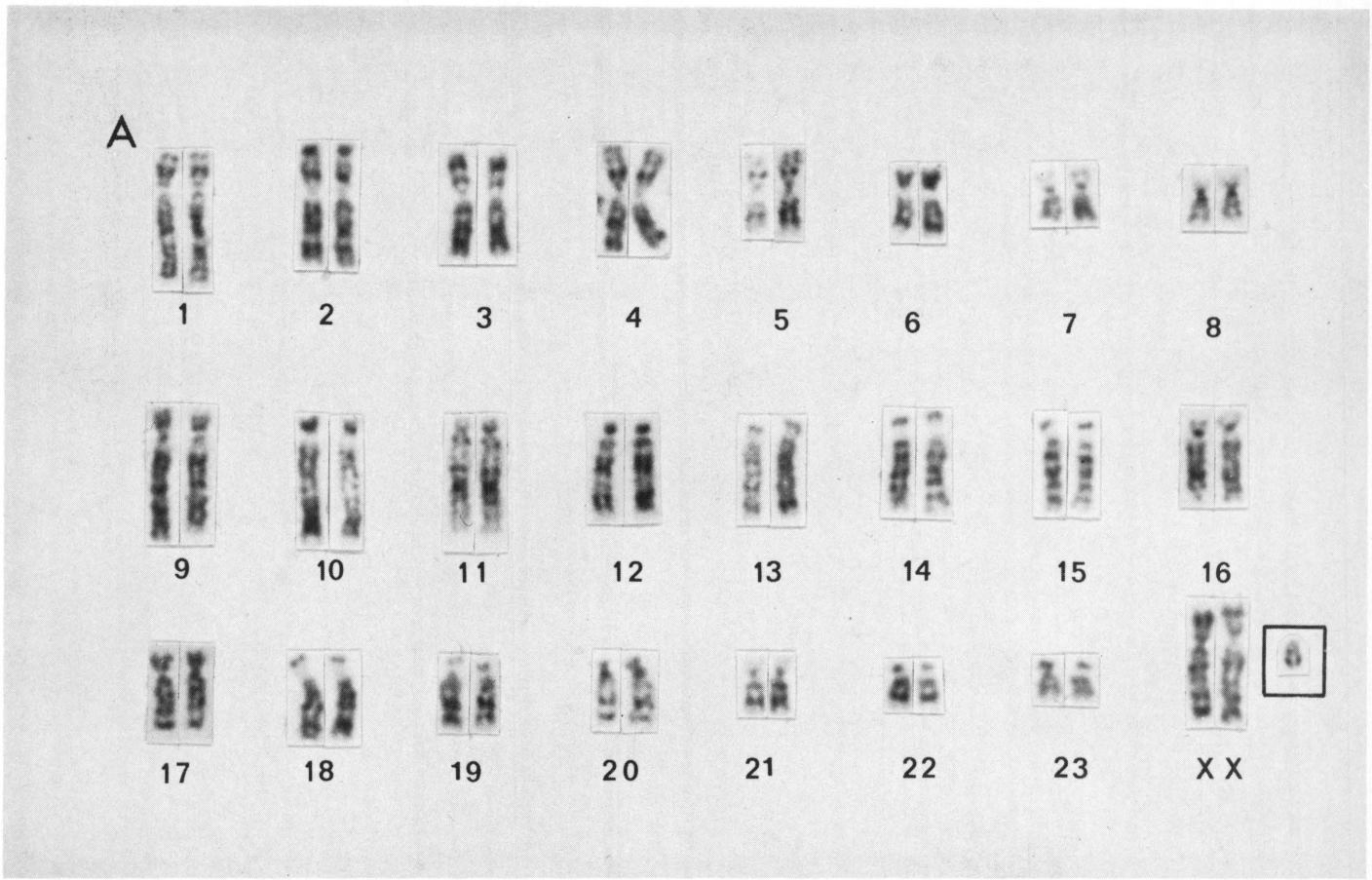

Fig. 20A: *G-banded karyotype of a female* L. capensis *(2n = 48). The inset shows the Y chromosome of this species.*

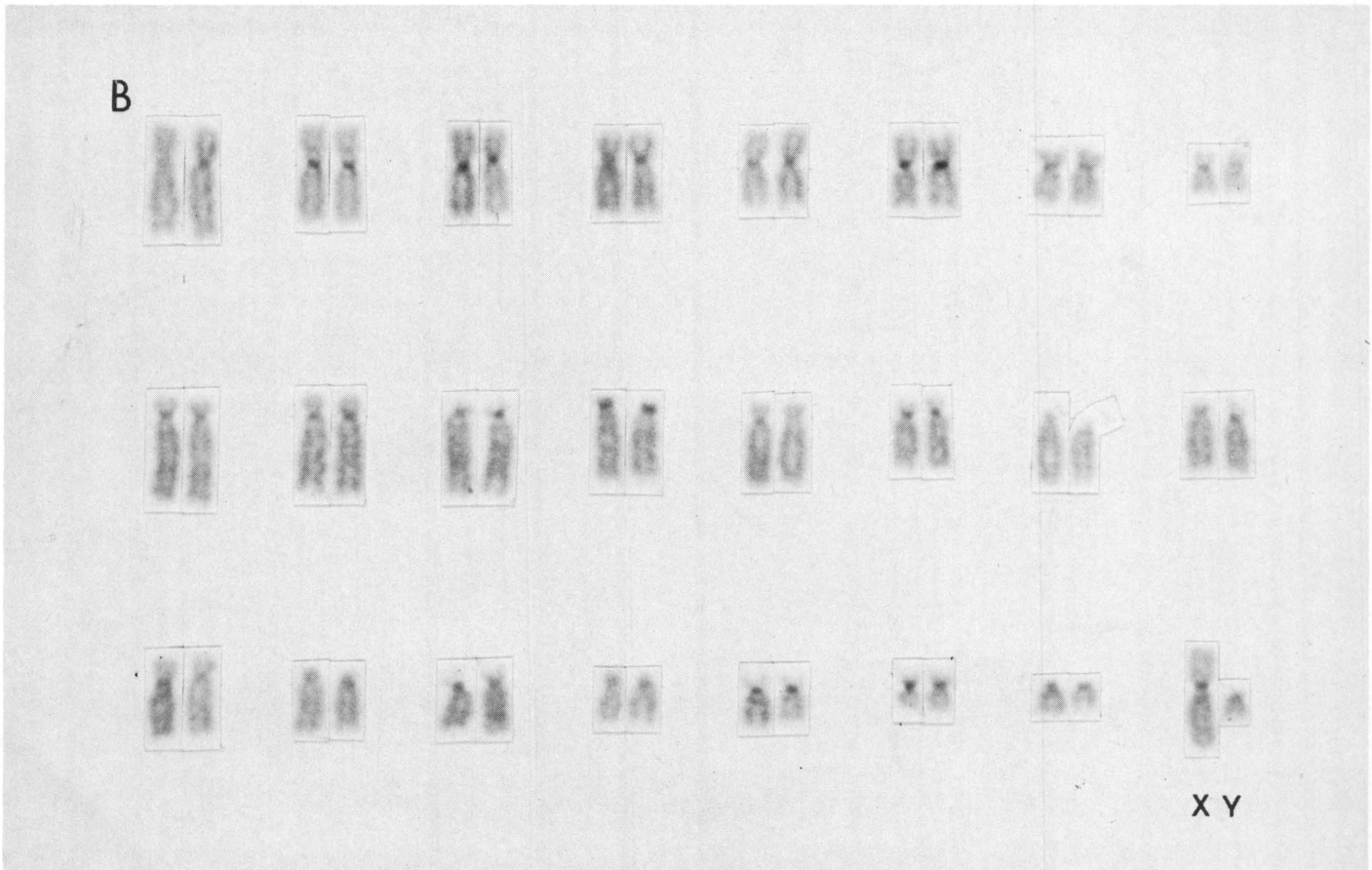

Fig. 20B: *G-banded karyotype of a male* L. capensis *(2n = 48).*

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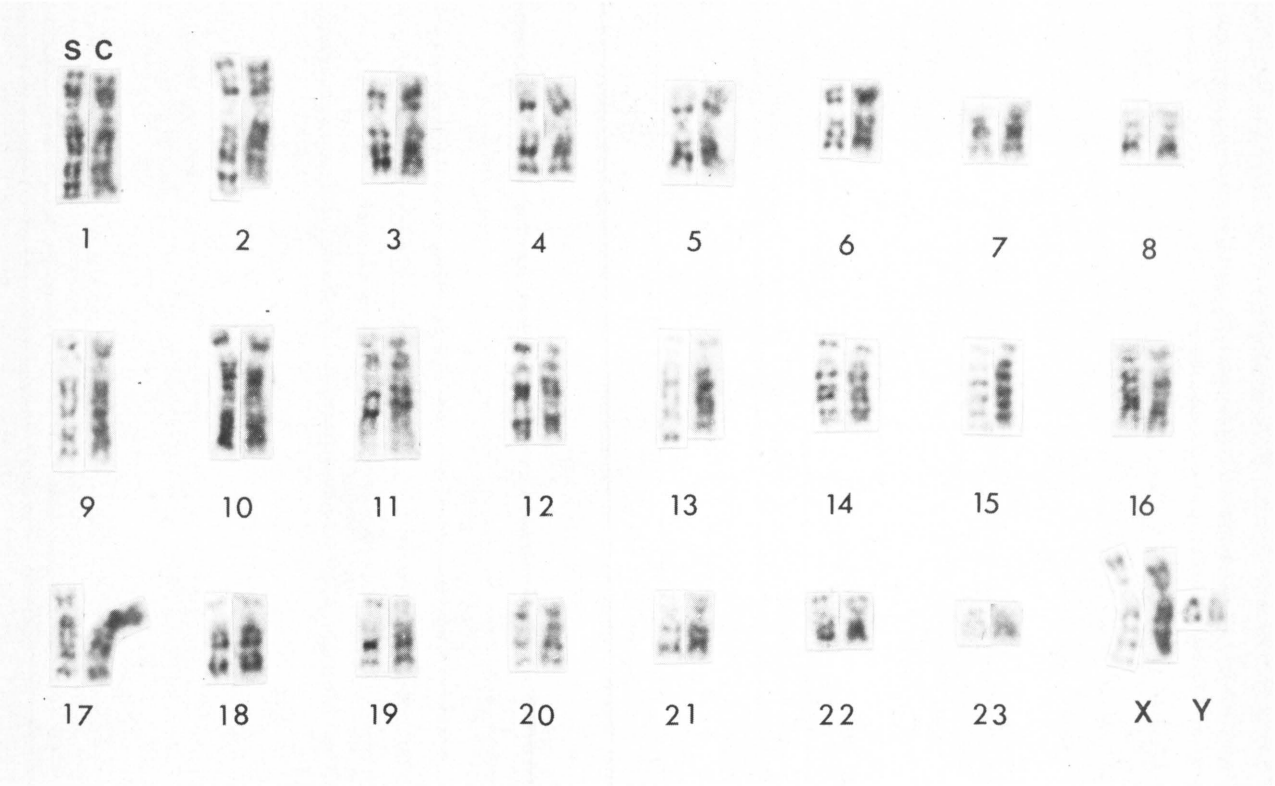

Fig. 21: *Comparison of the G-banded chromosomes of* L. saxatilis *(s) and* L. capensis *(c). The first chromosome in each pair is that of* L. saxatilis.

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B. *PRONOLAGUS:*

P. rupestris (2n = 42)

The G-banded chromosomes of *P. rupestris* are illustrated in Fig. 22A. The chromosomes have been numbered and ranked according to their relative lengths expressed as a percentage of a haploid female karyotype (Table 12). The male chromosomal complement consists of 11 pairs of metacentric or submetacentric autosomes (pairs 1–11) and nine pairs of subtelocentric and acrocentric chromosomes (pairs 12–20). The X is a medium sized submetacentric the Y a small telocentric.

Table 12:

Relative chromosome lengths of *P. rupestris* expressed as a percentage of a haploid female karyotype (n = 10). $\bar{X}$=Arithmetic mean; tSE = 95% Confidence limit.

Chromosome number	$\bar{X}$ length	tSE	Chromosome number	$\bar{X}$ length	tSE
1	9,3	0,45	12	6,4	0,16
2	7,2	0,18	13	6,3	0,32
3	6,4	0,32	14	5,3	0,11
4	6,0	0,14	15	4,6	0,14
5	5,2	0,27	16	4,5	0,32
6	4,8	0,16	17	4,1	0,23
7	4,5	0,23	18	3,5	0,34
8	4,2	0,36	19	2,6	0,45
9	3,2	0,25	20	2,1	0,14
10	2,7	0,23	X	5,1	0,16
11	2,2	0,09	Y	1,8	0,27

As in *Lepus*, the autosomes show a gradation in length without evidence of an abrupt size differential. The G-banding patterns allowed unequivocal identification of all the chromosomes, although pairs 19 and 20 which tended to be extremely sensitive to trypsin treatment, were usually largely G-band negative, making precise identification of homologues difficult. Furthermore, several of the autosomal pairs are very similar in banding pattern

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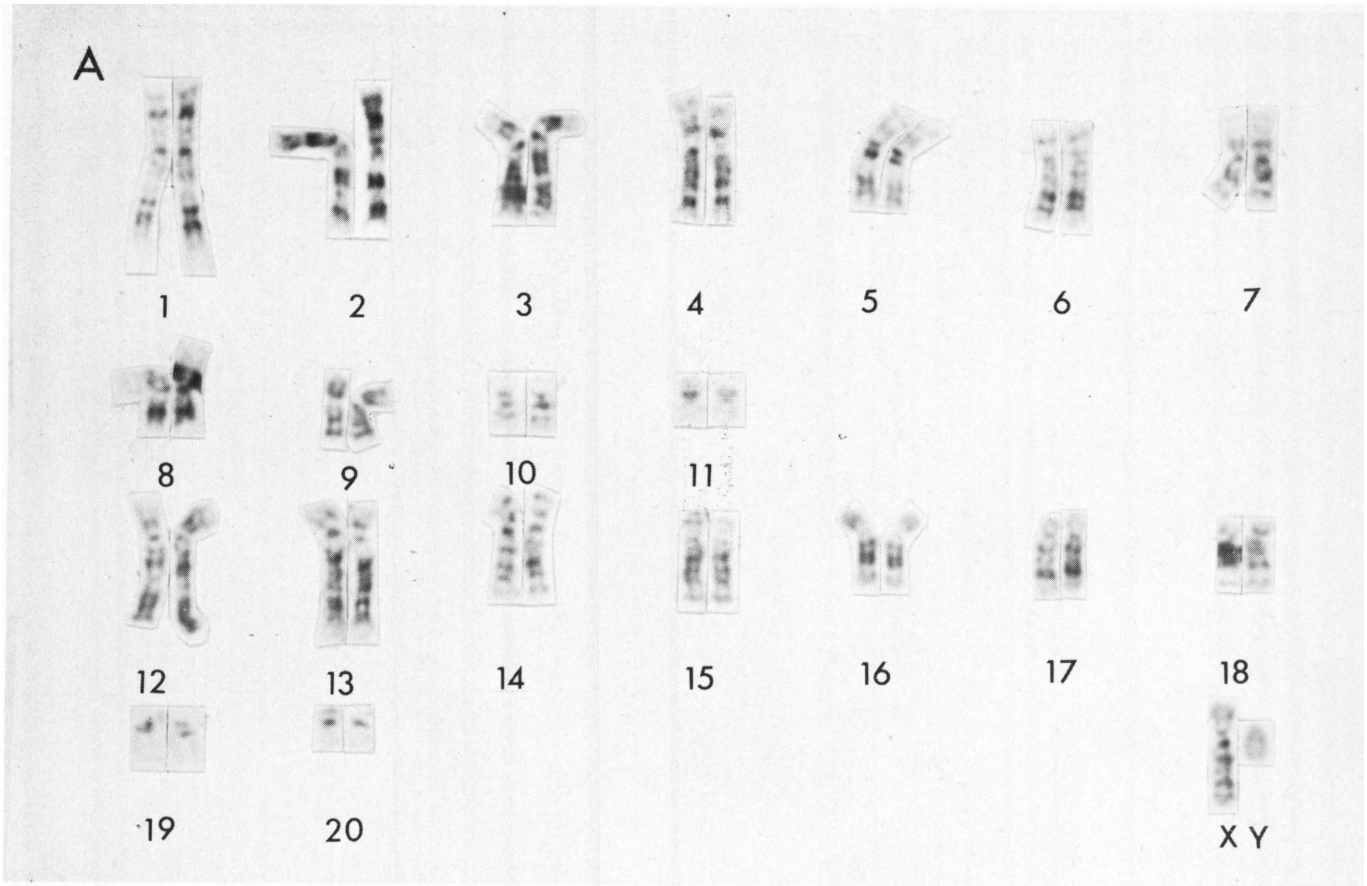

Fig. 22A: *G-banded karyotype of a male* P. rupestris *(2n = 42)*

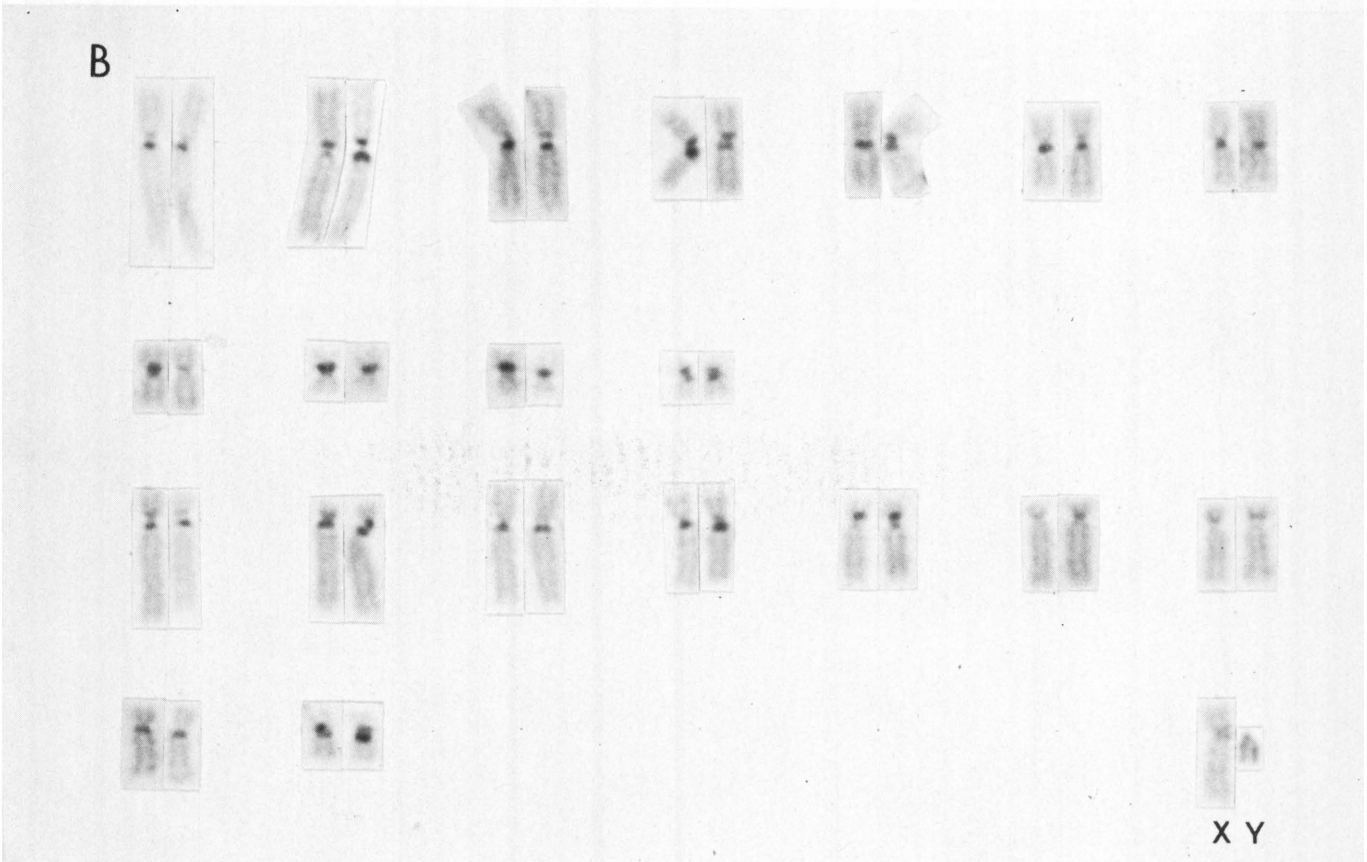

Fig. 22B: *C-banded karyotype of a male* P. rupestris *(2n = 42)*

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(pairs 10 and 11, 12 and 13), and are usually only distinguishable in good quality preparations. The centromeric regions of the chromosomes varied, several being G-band negative while others were G-band positive.

The C-banded chromosomes of *P. rupestris* are illustrated in Fig. 22B. The C-bands are localised around the centromeres with relatively large amounts of heterochromatin being clearly visible in all the chromosomes including the X and Y. The Y appeared to be largely heterochromatic in most preparations although not as intensely staining as that of some of the autosomes, while the X proved to have similar amounts of constitutive heterochromatin to the submetacentric autosomes of similar size. C-band heteromorphism was observed in most preparations (for example at positions 5, 8, 17 and 20), although this was by no means limited to *P. rupestris* (see below). No interstitial or telomeric C-bands were noted.

Chromosome analyses of this species conducted on 10 specimens from varying localities, failed to reveal any evidence of intraspecific G-band variation,while differences in the amounts of pericentric heterochromatin were sometimes observed between specimens, Fig. 22B representing the lower limits of material noted. However, these differences were slight, never associated with the development of heterochromatic chromosome arms and may be due in part, to differential chromosome contraction.

P. crassicaudatus (2n = 42)

The similarity in chromosome morphology and G-banding patterns to that of *P. rupestris* renders a detailed description of the *P. crassicaudatus* karyotype unnecessary. The chromosomes can be divided into two groups (Fig. 23A) and ranked following the format indicated by *P. rupestris.* Pairs 1–11 are metacentric to submetacentric autosomes, while 12–20 are subtelocentric or acrocentric in morphology. The sex chromosomes were similar to those of *P. rupestris.*

The C-banded karyotype is presented in Fig. 23B. In general the amount and distribution of constitutive heterochromatin was similar to that of the other *Pronolagus* species, with evidence of marked C-band heteromorphism in several pairs being particularly noticeable (for example at positions, 10, 11 and 16).

Since successful karyotype studies were only performed on a single representative (female) of this taxon, no analysis of possible intraspecific chromosomal variation could be made.

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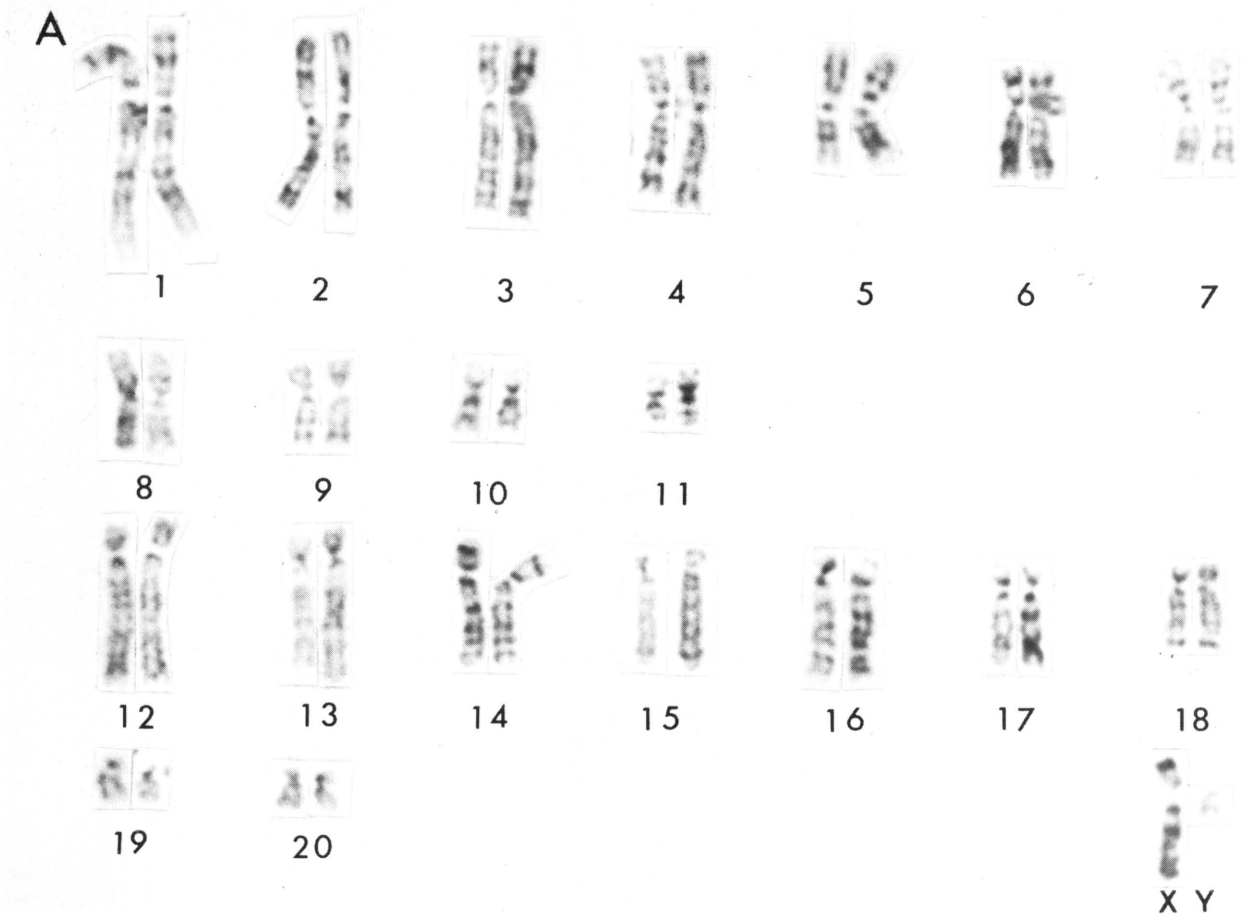

Fig. 24A: *G-banded karyotype of a male* P. randensis *(2n =42)*

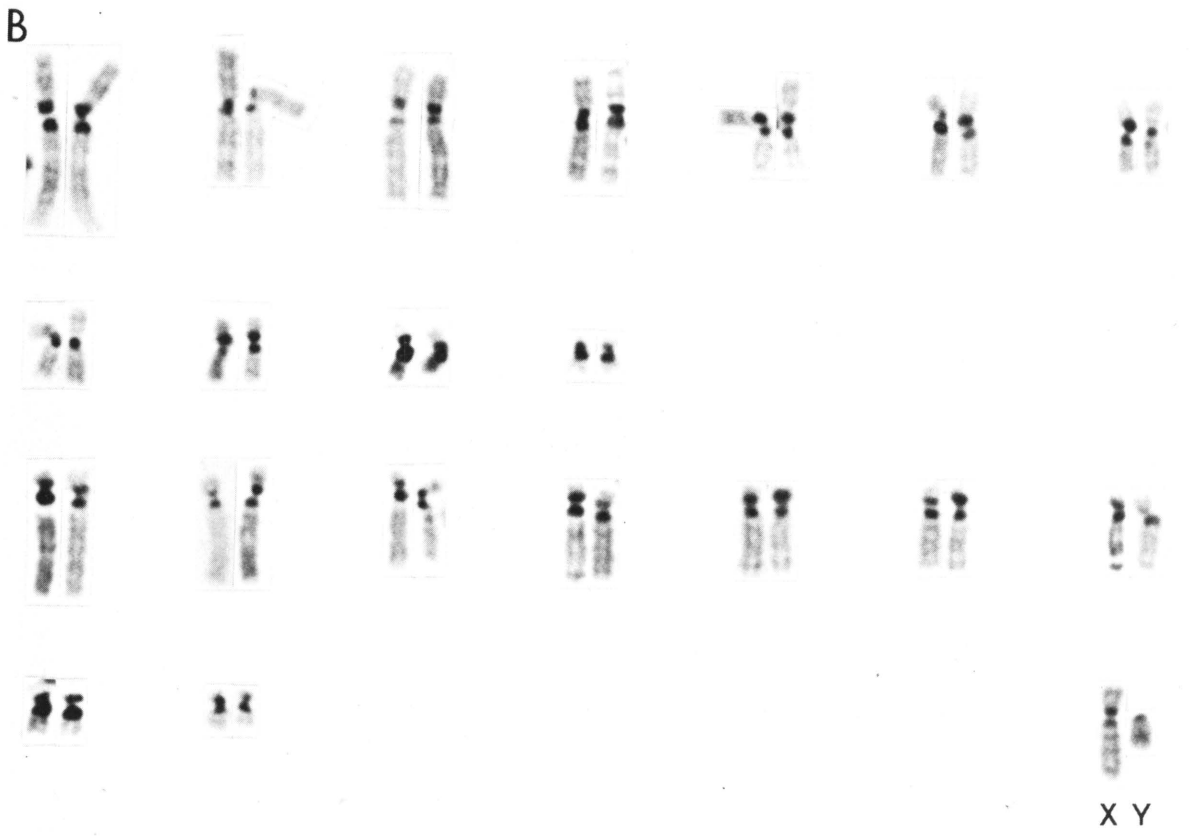

Fig. 24B: *C-banded karyotype of a male* P. randensis *(2n =42)*

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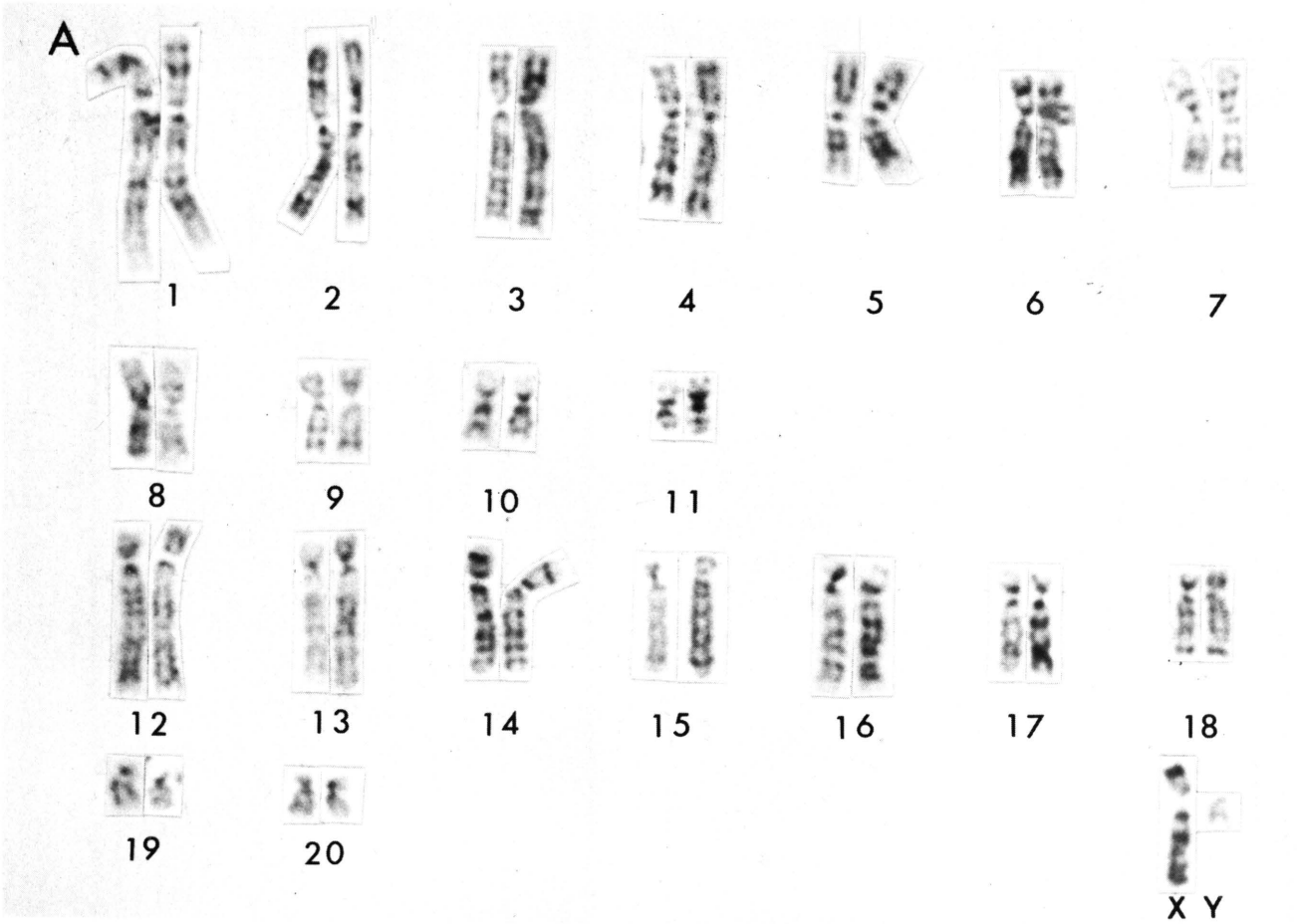

Fig. 24A: *G-banded karyotype of a male* P. randensis *(2n = 42)*

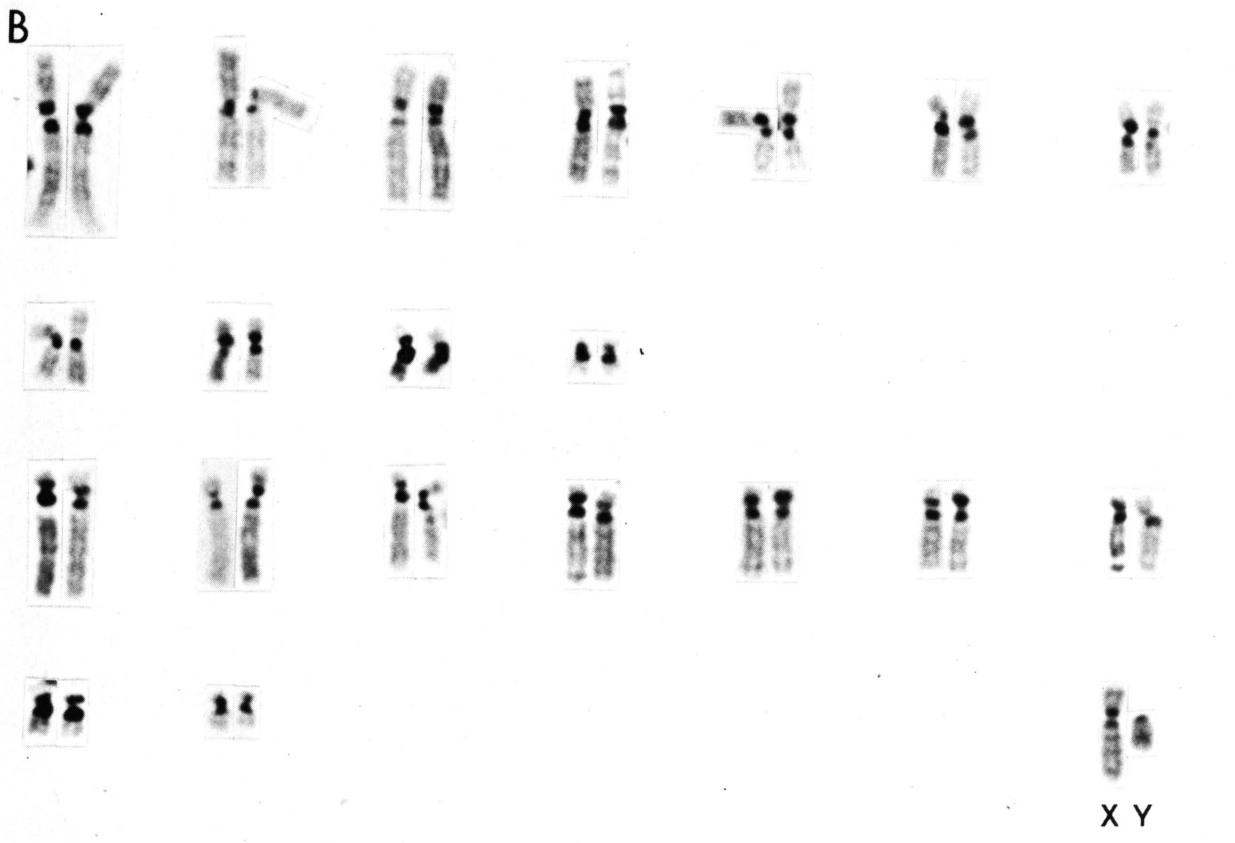

Fig. 24B: *C-banded karyotype of a male* P. randensis *(2n = 42)*

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P. randensis (2n = 42)

The G- and C-banded chromosomes of *P. randensis* (Figs. 24A and B) were similar to those of the two preceeding *Pronolagus* species, with autosomal pairs 1–11 being metacentric to submetacentric and those of 12–20 clearly subtelocentric or acrocentric. The same general observations which were made regarding the morphology of the sex chromosomes and the constitutive heterochromatin in the preceeding species hold for *P. randensis*. Observable C-band heteromorphism was also noted in this species.

No evidence of intraspecific G- or C-band variation was observed in the relatively small number of specimens examined (n = 3).

Interspecific comparisons:

A comparison of the G-banded chromosomes of *P. rupestris, P. crassicaudatus* and *P. randensis* is shown in Fig. 25. The karyotype uniformity of the genus is clearly reflected in both the morphology and the G-banding patterns of the chromosomes of the three species, which appear similar, if not identical, in all cases. No consistent differences were apparent in the amount and distribution of constitutive heterochromatin between species.

C. *BUNOLAGUS*

B. monticularis (2n = 44)

The G-banded chromosomes of *B. monticularis* are presented in Fig. 26A. The chromosomes have been numbered and the karyotype standardised based on the percentage contribution of each chromosome to the female genome (Table 13). The autosomes of the species can be grouped into two distinct categories, those with detectable short arms which are of metacentric, submetacentric and subtelocentric morphology (pairs 1–5), and a second larger group consisting entirely of telocentric autosomes (pairs 6–21). The X is a medium sized submetacentric chromosome and the Y a small acrocentric (detectable short arms).

The morphology of all the biarmed autosomes is distinctive and allows the identification of homologues from unbanded preparations. However, the telocentric autosomes show a gradation in size which, without the use of the G-banding patterns, could not be correctly

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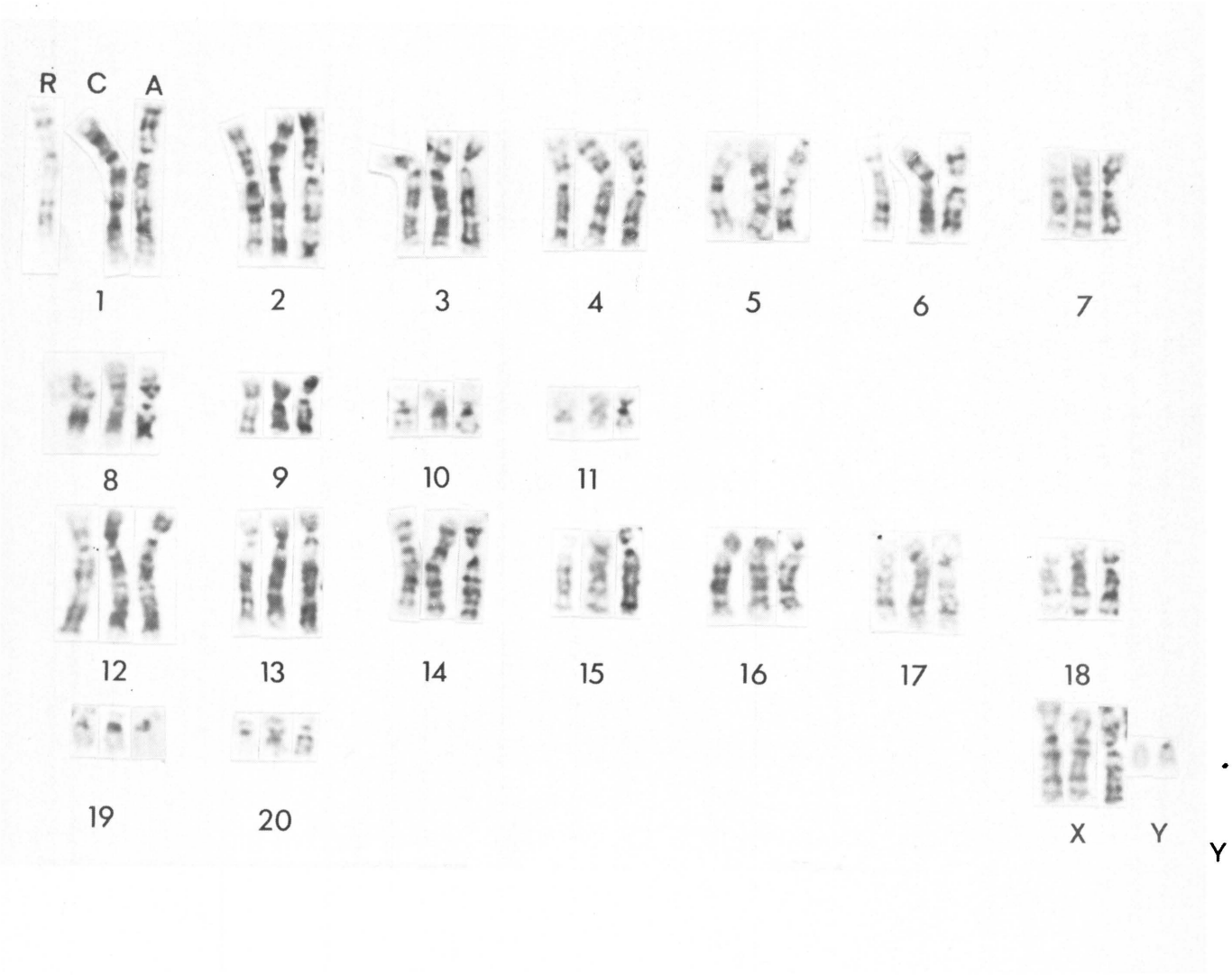

Fig. 25: *Comparison of the G-banded chromosomes of* P. rupestris *(R),* P. crassicaudatus *(C) and* P. randensis *(A). The Y chromosomes are those of* P. rupestris *and* P. randensis.

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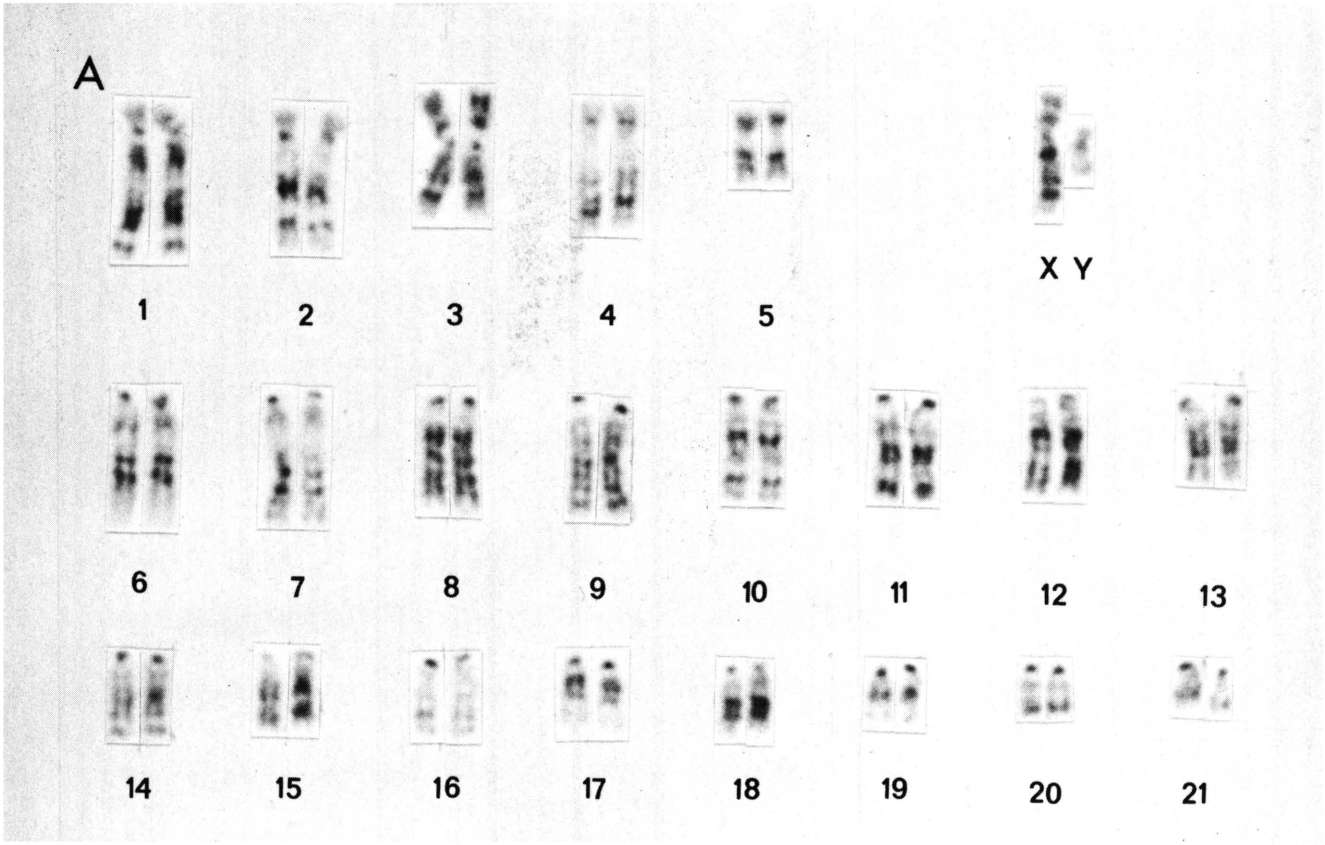

Fig. 26A: *G-banded karyotype of a male* B. monticularis *(2n = 44).*

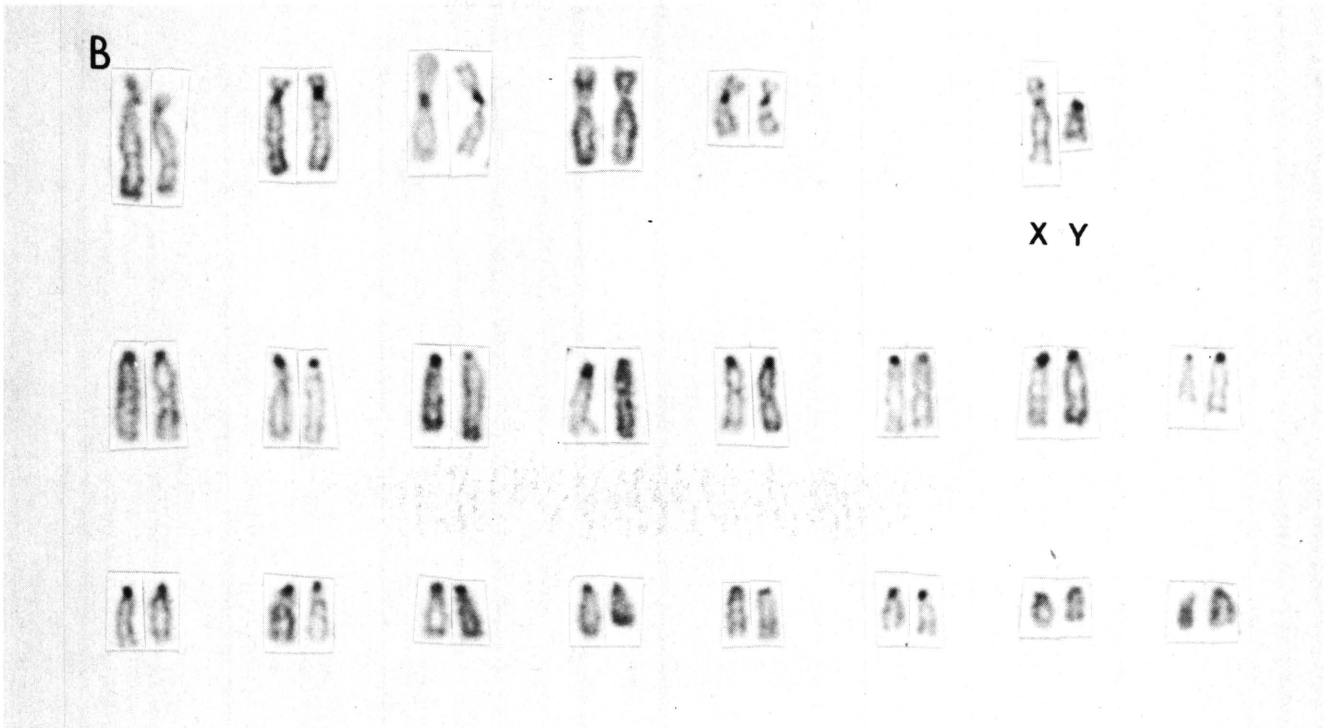

Fig. 26B: *C-banded karyotype of a male* B. monticularis *(2n = 44).*

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Table 13 :

Relative chromosome lengths of *B. monticularis* expressed as a percentage of a haploid female karyotype (n = 10). $\bar{X}$ = Arithmetic mean; tSE = 95% Confidence limit.

Chromosome number	$\bar{X}$ length	tSE	Chromosome number	$\bar{X}$ length	tSE
1	7,4	0,20	12	4,6	0,14
2	6,3	0,25	13	4,0	0,18
3	6,1	0,18	14	3,9	0,16
4	5,7	0,20	15	3,6	0,16
5	3,4	0,14	16	3,5	0,16
6	6,3	0,02	17	3,3	0,14
7	5,8	0,25	18	3,2	0,09
8	5,2	0,11	19	2,8	0,07
9	5,2	0,41	20	2,4	0,11
10	4,8	0,14	21	2,2	0,16
11	4,7	0,11	X	5,6	0,07
			Y	2,2	0,16

paired. Furthermore, high resolution of the banding patterns are necessary to distinguish many of the chromosomes, particularly pairs 19, 20 and 21. Unlike *Lepus*, the centromeric regions of most chromosomes are G-band positive.

The C-banded karyotype of *B. monticularis* is illustrated in Fig. 26B. As with the proceeding taxa the constitutive heterochromatin is located around the centromeres, with no evidence of interstitial or telomeric bands being present. All the chromosomes, with the exception of autosomal pair 4, show distinct C-bands. The absence of visible constitutive heterochromatin in this pair remained constant in all cells analysed. The amount of heterochromatin in the X chromosome appeared similar to that of the other species studied, while the Y was largely heterochromatic, with the short arms staining more darkly than the rest of the chromosome. Since only one specimen of this rare and endangered species (Robinson, 1981) was available for chromosome analysis, no indication of possible intraspecific chromosomal variation could be obtained.

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CHROMOSOME EVOLUTION

Chromosome evolution implies changes in the karyotype among related taxa. In order to illustrate the changes that have accompanied the diversification of the various leporid species, it was necessary to designate an ancestral karyotype for comparative purpose. To do this, data from several extralimital species have been included.

Ancestral karyotype:

Cytogenetic studies have shown that the constituent species of the genus *Lepus* appear to be characterised by a remarkably uniform karyotype (Table 10). In spite of their varying geographic origins all *Lepus* species thus far examined have 2n = 48. Furthermore, the conservativeness of the diploid number appears to be mirrored by the banding analyses. Studies on two African species (*L. saxatilis, L. capensis;* Robinson, in press and present study) and two European species (*L. europaeus, L. timidus;* Schröder, Suomalainen, Van der Loo and Schröder, 1978a) have shown no interspecific differences. This lack of variation is emphasised by the comparisons between *L. saxatilis, L. capensis* and *L. timidus* presented below. Additionally Schröder, Antoni and Van der Loo (1978b) found only minor differences (a single paracentric inversion) between *L. europaeus* and *L. californicus.*

Two other taxa, the Mexican volcano rabbit *R. diazi* and a cottontail species *S. bachmani*, also exhibit a diploid number of 48 (Table 10) which, based on existing data, represents the upper limit for the family. Stock (1976) clearly illustrated that all the *S. bachmani* chromosomes have G-band equivalents in *L. americanus*, while Van der Loo, Uribe-Alcocer and Schröder (1979) speculated that the G-banded karyotype of *R. diazi* was similar to that of *Lepus.* The only detectable difference in the karyotype between *S. bachmani* and *L. americanus* appeared to be in the amounts of constitutive heterochromatin, which was markedly greater in the former (Stock, 1976).

Chromosome comparisons between *R. diazi, L. saxatilis, L. capensis* and *L. timidus* are presented in Fig. 27. The excellent agreement in the G-bands between the taxa shows that their genomes are largely structurally homologous. Slight differences in chromosome morphology between *Lepus* (represented by three species) and *R. diazi* are noticeable in pairs 14, 15 and 19. In *Lepus* these chromosomes carry clearly detectable short arms which appear absent in *R. diazi.* Because of the small size of the short arms of these chromosomes in the former species, and the fact that they are largely G-band negative, it was not possible to ascertain the mechanisms responsible for producing the minor differences be-

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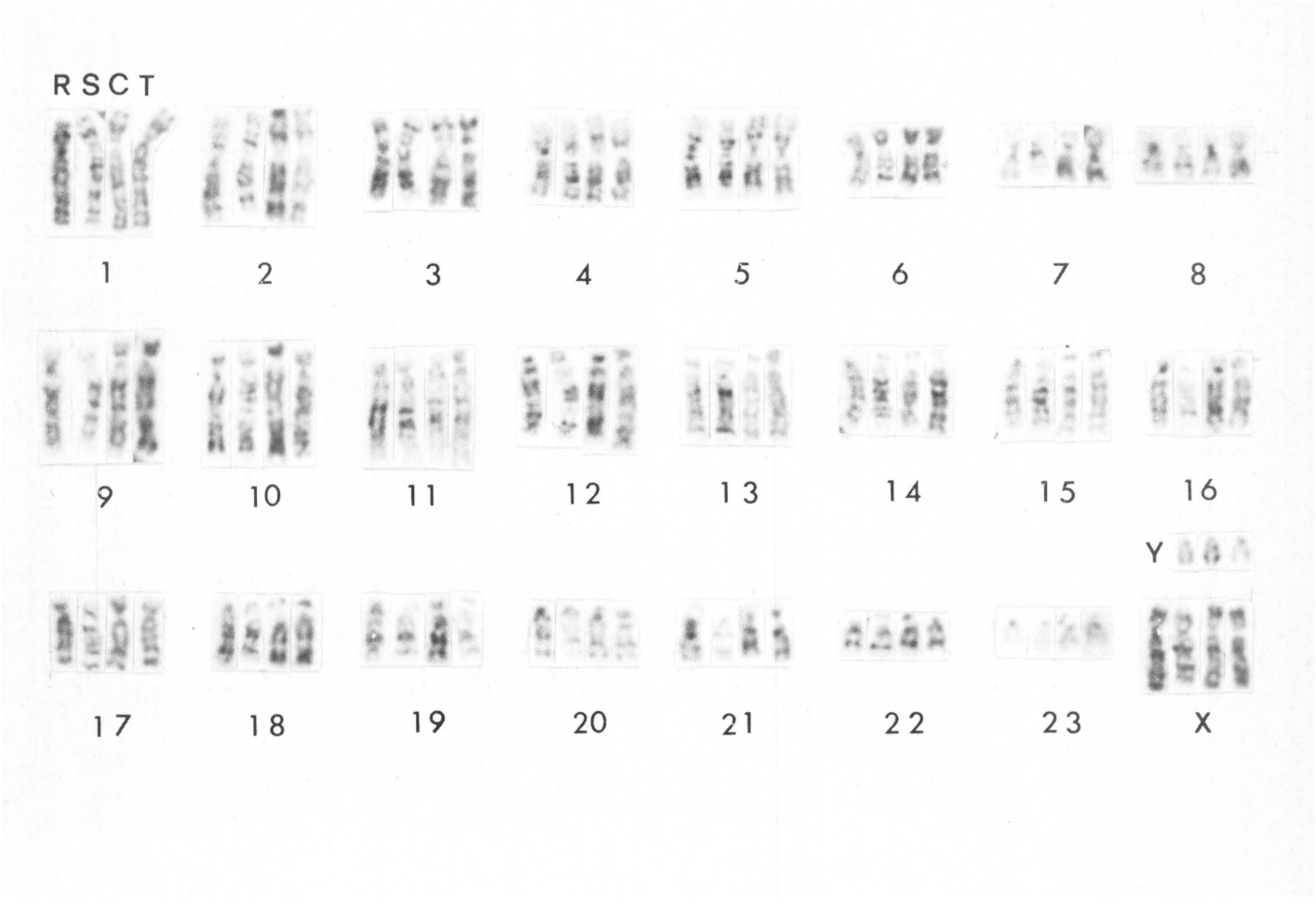

Fig. 27: *Comparison of the G-banded chromosomes of* R. diazi (R), L. saxatilis (S), L. capensis (C) *and* L. timidus (T). *Chromosome identification numbers are the standard numbers from the respective genomes. The Y chromosomes are those of* L. saxatilis, L. capensis *and* L. timidus; *no comparable data were available for* R. diazi *since a male specimen of this species was not available for analysis.*

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tween the karyotypes of the two genera. Other differences include the observably greater amounts of constitutive heterochromatin in the *R. diazi* genome and the more metacentric appearance of autosome 21, which is thought to be due to increased heterochromatin in the short arm of this chromosome (see Robinson, Elder and Lopez-Forment, 1981 for a detailed description of the *R. diazi* karyotype).

The remarkable similarity in chromosome morphology and G-banding patterns between representatives of the genus *Lepus, R. diazi* and *S. bachmani* seem unlikely to be due to chance alone. These taxa, based on fossil evidence involving the developmental patterns of the premolars (Hibbard, 1963), are thought to represent two distinct lineages which have been separated since the Pliocene. *Lepus* and *Sylvilagus* are, following Hibbard (1963), presumed to be more closely related, having diverged in the early Pleistocene.

It seems likely that the presence of a *Lepus*-like karyotype in three genera of two distinct leporid lineages indicates a common evolutionary origin, and points back to an ancestral karyotype from which all leporids may have been derived. Although minor modification of the *Lepus* karyotype may have taken place in the evolution of this genus, the close similarity of both chromosome morphology and G-banding patterns between *Lepus, Romerolagus* and a species of *Sylvilagus,* justifies the use of the *Lepus* karyotype as the ancestral form for the comparisons that follow.

Lepus (2n = 48) : *Pronolagus* (2n = 42):

As representative of these genera the chromosomes of *L. saxatilis* are compared with those of *P. rupestris* (Fig. 28). The G-banding patterns of the two species are easily matched and all the *Lepus* chromosomes have banding equivalents in the *Pronolagus* genome. The difference in diploid number between the genera has resulted from three fusions in *Pronolagus* which are not present in *Lepus,* each of which, once fixed, would have reduced the diploid number by two.

Chromosome 1 of *Pronolagus* corresponds to the two unfused *Lepus* chromosomes 11 and 19, chromosome 2 to *Lepus* chromosomes 12 and 18, while the third *Pronolagus* fusion product, chromosome 5, is represented by chromosomes 6 and 21 in *Lepus.* These rearrangement or translocation products have probably resulted from centromere – centromere fusions (Hsu, Pathak and Chen, 1975) and are widely referred to as Robertsonian translocations.

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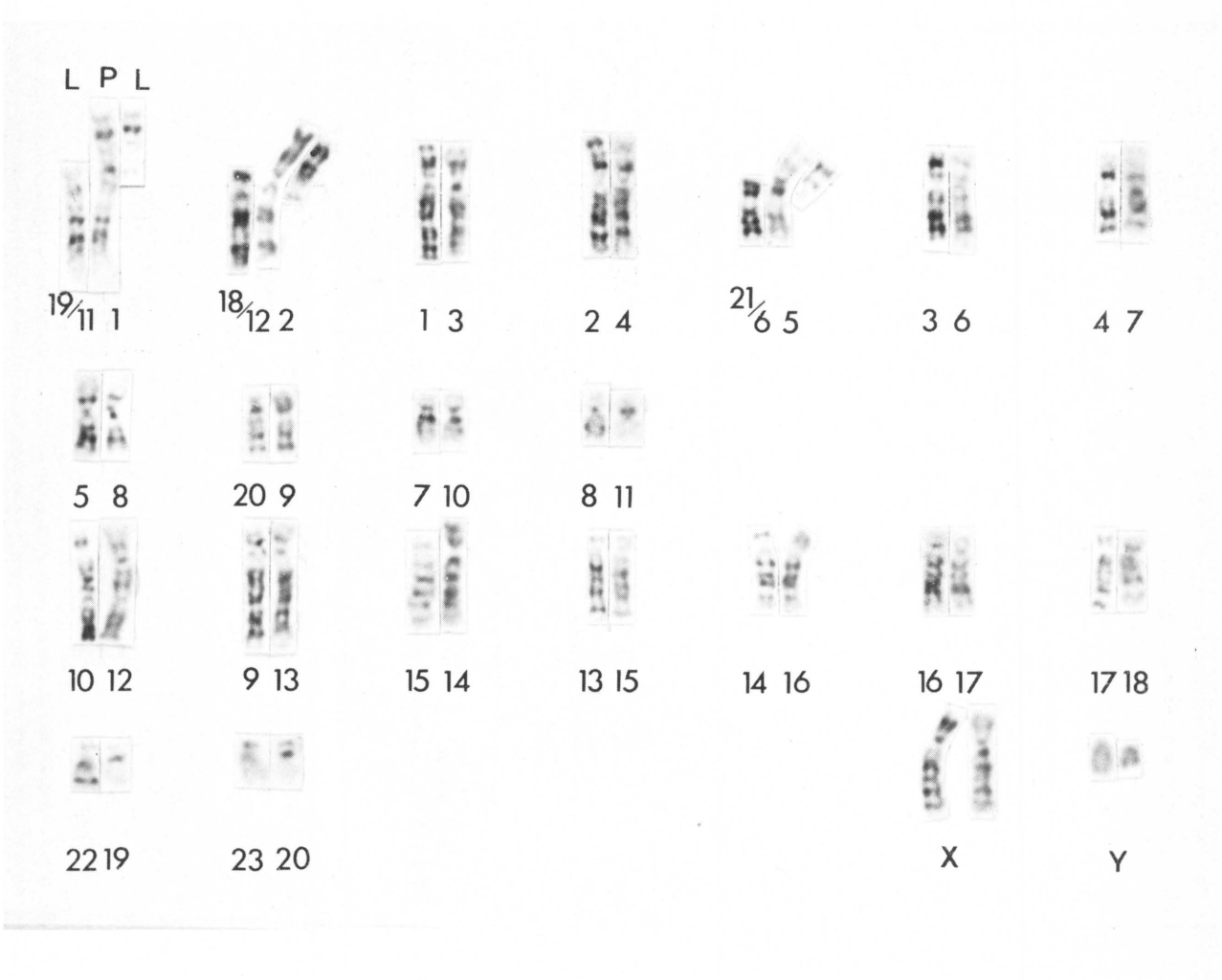

Fig. 28: *Comparison of the G-banded chromosomes of* L. saxatilis *(L) and* P. rupestris *(P). The first chromosome in each pair is that of* L. saxatilis. *Chromosome identification numbers are the standard numbers from the respective genomes.*

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In addition to the fusion differences between *Lepus* and *Pronolagus,* the latter is characterised by a pericentric inversion in one of the autosomes, chromosome 9. This inversion resulted in the formation of a submetacentric element in *Pronolagus,* compared to the acrocentric morphology of this chromosome in *Lepus.* Other differences in the karyotype of the two taxa include the longer p arms of the *Pronolagus* autosomal pair 14, which differs from *Lepus* by the presence of an extra G-positive band located distally, and the readily observable disparity in constitutive heterochromatin. The discrepancy in the length of the short arms of the *Pronolagus* chromosomes 12, 13 and 16 and their equivalents in *Lepus* 10, 9 and 14 are probably due to differences in heterochromatin. The morphology of the sex chromosomes is similar in both genera.

Lepus (2n = 48): *Bunolagus* (2n = 44)

A comparison between the G-banded karyotype of *L. saxatilis,* representative of the ancestral condition and those of *B. monticularis* is presented in Fig. 29. All the chromosomes, except numbers 17 and 18 of *Bunolagus,* and 4 and 5 of *Lepus,* have banding equivalents.

Chromosome 3 of *Bunolagus* represents a fusion between *Lepus* chromosomes 19 and 21, and chromosome 4, a similar fusion between *Lepus* chromosomes 8 and 16. The mechanism responsibility for these rearrangements are of the Robertsonian type, similar to those found in *Pronolagus.* A third fusion product was also evident in the *Bunolagus* genome. Chromosome 7 of *Bunolagus* is represented by two acrocentric *Lepus* elements, chromosomes 15 and 23. This rearrangement is of the type described as a centromere – telomere fusion (tandem fusion), which has been documented for cultured cells (Hsu *et al.,* 1975) and also the red muntjak (Liming, Yingying and Xingsheng, 1980) and cotton rats (Elder, 1980).

Interstitial heterochromatin would be expected following this form of rearrangement. However, its apparent absence in the *Bunolagus* fusion chromosome is probably indicative that the centromeric heterochromatin either did not participate, or persist, in the structuring of this autosome.

Should the hypothesis for the ancenstral karyotype and diploid number be valid, the three fusions which have occurred in the evolution of *Bunolagus* would have reduced the ancestral 48 to 42, a similar diploid number to that of *Pronolagus. Bunolagus* is characterised by 2n = 44. The increase in diploid number was traced to a single fission, the splitting of a biarmed chromosome into two telocentric elements. The submetacentric chromosome 1 of *Lepus* is represented by two telocentric autosomes, numbers 12 and 19 in *Bunolagus.*

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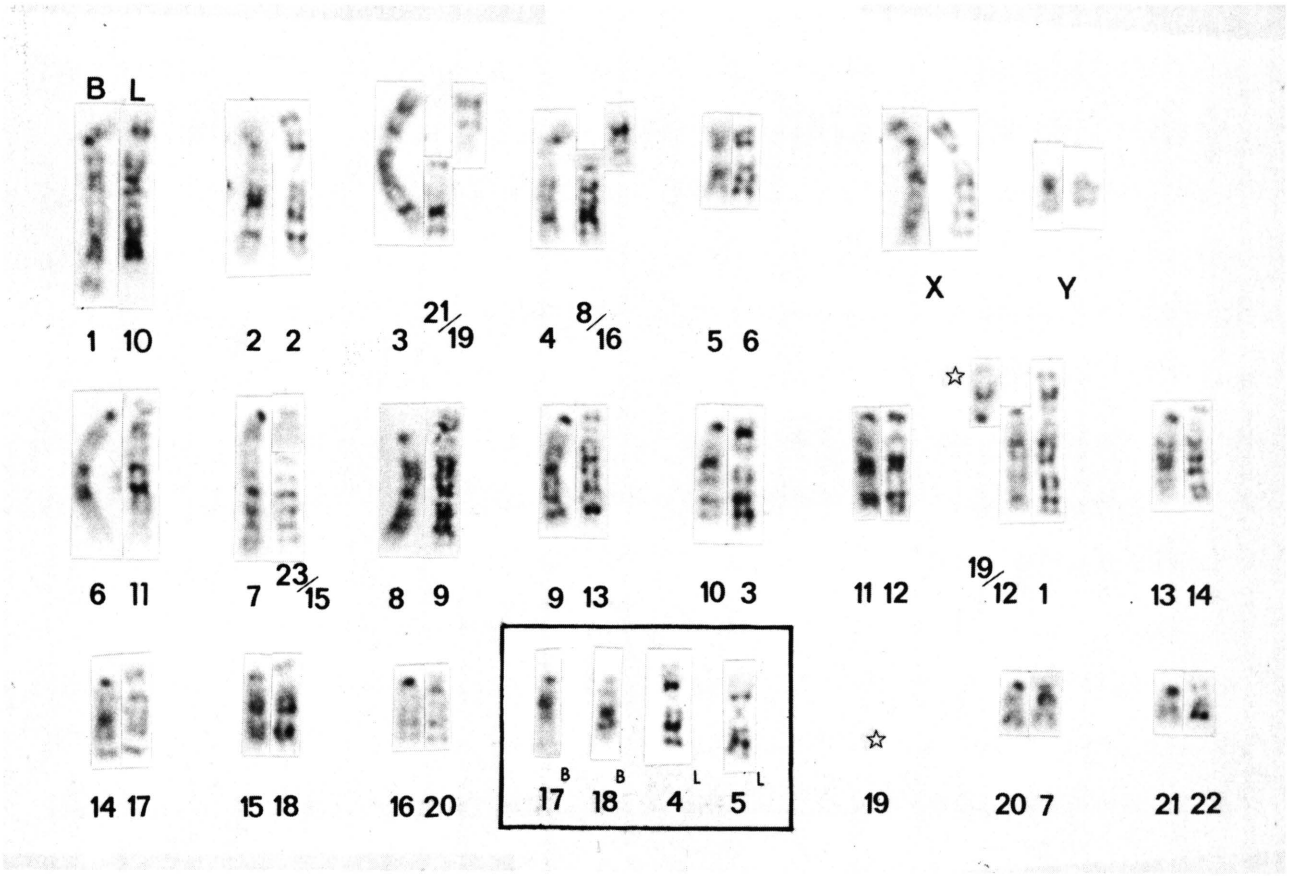

Fig. 29: *Comparison of the G-banded chromosomes of* B. monticularis *(B) and* L. saxatilis *(L). The first chromosome in each pair is that of* B. monticularis. *Chromosome identification numbers are the standard numbers from the respective genomes, while the box contains the unmatched chromosomes of the two species which have no apparent homology in the respective genomes.*

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Although the role of fission is generally regarded to be subordinate to that of fusion, it has nevertheless been reported in mole rats (Wahrman, Goitein and Nevo, 1969), root voles (Fredga and Bengström, 1970) and very conclusively by Dutrillaux *et al.* (1980b) in the primate subfamily Cercopithecinae. Although the two *Bunolagus* chromosomes 12 and 19 appear to be represented by a single submetacentric chromosome in all leporid species thus far examined using modern techniques (Stock, 1976; Schröder *et al.*, 1978a; Schröder *et al.*, 1978b; Schröder and Van der Loo, 1979; Van der Loo *et al.*, 1979; Hagelthorn and Gustavsson, 1979; Robinson, 1980; Robinson *et al.*, 1981; Robinson, in press and present study), it is interesting to note that the fused, or ancenstral form, is also represented by two unfused elements in the human karyotype (Dutrillaux, Viegas-Pequignot and Couturier (1980a). Dutrillaux (*pers. comm.* *) is of the opinion that the unique fission event in *Bunolagus* in fact represents a reverse mutation, the unfused form perhaps being similar to that found in the ancestral karyotype from which all mammals may have evolved.

Other differences in the karyotypes of the two species include the presence of an extra distal G-band in the long arm of *Bunolagus* chromosome 1 which is absent in other leporids, the altered morphology of the *Bunolagus* chromosome 10 which is due to a pericentric inversion in this genus, and the greater quantity of constitutive heterochromatin in the *Bunolagus* genome. The X chromosomes of the two taxa are of similar morphology and banding patterns, while the *Bunolagus* Y chromosome differs from its counterpart in *Lepus* by the presence of a heterochromatic short arm.

The lack of homology evident between the *Bunolagus* autosomes 17 and 18 and chromosomes comprising the *Lepus* genome, were similarly reflected in subsequent comparisons with *Oryctolagus* and *Sylvilagus* (represented by the species *S. aquaticus, S. floridanus* and *S. transitionalis*, unpublished observations, and *S. bachmani* Stock, 1976). It is possible that a series of complex structural rearrangements involving the two *Bunolagus* chromosomes are responsible for their altered morphology and lack of G-band homology with other lagomorph species.

The relatively small number of chromosomes with detectable short arms in the *Bunolagus* karyotype is striking. These structures are not heterochromatic in *Lepus* (Figs. 19B and 20B) and their absence in *Bunolagus* cannot be explained on the basis of

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heterochromatic loss. Because of their small size and the fact that they are largely G-band negative, it has not been possible to determine the mechanisms responsible for producing these changes. In certain groups of mammals however, there is a tendency towards similar structural changes establishing themselves in one chromosomal pair after another within the karyotype. This principle has been referred to as karyotypic orthoselection by White (1973) and is exemplified in species of *Peromyscus* (Pathak *et al.*, 1973), *Mus* (Capanna, Cristadi, Perlicone and Ressoni, 1975) and *Sigmodon* (Elder, 1978). It is not inconceivable that karyotypic orthoselection, perhaps involving repeated small pericentric inversions have been responsible, in part, for the unique structuring of the *Bunolagus* karyotype.

Constitutive heterochromatin:

While the amount and distribution of constitutive heterochromatin appears fairly constant within the polytypic South African genera *Lepus* and *Pronolagus*, noticeable differences in the amounts of C-banding material were evident between genera. Of these taxa *Pronolagus* appears to possess the largest quantity of constitutive heterochromatin followed by *Bunolagus* and *Lepus.*

These results support earlier observations that the different lagomorph taxa, at least at generic level, appear to possess differing amounts of C-heterochromatin (Earley and Mouton, 1973; Stock, 1976). While the significance of such differences are unknown, it is possible that karyotypic changes such as Robertsonian fusions, may be more difficult to achieve in those species characterised by small amounts of heterochromatin (Pathak and Wurster-Hill, 1977). This may be partially responsible for the remarkable lack of variation evident in the *Lepus* karyotype.

Phylogenetic affinities:

Karyotypic data can sometimes allow inferences regarding phylogenetic relationships. Structural rearrangements are regarded as unique events, and the probability that the same chromosomal change would occur twice is unlikely. Thus species which share the same derived chromosomal condition probably evolved from a common ancestor which exhibited this derived character.

Comparisons between the chromosomes of *Pronolagus* (2n = 42) and those of *Bunolagus* (2n = 44) clearly reveal the absence of any common structural rearrangement,

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implying that the changes which are evident in the genomes of the two taxa occurred subsequent to their separation.

Romerolagus chromosome 14 and its equivalent in *Bunolagus,* chromosome 13, lack detectable short arms, a characteristic not noted in any of the other taxa. However, the significance of this must be examined in the light of the extremely small size of the segment involved, the inability to identify the mechanisms responsible for this change, and the possible effects of karyotypic orthoselection in the structuring of the *Bunolagus* genome.

Additional chromosome comparisons were made between *Pronolagus, Bunolagus* and taxa peripheral to this investigation, *Oryctolagus* (2n = 44) and *Sylvilagus.* The latter genus was represented by three species for which sufficiently detailed banding preparations were available, *S. aquaticus* 2n = 38, *S. floridanus* 2n = 42 and *S. transitionalis* 2n = 46 (unpublished data; Robinson, Chapman and Elder, in preparation). Preliminary results indicate that *S. aquaticus* and *S. floridanus* share one chromosome mutation (fusion 13/15, Fig. 30), clearly indicating a closer phylogenetic affinity to each other, than to *S. transitionalis.* However, at generic level none of the numerous rearrangements which characterise the genomes of the various taxa were found to be common.

Several chromosomal trends were nevertheless evident from these comparisons. Figure 30 presents the fusion products identified in the various taxa. It is clear from these comparisons that although many of the same chromosomes have been involved in past fusion events, these have invariably been in different combinations. The similarities in the banding patterns between several of the chromosomes, notably the equivalents of *Lepus* 14 and 15, and their remarkable frequency (in different combinations) in the various fusion products, emphasis the need to base phylogenetic decisions on high quality preparations.

Possible factors influencing chromosomal evolution:

The remarkable uniformity in the karyotype of *Lepus* is in contrast to most other genera thus far analysed. Since karyotype variation is primarily an individual and once established, a population characteristic, the conditions under which a chromosome rearrangement can become fixed are severely limited. Wilson, Bush, Case and King (1975) point out that rearrangements are unlikely to become fixed in large outbreeding populations. Small deme size with some degree of inbreeding are probably essential for chromosome evolution.

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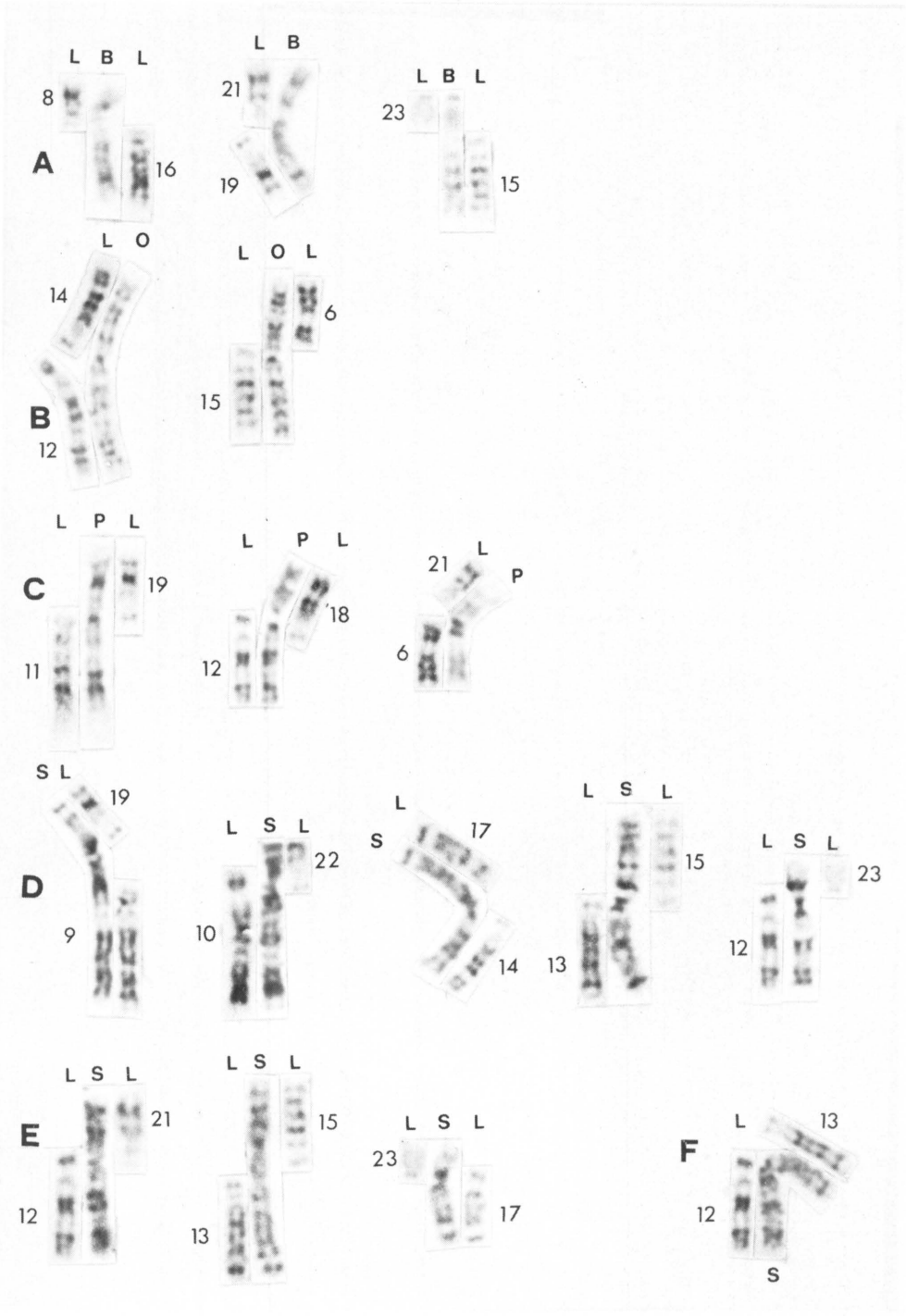

Fig. 30: *Comparison of the fusion chromosomes of: (A)* B. monticularis *(2n = 44), (B)* O. cuniculus *(2n = 44), (C)* P. rupestris *(2n = 42), (D)* S. aquaticus *(2n = 38), (E)* S. floridanus *(2n = 42) and (F)* S. transitionalis *(2n = 46). The corresponding unfused chromosomes of* L. saxatilis *(L), representative of the ancestral karyotype, are also presented. Note the high frequency of* Lepus *chromosome numbers 12, 14, 15, 19 and 23 in the different fusion products.*

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Recently Van der Loo *et al.* (1979) and Robinson (1980) speculated that the differences between the karyotypic uniformity of *Lepus* and most other taxa may be due, in part, to differences in social structure and mobility. *Lepus* tends to be solitary in habit and probably shows greater dispersion than is evident in other taxa. Another factor specifically pertinent to both *Pronolagus* and *Bunolagus* is their tendency towards habitat specificity. *Pronolagus* is narrowly confined to rocky situations which, in large parts of its range, resemble terrestrial islands, while *Bunolagus* is limited to suitable riverine scrub in the central and southern Karoo (Robinson, 1981; Robinson and Skinner, in press). In both taxa unsuitable habitat between populations may combine to create effective dispersal barriers. Many of these populations probably form small breeding units or demes which should be subject to drift. This situation would greatly facilitate the local spread of a *de nova* chromosomal rearrangement. Several other taxa with limited distributions and narrow habitat preferences such as *Caprolagus, Nesolagus* and *Pentalagus* may conceivably also show deviation from the ancestral karyotype, although the retention of the virtually unaltered ancestral genome in *Romerolagus* clearly indicates that generalisations can be erroneous.

TAXONOMIC CONCLUSIONS

Karyotype analyses conducted on specimens of the two phenetically distinct *Lepus* species, *L. capensis* and *L. saxatilis,* revealed no interspecific differences. A similar lack of variation was evident in *Pronolagus* and clearly indicates the limitations of this parameter for the delimitation of species within each genus.

However, the cytogenetic data is particularly pertinent to the taxonomy of *Bunolagus.* Based on available data, all the species of *Lepus,* the genus to which *Bunolagus* has been assigned in the past, possess 48 chromosomes which illustrate very little interspecific variation in gross morphology. Even more pertinently, this lack of variation has been mirrored to a large extent by the banding patterns of those species for which data are available. The presence of 44 chromosomes in *Bunolagus,* and in most cases their unique morphology, are considered to provide unusually strong support for generic status distinct from that of *Lepus.*

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CHAPTER 4

INCISOR MORPHOLOGY

INTRODUCTION

The enamel folding responsible for the notched appearance of the labial or outer surface of the principal upper incisor and the shape of the tooth in cross section, provide a valuable source of information for use in systematic studies in the Leporidae. Although differences in the enamel patterns of certain species of *Lepus* have been known for a considerable time (Forsyth Major, 1898), it appears that other than in Petter's work on African and Asian species of this genus (Petter, 1959; 1961; 1963; Petter and Genest, 1965), these criteria have not been widely used in comparative systematic investigations, particularly in genera other than *Lepus*.

In the present study, the reliability of characters such as the shape of the incisor in cross section, the pattern of the enamel invagination and the presence or absence of cement in this structure, are evaluated for use as systematic criteria, particularly with reference to the South African species.

MATERIAL AND METHODS

A single principal upper incisor was extracted from the skull of each field specimen collected, embedded in a polyester mounting medium (Poly-steel Filler; Sprayon Ltd, South Africa) and sectioned using a modified version of the Buehler Isomet low speed saw (Buehler Ltd, Evanston, Illinois, USA). Although teeth sections were not made from the Natal Museum *P. crassicaudatus* collection, incisors were extracted and processed as above for all museum specimens of *L. saxatilis* and *P. randensis* used. Due to the relatively small numbers of *Bunolagus* available in museum collections and for fear of slightly damaging the skulls during the extraction of the teeth, incisors were removed from only two of the specimens used in the morphometric analyses.

Each tooth section was placed on a microscope slide, immersed in a drop of Xylol and examined using bright field illumination. The Xylol tends to reduce the opacity of the sections,

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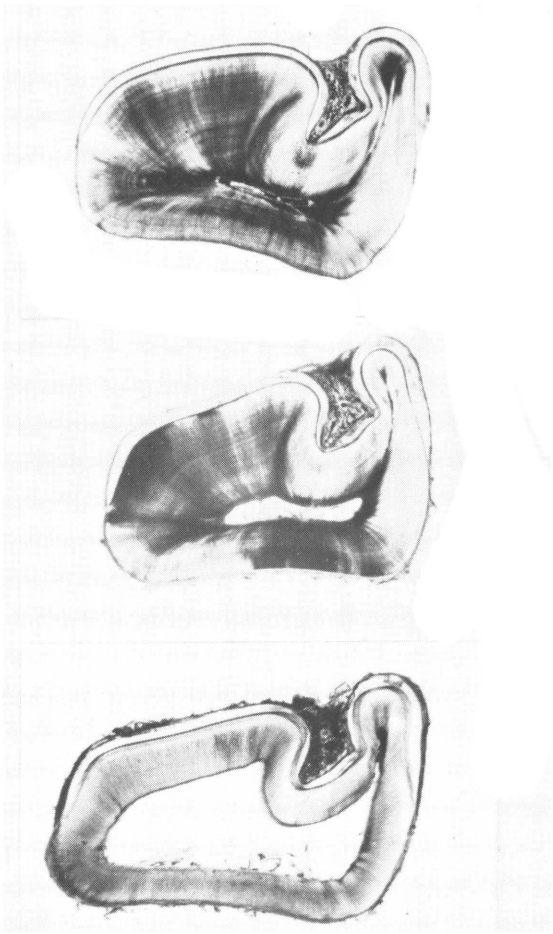

Fig. 31: *Cross section through a single principal upper incisor of* L. saxatilis *showing both the uniformity of the enamel fold and deposition of cement in this structure along the entire length of the tooth.*

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resulting in a more translucent preparation which is suitable for photomicrographic work. The photomicrographs of tooth sections were made with the aid of a Zeiss C35 M camera attachment mounted on a standard research microscope, using low power objectives (2,5X) and Ilford high contrast Pan F film.

RESULTS AND DISCUSSION

Variation with age, attrition and between incisors of the same specimen:

Although Forsyth Major (1898) proposed that the variation in tooth pattern may reflect an age component in that "... species whose incisors show the most complicated pattern in the adult have as yet no trace of this in very young animals, and *vica versa* in very old specimens complication tends to disappear again", no evidence of this was found in the test material. Comparisons of incisor cross sections between skeletally immature (some of which were very obviously juvenile even prior to ageing) and mature specimens from the same locality failed to reveal any significant differences.

Additional support for the reliability of the character was found by sequentially sectioning individual incisors along their entire lengths so as to ascertain whether attrition (and indirectly age since the incisors are evergrowing in the Leporidae) would alter the configurations in any way. Figure 31 illustrates the uniformity in both the enamel fold and deposition of cement along the entire length of a *L. saxatilis* tooth. A similar lack of variation in the incisors of the other taxa was also observed.

Slight differences between the left and right incisors of a single specimen were sometimes noticed. However, these were never so pronounced that depending on the tooth sectioned, ambiguous classification of the species could result. Similar minor differences between incisors of the same specimen have been previously reported (Forsyth Major, 1898 ; Petter, 1959).

Incisor tooth pattern variation in *L. capensis:*

The incisor patterns of *L. capensis* specimens utilised in the present study provide a reliable means of separating this taxon from other *Lepus* material. The enamel fold in the incisors of this species was consistently relatively shallow, always of simple configuration and without exception always characterised by the presence of cement. Figure 32 shows the characteristic "squareness" of the incisors of *L. capensis* in cross section as opposed to

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the more elongate form in *L. saxatilis* (Fig. 36 p. 107), as well as the extent of the individual variation illustrated by the test material.

The incisive enamel has played an important role in the taxonomy of *L. capensis.* In his earlier work, Petter (1959) was clearly of the opinion that in addition to the simple enamel fold (corroboration of which is provided in this study), the taxon was characterised by the absence of cement. He thereby confirmed an earlier observation by Forsyth Major (1898) who noted that *L. capensis* "differs scarcely from *L. europaeus*, Pall. by its minute enamel folding not filled with cement." In fact, in a subsequent paper Petter (1961) concluded that there appeared to be no reliable criteria for the recognition of *L. capensis* from *L. europaeus* and that in the palaearctic region the latter cannot be considered distinct from *L. capensis.* This has led to the synonomysing of the two species, (for example Hewson, 1977; Corbet and Hill, 1980) a decision not accepted by all workers (Angermann, 1972; Flux, *in litt.**1). Angermann (1972) has suggested as possible evidence against their being one species the fact that "the distribution of the two hares overlap east of the Caspian Sea, yet in this area they behave as separate species, that is they do not interbreed. The Cape hare is not only smaller but differs in coloration and in having long, naked ears".

In addition to Angermann's observations (Angermann, 1972), there does appear to be a very distinct difference in incisor pattern between *L. capensis* from the type locality and elsewhere in South Africa (Fig. 32), and those of *L. europaeus,* judging from the incisor patterns of the specimens*2 presented in the text (Fig. 33). The absence of cement in the enamel fold of *L. europaeus* observed by Forsyth Major (1898) and Petter (1959) is consequently confirmed. However, their conclusions pertaining to the similarity of the condition in *L. capensis,* at least in respect of specimens originating from South Africa, do not hold.

Although no reliable data are available on the extent of the variation in the enamel pattern in *L. capensis* outside South Africa, cross sections through the upper incisors of two specimens from northern Israel and the Negev desert respectively*3, appear similar in all respects (Fig. 34) to those of the typical *L. capensis.* The similarity in incisor patterns

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*1 Dr. J. Flux, Ecology Division, DSIR, Lower Hutt, New Zealand.

*2 Material supplied by the Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN, Scotland.

*3 Material supplied by Dr. Y. Yom-Tov, Department of Zoology, University of Tel-Aviv, Israel.

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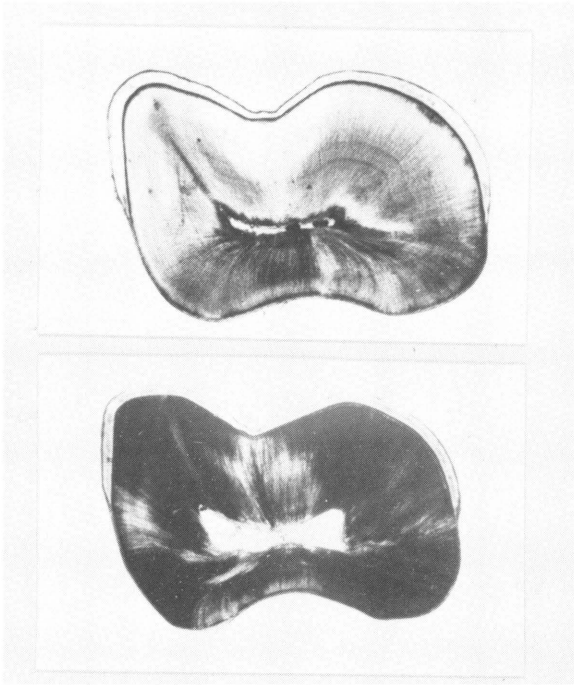

Fig. 33: *Cross section through the principal upper incisors of two* L. europaeus *specimens from Scotland showing the complete absence of cement in the enamel fold of this species.*

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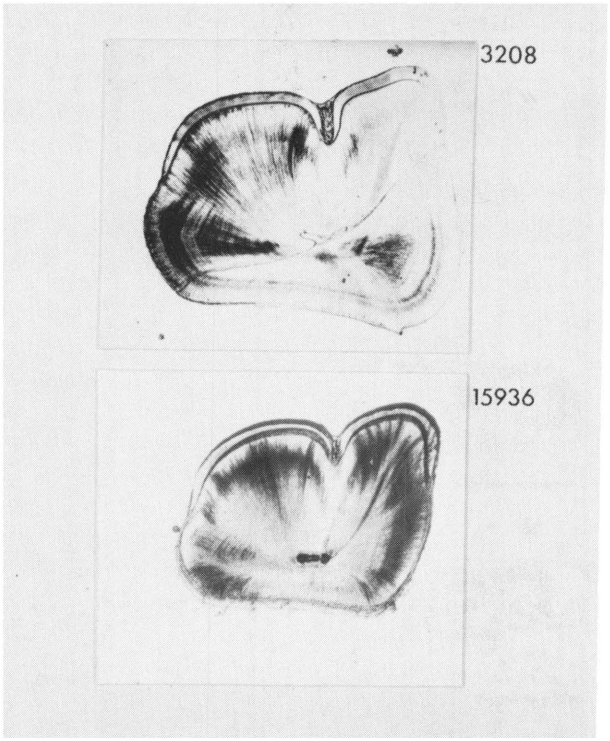

Fig. 34: *Cross section through the principal upper incisors of two* L. capensis *specimens from Israel. Specimen No. 3208 originated from northern Israel and No. 15936 from the Negev Desert.*

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between specimens of such divergent localities, is possibly indicative of the lack of variation in the incisor pattern of the species and may indicate the reliability of this character for the separation of *L. europaeus* from *L. capensis.*

The disagreement in the observations of this study and those of Forsyth Major (1898) and Petter (1959) relating to the presence of cement in the enamel fold of *L. capensis* may be attributable to differences in technique. Both Forsyth Major and Petter relied exclusively on superficial macroscopic investigations of the incisors, while tooth sections form the basis of this investigation. Support for this hypothesis was obtained by sectioning the teeth of specimens used by Petter and Genest (1965) in their study of the morphological variation and distribution of *L. capensis* in South West Africa. While clearly indicating that they regarded the typical *L. capensis* tooth pattern as being of simple configuration and without cement (in this respect similar to Petter's 1959 observations) they indicated that there appeared to be a gradual transition of this form northwards, through South West Africa to approach the deeper cement filled groove of *L. salai* Jentink, 1880 (= *L. capensis salai* Petter and Genest 1965). However, the variability in the depth of the lance shaped groove in specimens from the type locality alone (Fig. 32) renders the applicability of this observation to *L. salai* questionable.

The cross sections through the upper incisors of all the specimens utilised by Petter and Genest (1965) in their study with the exception of TM 8208, 10972 and 9583 which were not available, and TM 4985 and 12610 which lacked incisors, are presented in Fig. 35. The presence of cement in the enamel fold of all specimens including those regarded by him as not showing traces of this substance (for example TM 6926) is clearly illustrated. These results would seem to cast serious doubt on the consistent accurate scoring of cement in this species without the use of sectioned teeth, a trend noted in the preliminary investigations of the present study.

In view of these results, an extensive investigation of incisor morphology in *L. europaeus* using sectioning techniques in addition to a similar study on the north African forms of *L. capensis,* with emphasis on their area of sympatry east of the Caspian Sea, is indicated. Furthermore, a detailed examination to ascertain if the taxonomic affinities of the palaearctic forms are with the African *L. capensis,* particularly those from the type locality (Cape Province), appears long overdue.

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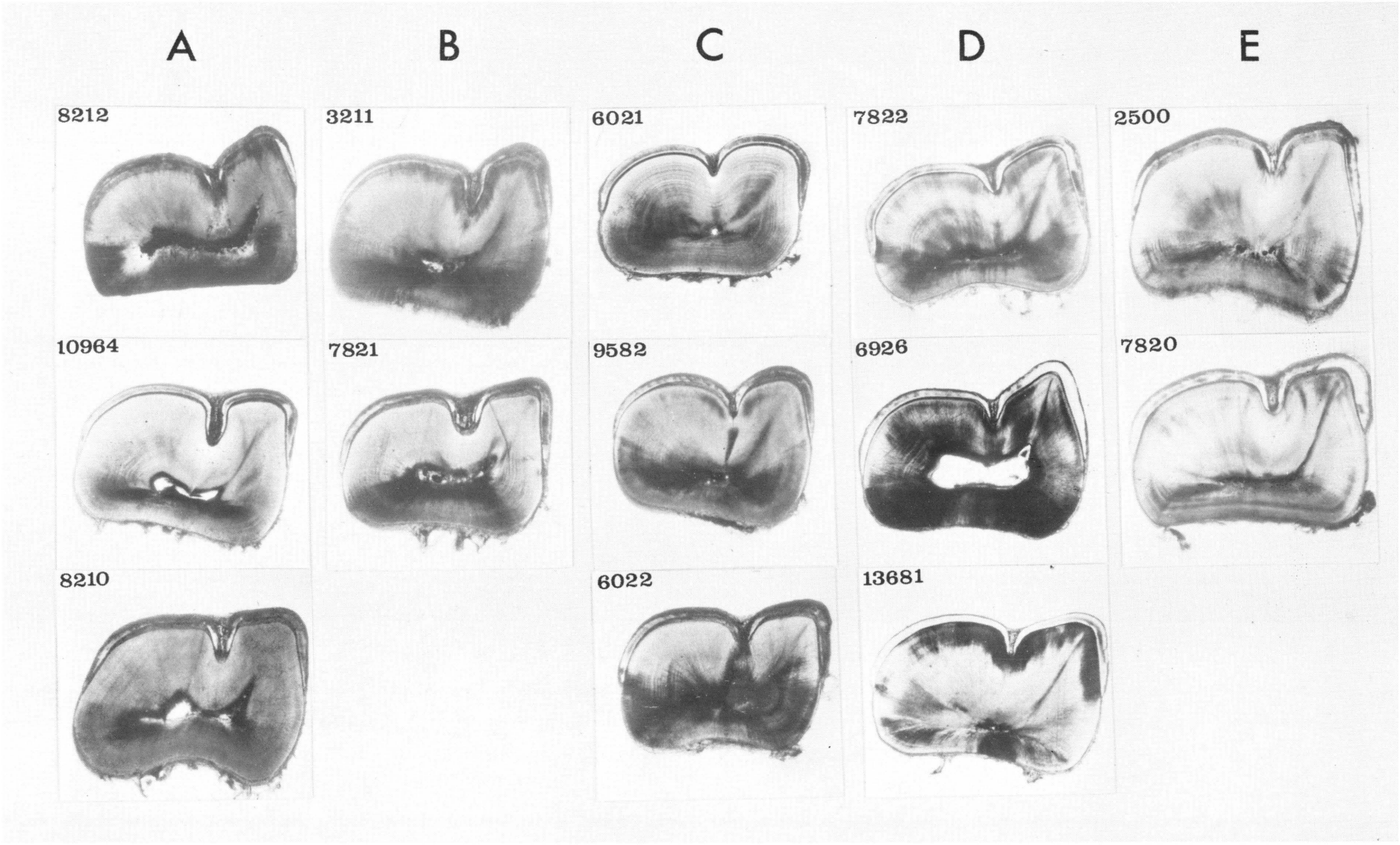

Fig. 35: *Cross section through the principal upper incisors of those* L. capensis *specimens utilised by Petter and Genest (1965), and grouped according to the results of their macroscopic investigations of this material. Specimens in column A were regarded by these authors as possessing a deep enamel fold characterised by the presence of cement, while those comprising C and D were reported as being of the typical* L. capensis, *not possessing cement (for example 6926: See Petter and Genest 1965). Specimens grouped in columns B and E were indicated as being intermediate in this character. The inconsistency of these results clearly illustrates the importance of tooth sections for accurate identification in this species.*

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Incisor tooth pattern variation in *L. saxatilis*:

Although the shape of the enamel fold in *L. saxatilis* shows far more intrapopulation variation than was evident in *L. capensis*, the use of this criterion provides an unequivocal means of separating the two species. Figure 36 shows the extent of the variation encountered in the incisor tooth patterns of *L. saxatilis* in South Africa. There appears to be a gradual, though not marked tendency towards increasing complexity of the groove from the southwestern parts of its geographic range extending northeastwards, mirroring to some extent the trend illustrated by the morphometric analyses.

Specimens originating from the southern and southwestern areas of the Cape Province tend to be characterised by a relatively simple lance shaped groove (Fig. 36A) which usually does not extend as deeply into the body of the tooth as is the case in the more northeastern areas. Consequently, there tends to be less cement present in the enamel fold of the incisors of specimens from the former areas. Incisors from specimens originating from the more central regions of the Cape Province, the Orange Free State, southern Natal and western Transvaal, usually possess a more convoluted fold, with the posterior portion of the invagination being expanded laterally (Fig. 36B and C). This results in a more bulbous appearance in that portion of the tooth which lies medial to the invagination, than was found in specimens from areas in the south and southwestern Cape Province. In the more northeastern regions (central and northern Natal and the remainder of the Transvaal), the general trend is towards complex invaginations in the majority of specimens (Fig. 36D), with a high percentage showing pronounced bifurcations of the fold.

Petter (1959, 1963) is of the opinion that *L. saxatilis* is characterised by a simple enamel groove, while *L. crawshayi* tends to be more complex in this structure. However, the degree of variation in specimens of this study, which using Petter's diagnostic key (Petter, 1972) are referable to *L. saxatilis* (principally A and B Fig. 36), is so great that it seriously questions use of this criterion in the delimition of these taxa.

Species comparisons and taxonomic conclusions:

In cross section, the principal upper incisor of *L. capensis* can be readily distinguished from those of *L. saxatilis* by their "squarer" appearance, particularly that section of the tooth which lies medial to the invagination, and the characteristic more abrupt infolding of the enamel layer where it turns into the invagination. Furthermore, the incisors of *L. capensis* are noticeably less robust than those of *L. saxatilis* being on average narrower

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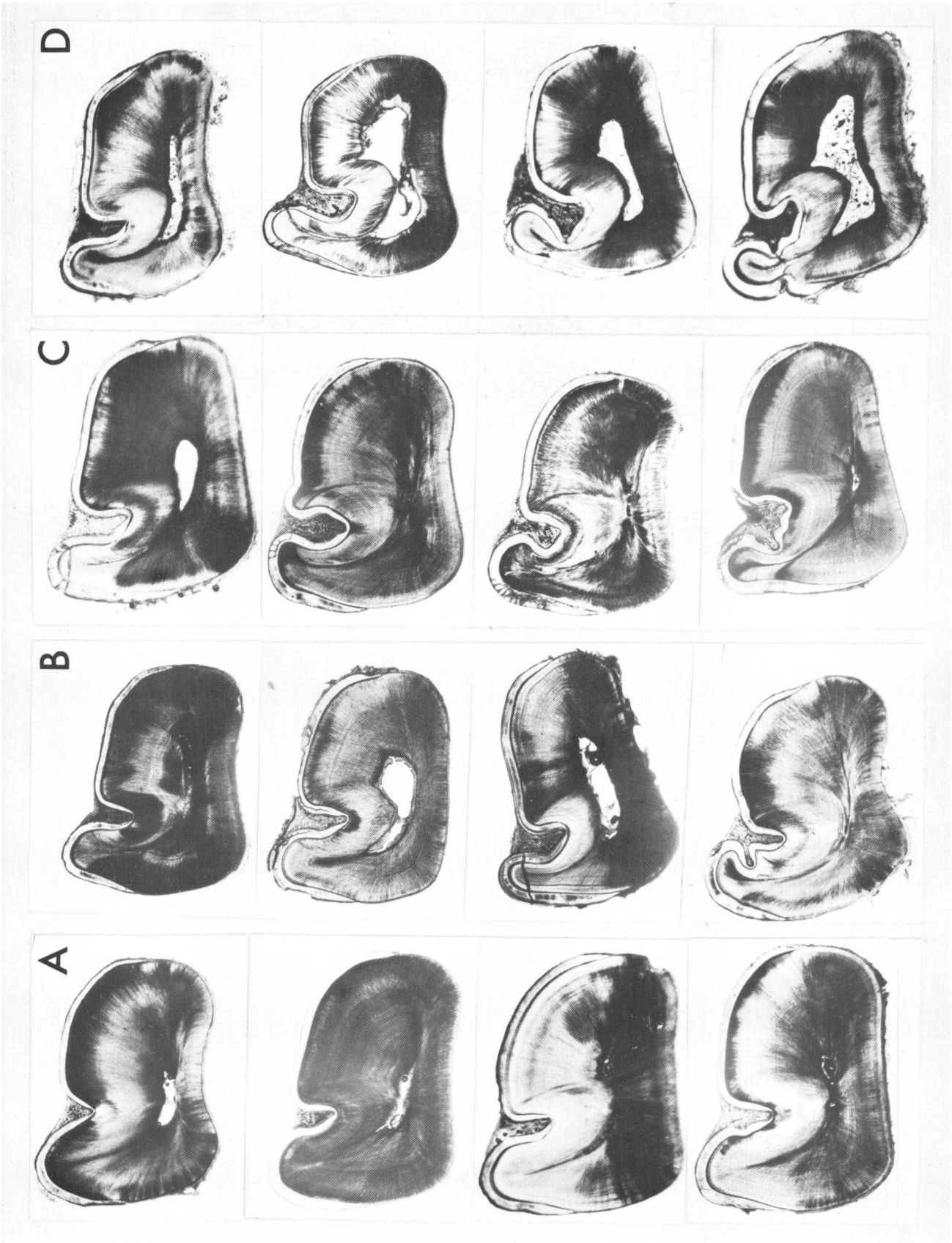

Fig. 36: *Variation in cross sections through principal upper incisors of* L. saxatilis *originating from: A = extreme southern and southwestern Cape Province; B = central Cape Province; C = Orange Free State, southern Natal and western Transvaal; D = central and northern Natal and remainder of Transvaal.*

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($\bar{X}$=2,5 mm observed range 2,3–2,8) than those of the latter ($\bar{X}$ = 3,1 mm range 2,6–3,6). The difference is particularly striking when dealing with adult material. The incisor tooth pattern characteristics of *L. capensis* clearly support the recognition of this species as a well deliniated taxon distinct from *L. saxatilis*, thereby confirming the results of the morphometric analysis.

Perceptable differences exist in the incisor tooth patterns of *L. saxatilis* particularly between those specimens taken from the southwestern Cape Province and those from further north. However, the extent of the variation between these extremes and the tendency towards increasing complexity of the invagination in a northeasterly direction, provides no support for Petter's recognition of the smaller northern forms as a species distinct from *L. saxatilis* (Petter, 1972), and consequently concurs with the results of the morphometric analyses.

Incisor tooth pattern variation in *Pronolagus*:

The most striking difference between *Pronolagus* and the South African representative of the genus *Lepus,* is the complete absence of cement in the incisor enamel fold of the former genus. The incisor tooth patterns and the extent of the variation in *P. crassicaudatus, P. randensis* and *P. rupestris* are presented in Fig. 37.

Species comparisons and taxonomic conclusions:

In *Pronolagus,* unlike *Lepus*, the source of variation lies with the shape of the incisors in cross section and not with the invagination itself. All three taxa are characterised by a simple, shallow enamel groove, which is closer in appearance to that of *L. capensis* than *L. saxatilis*, but characteristically without cement.

Within the respective species of *Pronolagus,* very little interpopulation and intrapopulation variation in incisor tooth pattern appears to occur (Fig. 37). However, distinct interspecific differences exist between *P. rupestris* and the remaining taxa, *P. crassicaudatus* and *P. randensis*, which are more similar with respect to this character. The obvious disparity in the appearance of the incisors between the latter two species and *P. rupestris* is due largely to the elongation of the tooth lateral to the invagination. This is reflected in the incisor breadth measurements of the *P. crassicaudatus* $\bar{X}$ = 3,2 mm ob-

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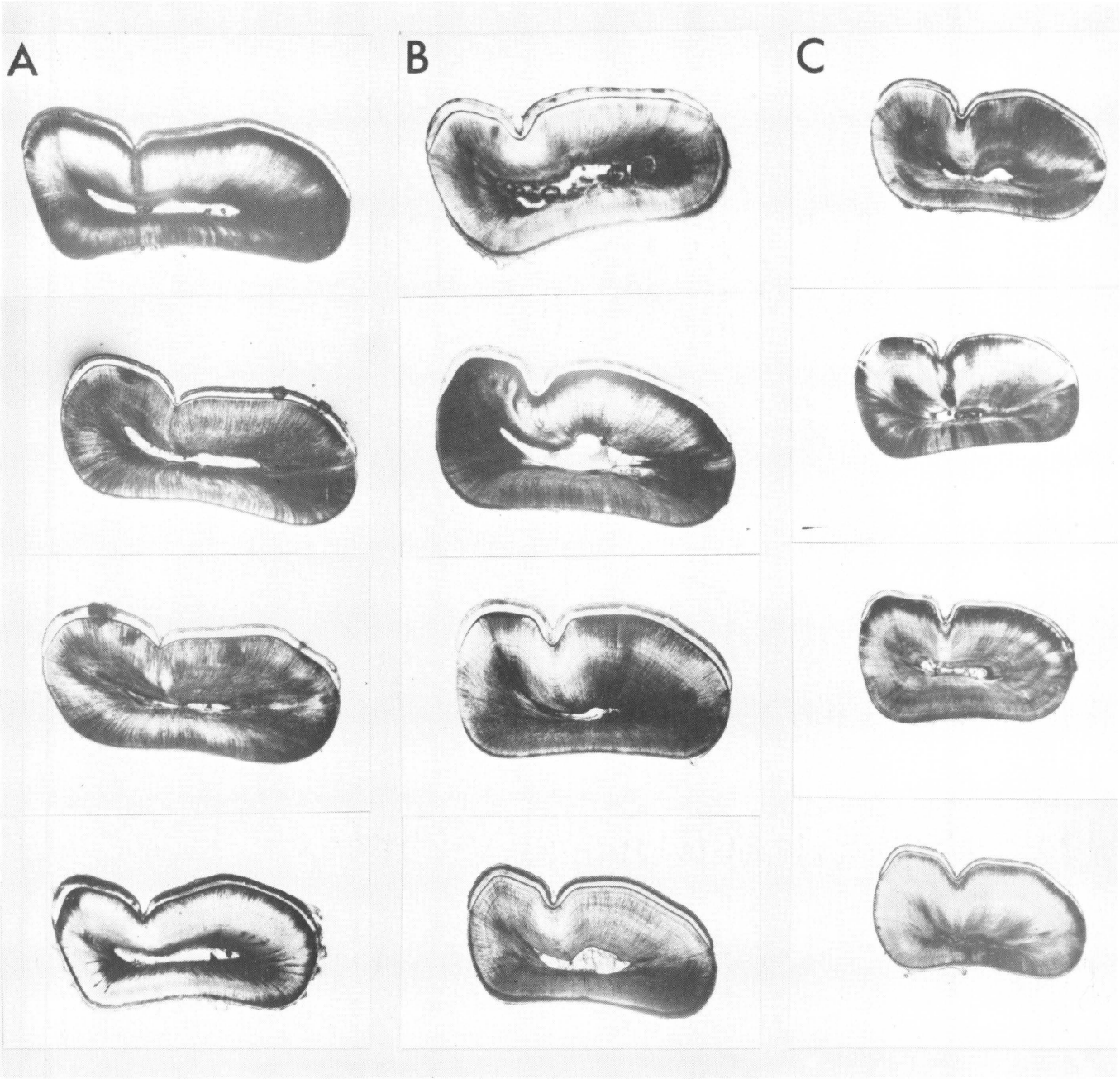

Fig. 37: *Variation in cross sections through the principal upper incisors of* P. crassicaudatus *(A),* P. randensis *(B) and* P. rupestris *(C).*

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served range 2,9–3,6) and *P. randensis* ($\bar{X}$ = 3,0 mm observed range 2,6–3,4) specimens used in the study, which on average are wider than those of *P. rupestris* ($\bar{X}$ = 2,5 mm range 2,3–2,8).

Both the width and shape of the incisors of *P. crassicaudatus* and *P. randensis* tend to be similar, however subtle differences in shape are evident in the sectioned teeth of the respective species. In cross section the *P. crassicaudatus* incisor tends to be flatter anterio-posteriorly than *P. randensis*. However, the difference is slight and may only prove to be of use when a series of incisors from both taxa are available.

The craniometric distinctiveness of *P. rupestris* illustrated in Chapter 2 is clearly reflected in the incisor tooth pattern data. However, this is not the case in *P. crassicaudatus* and *P. randensis*. Although well segregated in the morphometric analyses, the gross similarity of their incisor tooth patterns would make the deliniation of these taxa using only this criterion difficult.

Incisor tooth pattern variation in *Bunolagus*:

Incisor cross section of *Bunolagus* showing the typical pattern associated with the species and the degree of variation found in the two specimens used are presented in Fig. 38. Like *Pronolagus*, *Bunolagus* is also characterised by the absence of cement in the enamel fold. This supports Angermann's (1966) observations on *Bunolagus*.

Species comparisons and taxonomic conclusions:

Striking differences exist in the incisor tooth patterns between the South African *Lepus* and *Bunolagus*. The latter can be clearly distinguished by the absence of cement in the incisive fold, the extreme narrowness of the teeth ($\bar{X}$ = 2,1 mm observed range 2,0–2,2) and the thinner anterior covering of enamel. These data provide a highly reliable and practical means of cranially distinguishing *Bunolagus* from the South African *Lepus*.

Comparisons between the sectioned *Bunolagus* incisors and those of the *Pronolagus* species reveal that *P. rupestris* is closer to *Bunolagus* in this character than either *P. crassicaudatus* or *P. randensis*. However, the incisors of the two species differ in shape, particularly with regard to that portion of the tooth lateral to the invagination, which tends to be more rounded in *B. monticularis* than *P. rupestris*. Although this difference appears particularly evident in the test material, the small *B. monticularis* sample must be considered. With

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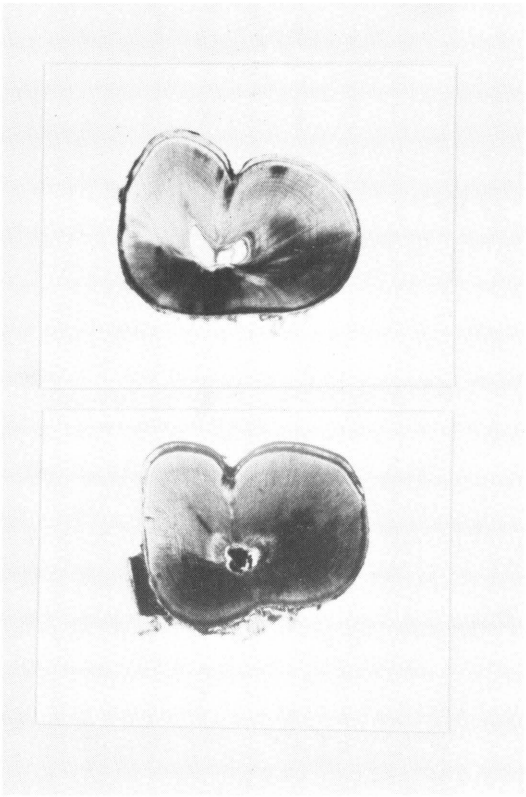

Fig. 38: *Cross sections through the principal upper incisors of two specimens of* B. monticularis, *showing the complete absence of cement in the enamel fold and the roundness of the tooth in section*

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larger sample sizes and possibly greater intraspecific variation in this character than present data suggests, the differences between *B. monticularis* and *P. rupestris* may not be as pronounced. The same basic shape differences that exist between *P. crassicaudatus* and *P. randensis* in respect of *P. rupestris* also hold, but even more pertinently for comparisons with *B. monticularis.*

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CHAPTER 5

SPERM MORPHOLOGY AND SILVER STAINING PATTERNS

INTRODUCTION

The use of sperm morphology as a taxonomic character although not new (for example Friend, 1936), does not appear to have been widely used in mammalian systematic studies. In addition to the early work on the British Muridae by Friend (1936), variation in sperm morphology among mammals was discussed by Bishop and Austin (1957) and Bishop and Walton (1960); they suggested that the morphology of the spermatozoa may be species specific.

While there is a considerable body of data relating to the genetics of spermatozoa shape and size differences between litters and strains of the laboratory mouse and rabbit (Beatty, 1958; Braden, 1959; Beatty and Napier, 1960a, 1960b; Beatty and Sharma, 1960), very little is known about the spermatozoa of free living mammal species. Systematically orientated studies include those of Hirth (1960) and Forman (1968) on Chiroptera, Hughes (1964; 1965) and Biggers and De Lamater (1965) on marsupials and Genoways (1973) on the rodent genus *Liomys*. There appears to be no published data relating to comparative systematic aspects of sperm morphology within the Leporidae.

Recently, Elder and Hsu (in press) using a modification of the ammoniacal silver staining procedure originally developed by Goodpasture and Bloom (1975) for the detection of nucleolar organizer regions in chromosomes, showed that silver staining patterns of mammalian spermatozoa reveal species specific and even strain specific differences. While the use of silver nitrate and other silver salts has been used to impregnate spermatozoa in the past (Hancock, 1957; Hancock and Trevan, 1957; Bishop and Walton, 1960), the differentiation of gross morphological features illustrated by Elder and Hsu (in press) is superior to that of other studies.

The aim of this part of the present investigation was to establish whether differences in the morphology and silver staining properties of spermatozoa exist in the South African Leporidae, and to ascertain if these differences would be of value in the systematic delimitation of the various taxa.

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MATERIAL AND METHODS

Testes with epidymes still attached were removed from all freshly killed mature males. Unfixed sperm smears using the conventional method for blood films were prepared as follows. A small incision was made in the cauda of one epididymis (the other being fixed, along with the testis, in Bouin's fluid for subsequent analyses), from which spermatozoa were extruded onto a clean microscope slide. Approximately 0,5 ml physiological saline (0,9% NaCl) was added to the semen, the suspension mixed gently, smeared and allowed to dry at room temperature before boxing.

Staining procedure:

Since silver nitrate tends to react with all organic material on a slide, an attempt was made to remove all unwanted background deposit by soaking the slides in a borate buffer for $\frac{1}{2}$ h at room temperature (0,1 M $Na_2 SO_4$ + 0,005 M $Na_2 B_2O_7$). This pretreatment helped greatly in the removal of slide artifact and considerably reduced unnecessary silver precipitation.

Slides were stained using the modified silver nitrate technique proposed by Elder and Hsu (in press). Two solutions were prepared:

(a) an aqueous silver nitrate solution comprising 1 g silver nitrate in 2 ml deionized water;

(b) a developing solution of 3% formalin first neutralised with sodium acetate and then adjusted to pH 5–6 using formic acid.

Approximately 0,01 ml of the neutralised formalin solution was added to the 50% aqueous silver nitrate which was then filtered through a Milipore syringe filter (0,22 µm porosity).

Individual slides were placed in moisture chambers constructed from plastic Petri dishes. Each dish contained a layer of absorbent filter paper at the bottom (to facilitate the retention of moisture in the chamber during incubation), as well as two glass grommets, used to suspend the slides above the wet filter paper.

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Sperm smears were flooded with the filtered aqueous silver nitrate solution, covered with 22 x 50 mm coverslips and positioned on the grommets. Distilled water sufficient to thoroughly wet the filter paper was added to the bottom of each chamber. The Petri dish lid was finally positioned over both the grommets and slide, and the moisture chamber and its contents incubated at 65°C for 1–3 h. The slides were continuously monitored during this period to ensure correct staining intensity, following which the cover slips were removed and the preparations thoroughly rinsed in distilled water.

Where possible silver stained preparations were made from at least one specimen of each representative species per collection locality. In addition to the silver stained preparations, a small percentage of the smears were stained by briefly immersing the slides (2–5 min) in a 2% giemsa solution diluted with 0,067 M phosphate buffer (Sörensen's buffer) pH 6,8.

Measurements and statistical comparisons:

Three measurements were recorded from each silver stained spermatozoon used for statistical comparisons. This was done using a Kellner 10 X eyepiece screwmicrometer and a 100 X oil immersion objective. These measurements, which are diagrammatically represented in Fig. 39, were length of the head including the acrosome, width of the head and length of the midpiece. The latter measurement included the neck, since this structure was not clearly differentiated in most silver stained spermatozoa. Measurements of the principal piece and end piece were not made since there appeared to be some distortion in these regions in certain preparations. Furthermore the end piece, which does not stain darkly with silver nitrate in the species examined, was not clearly visible, making accurate measurement of this portion of the flagellum difficult.

In the case of *L. saxatilis*, measurements of the spermatozoa and evaluations of the silver staining patterns were made from 15 specimens, each representing a collection locality for which suitable material was available. These localities were grouped into three broad geographic areas:

(a) the southwestern Cape Province (Calvinia, Nieuwoudtville, Springbok, Sutherland),

(b) the central areas of South Africa incorporating the Orange Free State (Rouxville, Winburg), western Transvaal (Bloemhof) and southern Natal (Matatiele) and

(c) the more northern sample areas of Natal (Babanango, Hilton, Magudu, Winterton) and the Transvaal (Derdepoort, Kaalplaats, Lydenburg).

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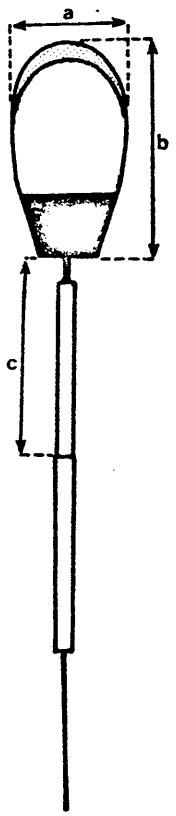

Fig. 39: *Diagrammatic representation of the spermatozoan measurements utilised in the present study: a = width of head; b = length of head including the acrosome; c = length of midpiece including the neck.*

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This was done in order to establish whether either quantitative or qualitative differences in sperm morphology could possibly provide support for Petter's (1972) recognition of the northern forms as a distinct species, *L. crawshayi.*

Mensural data for *L. capensis* and *P. rupestris* were derived in each case from specimens from five randomly selected collection localities, for which suitable material was available. Data on the remaining species was extremely limited, being restricted to a single specimen in both *P. crassicaudatus* and *B. monticularis* and two specimens in *P. randensis.*

Statistical analyses were performed on the University of Pretoria IBM 370 computer using the Statistical Analysis System (Version 79.3, SAS Institute Inc., Box 8000, Cary, North Carolina, USA). Comparisons between the means were computed using the analysis of variance procedure (ANOVA) in conjunction with Duncan's multiple range test.

RESULTS AND DISCUSSION

Shape of the acrosome:

Based on the shape of the acrosome, the spermatozoa from the South African Leporidae can be divided into one of two groups:

(a) Those with an acrosome which does not extend anteriorly much beyond the leading edge of the nucleus, being similar in morphology to that of the domestic rabbit *Oryctolagus* (see Bishop and Walton, 1960). This group includes *L. saxatilis* and *L. capensis.*

(b) The second group comprising the three species of *Pronolagus* and the monotypic *Bunolagus*, is characteristed by a relatively large acrosome which is very pronounced anteriorly.

This criterion provides unequivocable separation of the South African *Lepus* from the other endemic Leporidae. Statistical comparisons and the silver staining properties of the spermatozoa of the species incorporated in each group are presented below.

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GROUP A: *L. saxatilis* and *L. capensis:*

L. saxatilis: The results of an analysis of variance and Duncan's multiple range test of specimens representing three broad geographic areas are given in Table 14. Two of the three parameters tested, length and width of the head, showed significant differences between groups, while differences in the length of the midpiece, which was found to be highly variable, were nonsignificant.

Specimens from the more northern sample areas, which could possibly be classified as *L. crawshayi* using Petter's key (Petter, 1972; See Chapter 2), have sperm heads which are significantly broader than those of specimens from the more central and southern regions of South Africa. However, there appeared to be no consistent trend in either remaining quantitative character, nor in the silver staining patterns (Fig. 40). Consequently, the pooling of data from the different geographic regions for comparisons with *L. capensis* was considered justified.

Silver nitrate stained spermatozoa characteristic of *L. saxatilis* are shown in Fig. 40, while a typical giemsa stained preparation of the same species is shown in Fig. 41. The silver nitrate has differentiated a variety of structures which are not clearly visible in those preparations treated with giemsa. Furthermore, it is evident that no apparent differences exist in the silver staining patterns of specimens representative of the geographic regions referred to above.

One of the most striking features of the silver nitrate stain is its differentiation of the post acrosomal sheath into a strongly argentophilic band towards the middle of the sperm head, which is followed by a less densely staining region posteriorly. This differentiation, which is also clearly visible in *L. capensis* (Fig. 42) can easily be lost through understaining, a factor which results in only the outer borders of the sheath being darkly contrasted. Other structures which were heavily impregnated with silver were the outer fibres of the principal piece and annulus.

The acrosome which, as was previously indicated, is not pronounced in either South Africa *Lepus* species or in several extralimital species (Dott, *in litt.* *), is usually lightly

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Table 14:

Results of an analysis of variance and Duncan's multiple range test conducted on spermatozoan data obtained from specimens of *L. saxatilis* originating from localities which have been grouped into three broad geographic areas (*Southern* = Calvinia, Nieuwoudtville, Springbok, Sutherland; *Central* = Matatiele, Rouxville, Bloemhof, Winburg; *Northern* = Babanango, Derdepoort, Hilton, Kaalplaats, Lydenburg, Magudu, Winterton). Vertical lines to the left of the array of means connect maximally nonsignificant subsets at the 5% level;
P = level of significance.

Parameter measured	Number of sperm measured	Ranked means (µm) and corresponding geographic areas		P
Length of head	105	6,8	*Northern*	0,008
	60	6,8	*Southern*	
	60	6,6	*Central*	
Width of head	105	3,8	*Northern*	0,002
	60	3,7	*Southern*	
	60	3,6	*Central*	
Length of midpiece	60	11,5	*Southern*	0,08
	105	9,6	*Northern*	
	60	9,3	*Central*	

stained by silver nitrate, as was the midpiece. The neck region, which was usually distinguishable in giemsa stained preparations being giemsa negative in most cases, was frequently not clearly differentiated following silver staining.

L. capensis: The silver staining properties of *L. capensis* spermatozoa (Fig. 42) in respect of the post acrosomal sheath, acrosome, midpiece, principal piece and annulus are similar to those described for *L. saxatilis*. However, one of the most striking and characteristic features of *L. capensis* spermatozoa is the presence of usually two or more strongly argentophylic bead-like structures on the equatorial segment.

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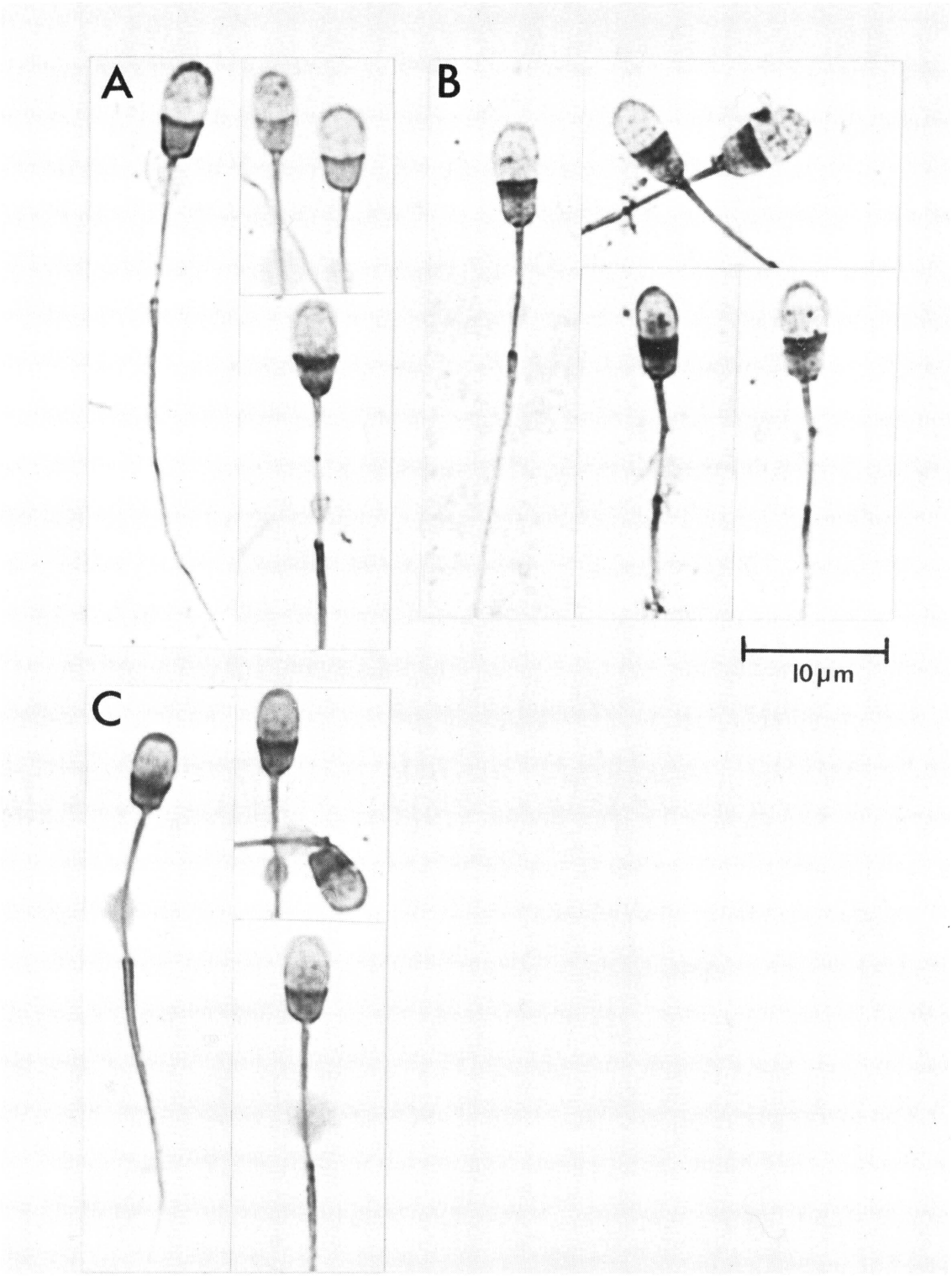

Fig. 40: *Silver stained spermatozoa characteristic of* L. saxatilis *specimens originating from: (A) the Cape Province, (B) the Orange Free State, southern Natal and western Transvaal and (C) the central and northern regions of Natal and the remainder of the Transvaal.*

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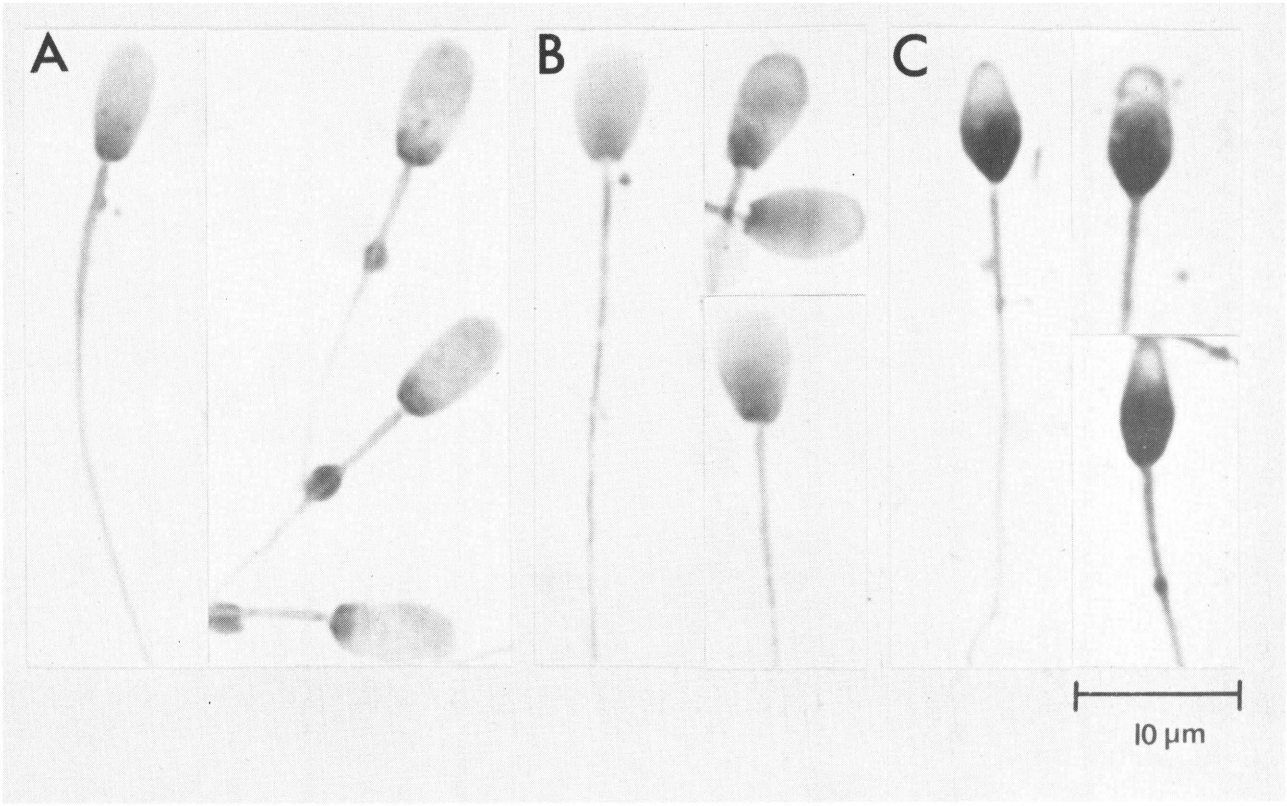

Fig. 41: *Giemsa stained spermatozoa of* L. saxatilis *(A),* L. capensis *(B) and* P. rupestris *(C).*

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These structures which are giemsa negative (Fig. 41), have also been observed in certain, but not all, strains of laboratory mice examined by Elder and Hsu (Elder, *in litt.* *). This coupled to the fact that these structures were invariably present in the silver stained *L. capensis* preparations examined, clearly indicate that they are not artifacts.

While no reference could be found to the presence of similar structures in other species, Dott (*in litt.*) has suggested that the silver may be deposited in pits which are sometimes visible in spermatozoa that are examined under the electron microscope. He is further of the opinion that the deposition of silver in the region of the equatorial segment "is displaying areas of the spermatozoa which are occupied by material which is of lower viscosity than its surroundings."

Alternatively, it is possible that the visualization of these structures in *L. capensis* and their obvious absence in *L. saxatilis* may be due, in part, to underlying biochemical differences between the taxa.

While the basis for the silver staining reaction has been attributed to both acidic and basic proteins by different authors (Hubbel, Rothblum and Hsu, 1979; Jones and Wallace, 1980), Buys and Osinga (1980) have suggested that the reaction in the nucleolar organizer regions of mitotic chromosomes may depend on protein bound sulphydryl and disulphide groups. Elder and Hsu (in press) have extended this argument to silver staining patterns obtained on 12 species of mammals. They have shown that in most cases the silver tends to impregnate those regions of the sperm which are rich in disulphide and sulphydryl moeities, although they do suggest that other cytochemical reactions may be important, particularly in the post acrosomal sheath which is not characterised by an overly high sulphydryl content (Elder and Hsu, in press).

Comparisons of the spermatozoa of *L. saxatilis* and *L. capensis*:

In addition to the striking differences of the silver stained spermatozoa of *L. saxatilis* and *L. capensis* with regard to the argentophilic bead-like structures discussed above, the two species differ markedly in the shape of the basal portion of post acrosomal sheath. This structure, which tends to be characteristically flatter in *L. capensis*, has a more convex appearance in *L. saxatilis* (Figs. 40 and 42).

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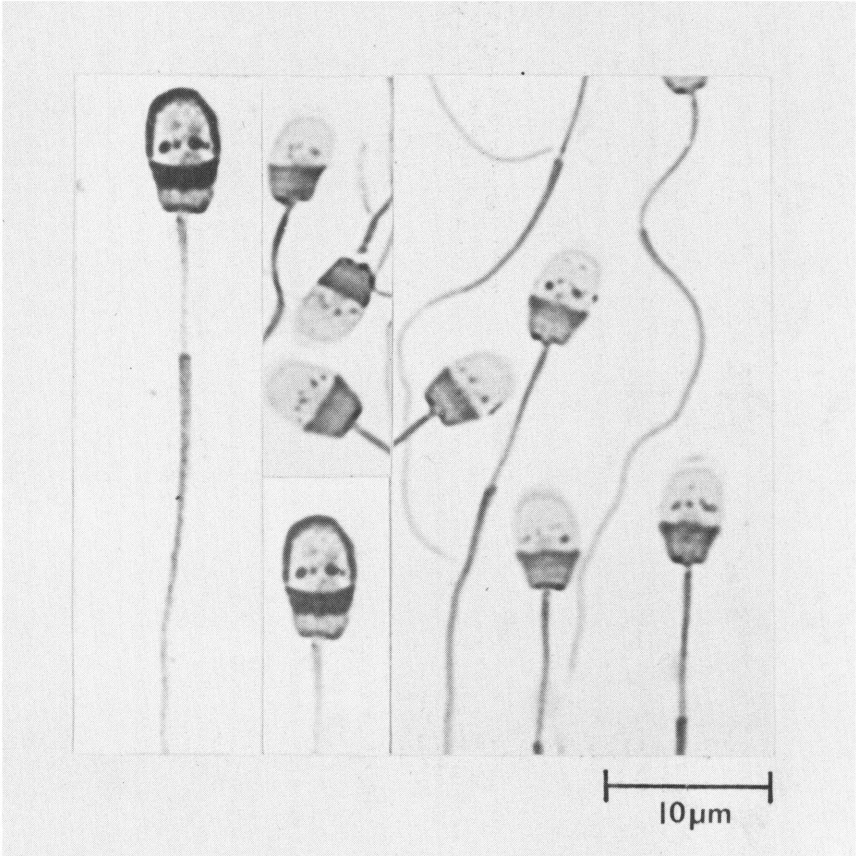

Fig. 42: *Silver stained spermatozoa of* L. capensis *showing the characteristic argentophilic bead-like structures on the equatorial segment of this species.*

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The results of an analysis of variance performed on the spermatozoan mensural data obtained from specimens of each species are presented in Table 15. These results illustrate consistent significant quantitative differences in the spermatozoa of the two species. *L. saxatilis* is characterised by spermatozoa which have significantly greater head and midpiece lengths than *L. capensis*, while the converse holds for the width of the sperm head, *L. capensis* being significantly wider in this parameter.

Table 15:

Results of an analysis of variance conducted on spermatozoan data obtained from specimens of *L. saxatilis* and *L. capensis*. P = Level of significance.

Parameter measured	Number of sperm measured	Mean (μ m) and corresponding species		P
Length of head	225	6,7	*L. saxatilis*	0,0001
	75	6,5	*L. capensis*	
Width of head	75	4,0	*L. capensis*	0,0001
	225	3,7	*L. saxatilis*	
Length of midpiece	225	10,0	*L. saxatilis*	0,03
	75	8,5	*L. capensis*	

GROUP B: *P. crassicaudatus, P. rupestris, P. randensis* and *B. monticularis:*

The silver staining properties of the species comprising this category (those with prominent acrosomes: *P. crassicaudatus, P. rupestris, P. randensis, B. monticularis*) are in most cases very similar, making accurate distinction between the sperm of these taxa highly unreliable (Figs. 43 and 44).

The deposition of silver in the post acrosomal regions of the four species is similar to that shown for *Lepus*, although the argentophilic band in the anterior portion is not as pronounced. Other morphological features of the sperm which were differentiated with silver in *Lepus*, such as the acrosome, midpiece, annulus and the dense outer fibres of

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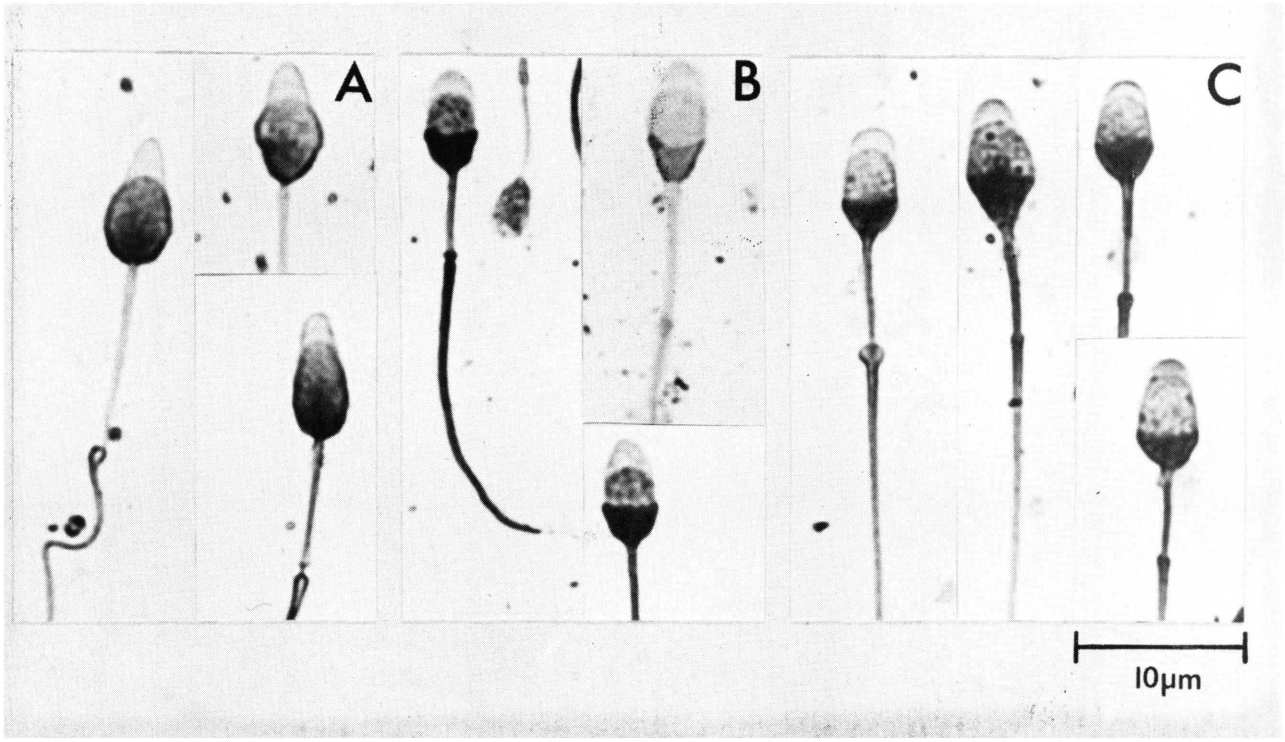

Fig. 43: *Silver stained spermatozoa of* P. crassicaudatus *(A),* P. randensis *(B) and* P. rupestris *(C) clearly showing the pronounced acrosomes of these species.*

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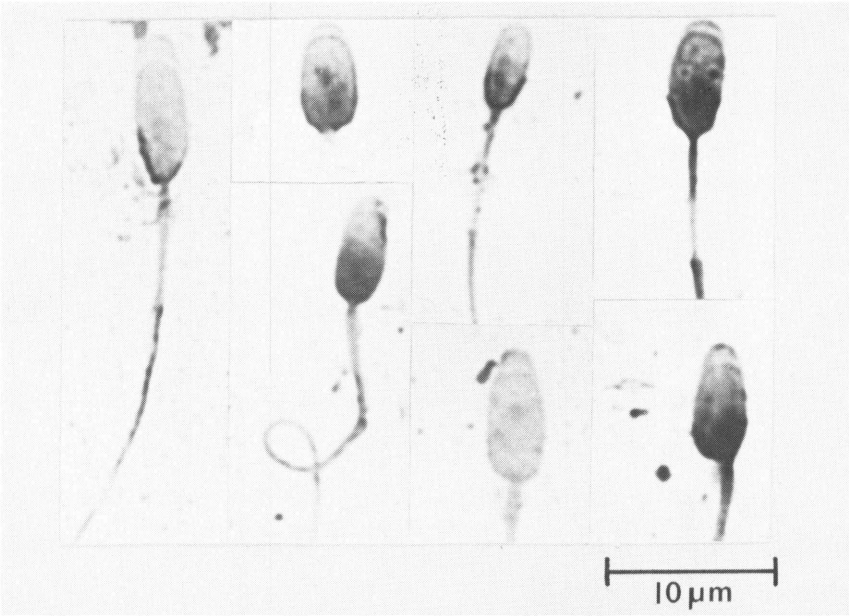

Fig. 44: *Silver stained spermatozoa of* B. monticularis *showing pronounced acrosomes.*

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the principal piece, are similarly stained in the other South African Leporidae.

Giemsa stained spermatozoa of *P. rupestris* are presented in Fig. 41 (p. 121). Unfortunately the small sample sizes in the other taxa (*P. crassicaudatus* n = 1, *P. randensis* n = 2, *B. monticularis* n = 1) precluded the examination of similar preparations in these species. The neck region which was not clearly differentiated by the silver nitrate in either *Pronolagus* or *Bunolagus* was however visible in most giemsa preparations of *P. rupestris*. The staining properties of this region therefore seem similar to that of *Lepus*.

Comparisons of the spermatozoa of the three *Pronolagus* species and *Bunolagus*:

Although no accurate distinction based on qualitative characters could be made from the spermatozoa of the four species comprising this group, quantitative differences are apparent. However, these must be viewed with caution since in most cases these are based only on one or two specimens.

Table 16:

Results of an analysis of variance and Duncan's multiple range test conducted on spermatozoan data obtained from specimens of *P. crassicaudatus*, *P. randensis* and *B. monticularis*. Vertical lines to the left of the array of means connect maximally nonsignificant subsets at the 5% level; P = level of significance

Parameter measured	Number of sperm measured	Mean (µm) and corresponding species		P
Length of head	75	6,8	*P. rupestris*	0,0001
	30	6,7	*P. crassicaudatus*	
	30	6,6	*B. monticularis*	
	30	6,4	*P. randensis*	
Width of head	30	3,8	*P. crassicaudatus*	0,0004
	75	3,7	*P. rupestris*	
	30	3,6	*P. randensis*	
	30	3,6	*B. monticularis*	
Length of midpiece	30	8,8	*P. crassicaudatus*	0,0001
	30	7,4	*B. monticularis*	
	75	7,1	*P. rupestris*	
	30	6,4	*P. randensis*	

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The result of an analysis of variance conducted on the four taxa are presented in Table 16. The results indicate that the *P. crassicaudatus* spermatozoa differ from those of the other species by having a significantly wider head and longer midpiece. Conversely, *P. randensis* has spermatozoa which are of the smallest of the species examined in respect of these parameters. The two remaining species *P. rupestris* and *B. monticularis* differ significantly from each other in both the length of the sperm head and midpiece, while neither taxon differs significantly in spermatozoan head width.

TAXONOMIC CONCLUSIONS:

The difference in acrosome shape between *Lepus* and *Bunolagus* is particularly pertinent and is considered to provide strong support for the currently accepted generic status of the latter. Based on the existing data, *Bunolagus* appears closer in sperm morphology to *Pronolagus,* than either *Lepus* or *Oryctolagus.*

The silver staining properties of the South African *Lepus* provide unequivocal evidence of two phena, corresponding to the species *L. saxatilis* and *L. capensis.* In addition, this distinction is further enhanced by consistent quantitative differences in sperm morpholgy, all of which support the classification based on morphometric and incisor tooth pattern data.

Although the silver staining properties of the *P. crassicaudatus, P. randensis, P. rupestris* and *B. monticularis* spermatozoa appear similar, quantitative differences in sperm dimensions are evident. Unfortunately, pending further investigations involving larger numbers, the small sample sizes for the majority of these species must temper any conclusions regarding the taxonomic significance of these differences.

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CHAPTER 6

ELECTROPHORESIS

INTRODUCTION

Electrophoretic techniques have been applied widely in mammalian systematic studies to help clarify taxonomic relationships between closely related species and to study the variation both within and between populations. The majority of this research has undoubtedly centered on small mammal taxonomy as exemplified in the extensive investigations of the rodent genera *Mus* and *Peromyscus* (Selander, Hunt and Yang, 1969; Selander and Yang, 1969; Selander, Smith, Yang,Johnson and Gentry, 1971; Smith, Selander and Johnson, 1973; Johnson and Packard 1974; Berry, 1977 and references therein).

Considering the ease with which material can be obtained from certain species, relatively little electrophoretic research appears to have been carried out using feral lagomorphs. Johnson (1968) studied the serum proteins and haemoglobin of *Brachylagus* and four species of *Sylvilagus.* More recently Chapman and Morgan (1973) used electrophoresis as a systematic aid in their work on *S. audobonii, S. floridanus* and *S. transitionalis,* while Glover, Smith, Ames, Joule and Durbach (1976) studied genetic variation in populations of *Ochotona princeps* (Family Ochotonidae) using similar techniques. Genetic variation in feral populations of *Oryctolagus* has been analysed by Coggan, Baldwin and Richardson (1974), Richardson, Rodgers and Hewitt (1980) and Richardson (1980).

In the present study, electrophoretic investigations were conducted to ascertain whether biochemical data would be of value in the delimitation of the South African Leporidae and to assess where possible, the degree of congruence between this and the other systematic parameters used.

MATERIAL AND METHODS

This study is based on blood samples from 203 specimens representative of all the South African leporid species with the exception *B. monticularis.* Blood was drawn by cardiac puncture immediately after shooting, using a 21 gauge needle and 10 ml syringe

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with heparin as anticoagulant. Samples were separated into plasma and erythrocytes and deep frozen at approximately $-20^{o}C$. Prior to freezing, the erythrocytes were washed with a 0,9% saline solution to remove all traces of plasma, then lysed by adding one volume of distilled water and one drop of saponine to one volume of erythrocytes.

Plasma and haemolysate samples were absorbed on 7 x 4 mm pieces of Whatman number three filter paper, which were placed into a transverse cut made 5 cm from the cathodic edge of each gel. The gels were prepared using a 14% starch concentration (35 g Connaught hydrolysed starch: 250 ml buffer) and poured in 13,5 cm x 20 cm x 0,6 cm perspex molds. During the electrophoresis the gels were covered with plastic sheeting and damp cheese cloth to prevent overheating or alternatively, especially in the case of the erythrocytic enzymes, electrophoresis was conducted in a cold room at $4^{o}C$. Following electrophoresis, 3 mm slices were cut from each gel and incubated in the appropriate staining solution.

Fourteen proteins were initially examined, however, only 9 of these appeared with sufficient consistency and clarity to be scored; these are listed in Table 17 together with the corresponding buffer and stain procedures used. The five proteins which could not be recognised as comprising a distinct band or bands and which had to be excluded were the cathodic form of carbonic anhydrase (CA-2), glucose-6-phosphate dehydrogenase (G-6 PD), isocitrate dehydrogenase (IDH), the most anodal form of malate dehydrogenase (MDH-3), and phosphohexose isomerase (PHI). It seems likely that the inconsistency and lack of clarity found in these proteins are attributable to denaturation changes. These probably resulted from unavoidable and, in some exceptionally remote areas, protracted delays before blood samples collected in the field could be suitably frozen.

Genotype frequencies were calculated in suitable polymorphic systems. The numbers of individual genotypes expected under Hardy Weinberg equilibrium were computed and compared to the number of phenotypes actually observed. The chi-square test was used to test for genetic equilibrium. Systems characterised by absent or too few phenotypic classes (for example transferrin) were not tested for Hardy Weinberg equilibrium since little meaningful data can be derived from these computations.

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Table 17:

Proteins studied and references to buffers and stains employed together with optimum running conditions for test material

Protein examined and source	Buffer system, stain and running conditions
1. *Albumin (ALB):* Plasma diluted 1:5 with distilled H_2O	**Buffer:** Lithium hydroxide (Selander *et al.*, 1971 buffer system 2) **Stain:** 5% amido black 10 B and nigrosin solution. To visualise bands gel distained in water/methanol/acetic acid destainer (4:4:1) **Potential:** 350 V for 5h or until borate line reaches the anodal sponge.
2. *Transferrin (TRF):* Undiluted plasma	As for albumin
3. *Carbonic anhydrase (CA-1):* Haemolysate supernatant (0,2 ml ethanol + 0,4 ml haemolysate + 0,2 ml chloroform – thoroughly mixed in Vortex mixer, refigerated for 30 min. and centrifuged at 3000 rpm to obtain clear supernatant)	**Buffer:** See Schoeman (1977) **Gel buffer:** 20 ml stock solution A + 20 ml stock solution B + 210 ml deionised H_2O. (Stock A: 10,5 g monohydrate citric acid / 1000 ml; B: 23,0 g tris-hydroxymethyl-aminomethane / 1000 ml) **Bridge buffer:** 18,55 g boric acid + 4,0 g sodium hydroxide/ 1000 ml H_2O **Stain:** As for albumin **Potential:** 150 V for 30 min, remove paper inserts then 300 V until borate line reaches the anodal sponge.
4. *Esterase–1 (ES–1):* Haemolysate	**Buffer:** Tris hydrochloric acid (Selander *et al.*, 1971 buffer system 2) **Stain:** Esterase haemolysate stain (Selander *et al.*, 1971) but substitute α napthyl acetate for α-napthyl propionate and Fast Blue B Salt for Fast Garnet GBC **Potential:** 250 V for 1 h.

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Protein examined and source	Buffer system, stain and running conditions
5. *Esterase 2 (ES–2):* Haemolysate supernatant (See carbonic anydrase)	**Buffer:** As for carbonic anydrase **Stain:** As for esterase-1 **Potential:** 150 V for $\frac{1}{2}$ h.
6. *Haemoglobin (HB):* Haemolysate diluted approximately 1:10 with distilled H_2O	**Buffer:** Discontinuous Tris-boric acid (Buschmann, 1963): See Kristjansson (1963) for bridge buffer **Stain:** Staining not necessary **Potential:** 200 V for $1\frac{1}{2}$ h.
7. *Indophenol oxidase (IPO):* Haemolysate	As for PGD. IPO usually appears in gels stained for dehydrogenase activity (G–6PD, PGD, MDH) and can be clearly visualised by overstaining for one of these enzymes.
8. *Malate dehydrogenase (MDH–1):* Haemolysate	**Buffer:** Continuous tris-citrate (Selander *et al.*, 1971 buffer system 4) **Stain:** See Harris and Hopkinson (1976) **Potential:** 200 V for 3 h.
9. *6-Phosphogluconate dehydrogenase (PGD):*	**Buffer:** Tris-maleate (Selander *et al.*, 1971 buffer system 9) **Stain:** See Harris and Hopkinson (1976) **Potential:** 100 V for 5 h.

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RESULTS AND DISCUSSION

Monomorphic proteins: Six of the nine proteins which could be consistently scored in each specimen of this study proved to be monomorphic in both *Lepus* and *Pronolagus.*

Albumin: This protein was present as a single intensely staining band situated anodally to all the other observed plasma proteins. While no interspecific variation was apparent, marked differences in the mobility of this fraction were observed between the genera. Direct side by side comparisons on the same gel indicate that the *Pronolagus* albumin is slower migrating than that of *Lepus.*

Carbonic anhydrase: Two carbonic anhydrase systems designated CA-1 and CA-2 were noted on tris citric acid gels stained with a nonspecific amido black nigrosin solution. The positions of both systems were verified as suggested by Schoeman (1977). Treatment of test gels with a 10^{-5}M acetazalomide solution, although failing to completely inhibit the staining of the enzyme, resulted in a distinct lightening of the otherwise darkly staining bands.

The conspicuous anodal CA-1 system was determined as being monomorphic in the respective species, while the cathodally migrating CA-2 appeared polymorphic. However, the poor resolution of the latter made the accurate scoring of phenotypes highly unreliable, resulting in the exclusion of the CA-2 enzyme from detailed analyses. It is interesting to note however, that a similar CA-2 polymorphism was noted in feral *Oryctolagus* populations (Richardson *et al.*, 1980).

Although no interspecific variation was evident in the *Pronolagus* CA-1, this system was found to be most useful for the delimitation of species within *Lepus.* All *Lepus* specimens examined in the present study could be assigned to one of two phenotypic classes: aa, represented by a single dark staining anodal band characterised all specimens regarded as belonging to *L. saxatilis,* while the smaller *L. capensis* were recognised by the complete absence of a band, oo (Fig. 45). The latter phenotype is presumed to be the result of the expression of a null or silent allele CA-1^{o}, and since the heterozygous condition (a single band of reduced intensity) was never observed, it can be concluded that the alternative alleles are fixed in each species.

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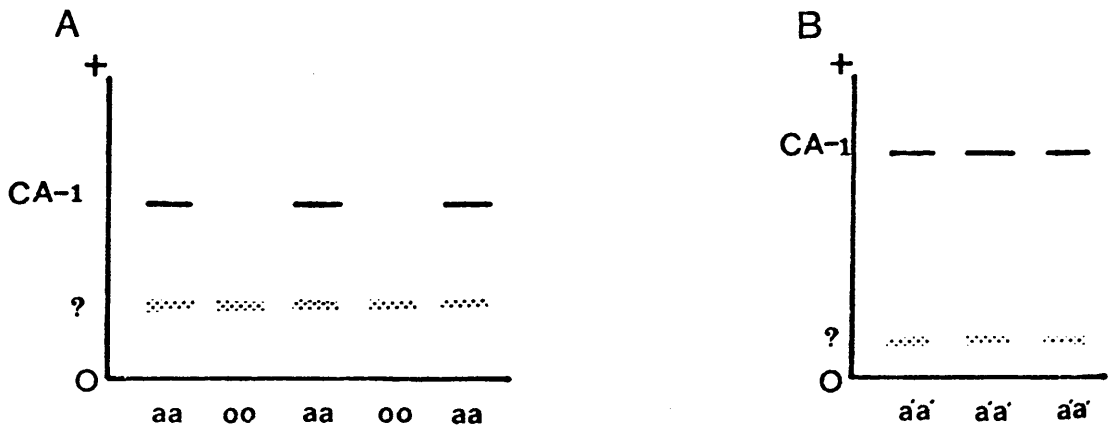

Fig. 45: *Electrophoretic variation in carbonic anhydrase (CA-1) of the South African* Lepus *(A: aa* =L. saxatilis; oo =L. capensis*) and the corresponding position of the faster migrating* Pronolagus *system (B). Also shown are the approximate positions of the unknown erythrocytic protein which was visualised when staining for CA-1 in both genera.*

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It is possible that the expression of CA–1^{O} as a silent phenotype in *L. capensis* is restricted to red blood cells and that detailed investigations of other tissues (kidney, liver) may reveal the presence of the enzyme in this species. This would parallel the situation described by Daly (in Richardson, *et al.*, 1980) for the ES–1 locus, where the allele is expressed as a null phenotype in red blood cells, but as a B phenotype in liver homogenates.

In addition to the conspicuous monomorphic anodal CA–1, a faint inconsistant but obviously polymorphic protein system of similar mobility to that of ES–1 was noted (Fig. 45). Attempts to establish any homology between the ES–1 phenotypes and those of the unknown erythrocytic protein within specimens, were confounded due to the poor resolution of the latter. The system was however completely insensitive to acetazalomide inhibition.

Marked intergeneric differences in the mobility of CA–1 were observed. In side by side comparisons between the two genera tested, the *Pronolagus* CA–1 fraction was invariably anodal to that of *Lepus*.

Haemoglobin: Haemoglobin was monomorphic in both *Lepus* and *Pronolagus* being represented by a single band in all specimens examined. As with albumin and carbonic anhydrase, marked differences in the mobility of the protein exist between the two genera, with the *Pronolagus* fraction characterised by greater mobility than that of *Lepus*. No interspecific differences were observed in either genus.

Indophenol oxidase: This prominent monomorphic protein appears on all gels that were stained for dehydrogenase activity, then left in the light. Due to the thickness of the IPO bands in both genera, the possibility that the system may in fact be represented by several bands of closely similar mobility cannot be discounted. However, despite the use of different buffer systems more detailed resolution of these bands could not be obtained.

Malate dehydrogenase: Two forms of this enzyme MDH–1 and MDH–3 both apparently NAD dependent, were observed in haemolysates of specimens of both *Lepus* and *Pronolagus*. MDH–3, which is located more anodally than MDH–1, was too irregular to be scored confidently in most samples. MDH–1, despite some sub-banding, was determined to be monomorphic. Migration rates were uniform in both genera.

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6-Phosphate dehydrogenase : Using sodium phosphate gels, PGD appears as a single anodal band in haemolysates of all specimens. Mobility differences between PGD of *Lepus* and *Pronolagus* are clearly distinguishable, with the enzyme located more anodally in the latter genus.

Polymorphic proteins: Three protein systems were found to be polymorphic in the test material, each being represented by bands of differing mobility.

Transferrin: Extensive transferrin polymorphism was shown involving seven phenotypes in *Lepus* and four in *Pronolagus* (Fig. 46). Homozygous phenotypes which are characterised by a single band, are for the most part absent in the sample material of both genera, indicating the relative rarity of these fractions, while heterozygotes have two bands of differing mobility. These configurations are suggestive of a monomeric structure.

As with most of the monomorphic systems previously described, intergeneric differences in the migration of transferrin were noted. In side by side comparisons it would appear that *Lepus* migrates further than *Pronolagus*, although this was obscured to some extent by the polymorphism exhibited in both taxa.

Five alleles were present in the *L. capensis* material used in this study, four of which are shared by *L. saxatilis* (Table 18). One allele, TRF-1^{e}, present in the heterozygous condition (de) in a single *L. capensis* specimen, was not found in *L. saxatilis.*

Table 18:

Allele frequencies at the TRF-1 locus among specimens of *L. saxatilis* and *L. capensis.*

		TRF-1				
Species	Sample size	TRF-1^{a}	TRF-1^{b}	TRF-1^{c}	TRF-1^{d}	TRF-1^{e}
L. saxatilis	117	0,74	0,03	0,01	0,21	–
L. capensis	46	0,85	0,05	0,01	0,08	0,01

Three transferrin alleles were noted in *Pronolagus* specimens used in this study (Table 19). One of these TRF$-1^{c'}$ was absent in *P. randensis* since all four specimens representative of this species were heterozygous a'b'. The TRF$-1^{a'}$ allele predominates in material representative of *P. crassicaudatus* and *P. rupestris.*

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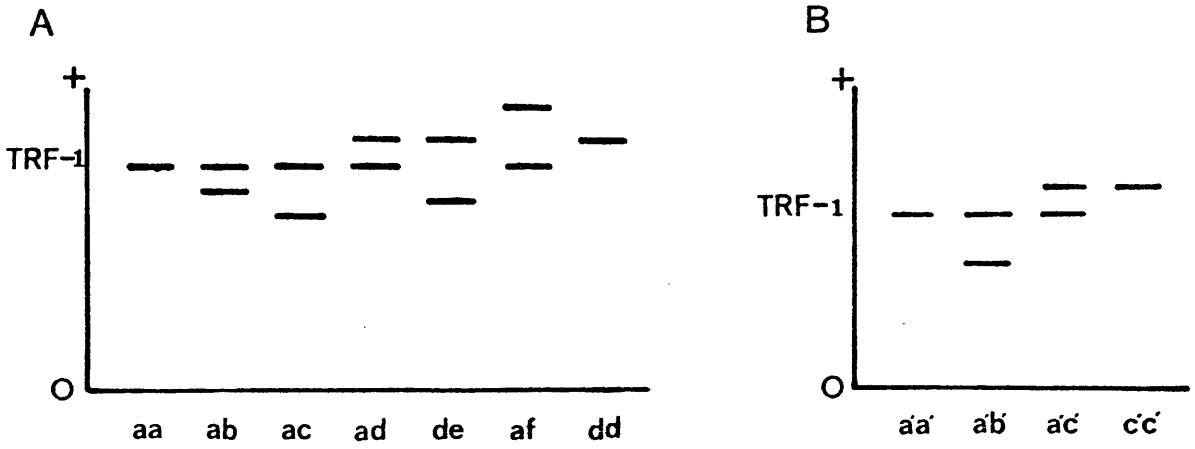

Fig. 46: *Electrophoretic variation in transferrin among specimens of* Lepus *(A) and* Pronolagus *(B). Observable differences in the migration rate of transferrin were evident between the two genera.*

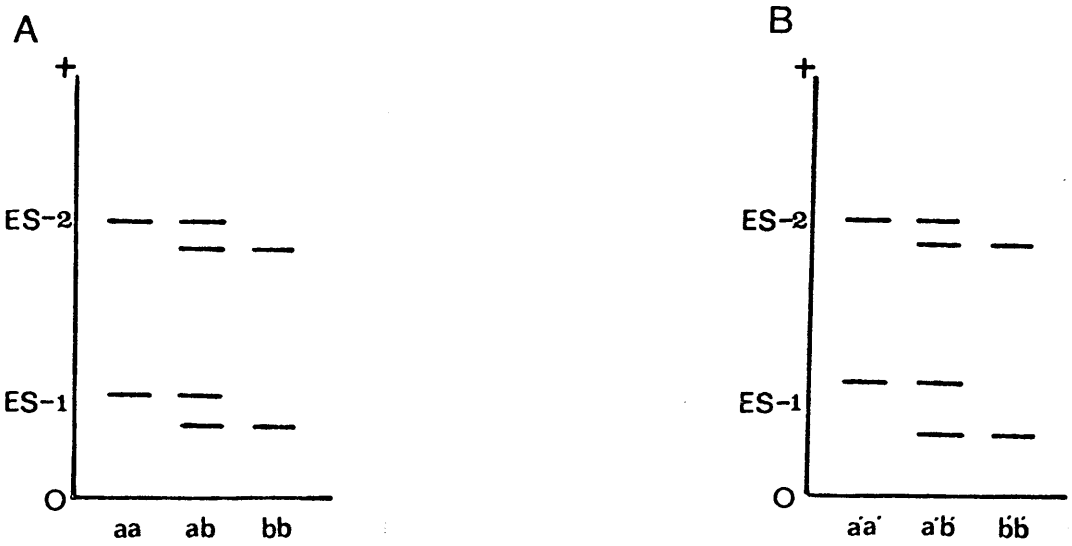

Fig. 47: *Electrophoretic variation in esterase systems 1 and 2 among specimens of* Lepus *(A) and* Pronolagus *(B). Slight differences in the migration rate of ES–1 were evident between representatives of the two genera. This was most pronounced in the width of the heterozygote* a'b'.

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Table 19:

Allele frequencies at the TRF–1 locus among specimens of *P. crassicaudatus, P. rupestris* and *P. randensis.*

		TRF–1		
Species	Sample size	TRF–$1^{a'}$	TRF–$1^{b'}$	TRF–$1^{c'}$
P. crassicaudatus	12	0,79	0,04	0,17
P. rupestris	24	0,85	0,08	0,06
P. randensis	4	0,50	0,50	–

Esterase–1: Haemolysates electrophoresed using a tris-HC1 buffer revealed a prominent esterase system which migrates only a short distance from the origin, but which is situated anodally to the haemoglobin bands (Fig. 47). Homozygotes are characterised by a single band while in the heterozygous condition, two bands are evident indicating a monomeric structure for the protein. This variation is attributed to the products of two codominant alleles segregating at a single locus. Slight intergeneric differences in the mobility of the fractions exist, with the *Pronolagus* phenotypes a' a' and b' b' respectively faster and slower than the equivalent *Lepus* bands. These differences although small in respect of the homozygotes were particularly noticeable in the heterozygous condition, with the distance between the two bands being clearly wider in *Pronolagus* than *Lepus.*

In both *L. saxatilis* and *L. capensis* the predominant allele at the ES–1 locus was ES–1^{b}. All three phenotypes were represented in the test material and their distribution conforms to that expected under Hardy Weinberg conditions (Table 20).

Table 20:

Allele frequencies and distribution of the three ES–1 phenotypes in *L. saxatilis* and *L. capensis.*

Species and sample size		Phenotypes			Allele frequencies		
		aa	ab	bb	ES–1^{a}	ES–1^{b}	Chi-square
	Observed	8	44	65			
L. saxatilis					0,26	0,74	0,038
n = 117	Expected	7,91	45,02	64,07			
	Observed	5	24	17			
L. capensis					0,37	0,63	0,658
n = 46	Expected	6,30	21,45	18,26			

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In the case of the *Pronolagus* species, not all the expected ES–1 phenotypes were represented in the test material of the different taxa. Both *P. crassicaudatus* (n = 12) and *P. randensis* (n = 4) were characterised entirely by specimens that were heterozygous at this locus, while all three phenotypes were evident in the slower *P. rupestris* sample, probably indicating that the discrepancies in the former species are due to the vagaries of sampling. The distribution of the observed phenotypes for the ES–1 locus in *P. rupestris* conform closely to that expected from the Hardy Weinberg equilibrium (Table 21).

Esterase–2: The variation found in haemolysate supernatants electrophoresed on tris citrate gels is illustrated in Fig. 47. This anodal esterase system designated ES–2, appears to be regulated by two codominant autosomal alleles, with homozygotes possessing one band and heterozygotes two, a configuration which is considered indicative of a monomeric protein structure (Harris and Hopkinson, 1976). No intergeneric differences in the mobility of the ES–2 phenotypes were noted.

The predominant allele in both *L. saxatilis* and *L. capensis* was found to be ES–2^{a}. While the distribution of the three ES–2 phenotypes in *L. capensis* follows that expected from the Hardy Weinberg equilibrium, those of *L. saxatilis* do not conform (Table 22). This may be indicative of some isolation between populations. However, as this was not substantiated by any of the other systems it was not considered sufficient cause to treat the *L. saxatilis* populations as distinct entities.

Table 21:

Allele frequencies and distribution of the three ES–1 phenotypes in *P. crassicaudatus*, *P. rupestris* and *P. randensis*

Species and sample size		Phenotypes			Allele frequencies		
		a^1a^1	a^1b^1	b^1b^1	ES–1^{a^1}	ES–1^{b^1}	Chi-square
P. crassicaudatus n = 12		–	12	–	0,50	0,50	
	Observed	1	10	13			
P. rupestris					0,25	0,75	0,296
n = 24	Expected	1,50	9,00	13,50			
P. randensis n = 4		–	4	–	0,50	0,50	

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Table 22:

Allele frequencies and distribution of the three ES–2 phenotypes in *L. saxatilis* and *L. capensis*.

Species and sample size		Penotypes			Allele frequencies		
		aa	ab	bb	ES–2^a	ES–2^b	Chi-square
	Observed	28	78	11			
L. saxatilis					0,57	0,43	15,296
n = 117	Expected	38,01	57,35	21,63			
	Observed	20	24	2			
L. capensis					0,70	0,30	2,526
n = 46	Expected	22,54	19,32	4,14			

Three ES–2 phenotypes were represented in the *P. crassicaudatus* and *P. rupestris* samples while the four *P. randensis* specimens proved to be heterozygous at this locus (Table 23). Analysis of genetic equilibrium showed that the observed distribution of ES–2 phenotypes in the two species tested, *P. crassicaudatus* and *P. rupestris*, conforms to that expected from the Hardy Weinberg equilibrium.

It is interesting to note that unlike ES–1 the predominant allele in *P. crassicaudatus* and *P. rupestris* differ, with ES–$2^{b'}$ being more frequent in the former and ES–$2^{a'}$ in the latter. However, considering the small number of specimens in each category this difference is probably attributable to sampling artifact.

TAXONOMIC CONCLUSIONS

Although small sample sizes and in particular the low number of suitable polymorphic systems resulting from this study curtailed detailed investigations of genetic similarities between taxa (See Ferguson, 1980), the electrophoretic data do provide valuable information, albeit limited, on some aspects of the taxonomy of the species examined.

Marked intergeneric differences in the mobility of albumin, carbonic anhydrase, haemoglobin, 6-phosphate dehydrogenase and transferrin provide strong support for the generic status of *Lepus* and *Pronolagus*.

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Table 23:

Allele frequencies and distribution of the three ES–2 phenotypes in *P. crassicaudatus*, *P. rupestris* and *P. randensis*.

Species and sample size		Phenotypes			Allele frequencies		
		ES–$2^{a'}$	ES–$2^{a'b'}$	ES–$2^{b'}$	ES–$2^{a'}$	ES–$2^{b'}$	Chi-square
	Observed	1	8	3			
P. crassicaudatus					0,42	0,58	1,650
n = 12	Expected	2,12	5,85	4,04			
	Observed	8	13	3			
P. rupestris					0,60	0,40	0,421
n = 24	Expected	8,64	11,52	3,84			
P. randensis		–	4	–	0,50	0,50	
n = 4							

Of the nine proteins examined only CA–1 proved useful in species identification, this being restricted to the genus *Lepus*. Based on this system, all the *Lepus* specimens analysed could be assigned to one of two groups corresponding to the species *L. capensis* and *L. saxatilis*, while no evidence was found to support Petter's contention (Petter, 1972) that the northern forms of *L. saxatilis* should be regarded as a distinct species, *L. crawshayi*. These data thereby confirm conclusions drawn from results obtained for the comparative incisor, sperm and morphometric analyses.

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CHAPTER 7

INTERSPECIFIC COMPARISONS

INTRODUCTION

One of the aims of the present investigation was to provide a reliable identification scheme to the South African Leporidae. Of importance in the construction of keys for the identification of these species are comprehensive descriptions of pelage characteristics and indications of the variability in cranial and external body measurements data observed in the respective taxa. This information is provided in the descriptions and comparison that follow, and which are buttressed by a synthesis of the systematic parameters presented in preceeding chapters.

MATERIAL AND METHODS

Descriptions of pelage characteristics were made largely from field specimens collected during the course of this study, supplemented where necessary by museum material and colour slides from freshly culled specimens.

Univariate analyses generating standard statistics including the mean, range, standard error, standard deviation and coefficient of variation were computed using the CONDESCRIPTIVE subprogramme of the Statistical Package for the Social Sciences (SPSS; Nie *et al.*, 1975). Computations were made on all external and cranial measurements available for the different species, and include specimens lacking full data sets. These data are presented in Appendix II. Unlike the other taxa, the clinal variation illustrated by most characters in *L. saxatilis* confounded calculations of overall mean values for this species. Consequently, standard statistics were computed for specimens from the respective sample localities. The localities were then ranked in accordance with the pattern of variation illustrated in the measurement, greatest length of the skull.

In order to provide an additional means of species identification using cranial measurements alone, use was made of the two-group discriminant function analysis provided by the DISCRIMINANT subprogramme of the SPSS. This analysis provides a reliable means of species identification which can be used independently of the other diagnostic features presented in this study, and is particularly pertinent to museum taxonomy where fresh

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material for many of the investigatory procedure (sperm morphology, karyology, electrophoresis) is not available.

Standardised and unstandardised coefficients and discriminant scores generated by the programme are presented in the text, as are the histograms of discriminant scores resulting from each two group analysis. As indicated by Dippenaar (1980), an unknown specimen can be identified using the results of the analysis, the methodology of which is most clearly illustrated by way of example. Assume that the accurate species identification of a *Lepus* specimen is to be resolved. This can be done by recording the suite of selected cranial measurements which, based on the discriminant function analysis of material used in this study, provide maximum separation between groups, in this case *L. saxatilis* and *L. capensis*. The selected measurements from the unknown are multiplied by the corresponding unstandardised coefficients, summated and added to the constant. The unknown's resultant discriminant score is then plotted in the accompanying *L. saxatilis: L. capensis* histograms. In this way, the unknown will group within the species with which it has the greatest affinity.

The collection localities of specimens utilised in this study are not comprehensive enough to permit detailed descriptions of species distribution patterns. However, by combining data from this study with the results of mammal surveys in the provinces by Pringle (1974; Natal), Lynch (1975; Orange Free state), Rautenbach(1978a; Transvaal), Stuart and Lloyd (1979; Cape Province), an attempt has been made to illustrate the broad geographic areas associated with each species.

KEYS, SPECIES COMPARISONS AND DISTINGUISHING CHARACTERS:

Key to the genera (minimum and maximum values are $\bar{X} \pm 2SD$):

1. Tail white below, black above; groove on anterior surface of principal upper incisor invariably filled with cement; chromosomal complement 2n=48; spermatozoa characterised by cap-like acrosome, not pronounced anteriorly.

   ——— *LEPUS*

1[1] Tail not as above being uniformly reddish brown, brown or dark brown; groove on anterior surface of principal upper incisor never filled with cement; chromosomal complement never 2n=48; spermatozoa characterised by relatively large acrosome, pronounced anteriorly.

   ——— 2

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2. Ears short (63 – 106 mm); no evidence of black stripe running along lower margins of jaw towards base of ear; mesopterygoid space always narrower than minimal length of hard palate (38,6 to 87,7% of length of hard palate); no interparietal bone present in adults; chromosomal complement 2n=42; relatively widely distributed throughout South Africa where suitable habitat occurs, occupying rocky outcrops or koppies.

——— *PRONOLAGUS*

Ears long (109 – 124 mm); dark brown stripe running along lower margins of jaw towards base of ears; mesopterygoid space always wider than minimal length of hard palate (124,9 to 180,5% of hard palate); well defined interparietal bone in adults; chromosomal complement 2n=44; limited distribution occupying riverine scrub in central and southern Karoo, Cape Province.

——— *BUNOLAGUS*

GENUS *LEPUS* LINNAEUS:

Lepus Linnaeus, *Syst. Nat.* 10th ed. 1:57, 1758. Genotype *L. timidus.*

L. capensis Linnaeus, *Syst. Nat.* 10th ed. 1:58, 1758.
Type locality: Cape of Good Hope (Cape Province).

Description:

Smaller species (Appendix II, Table 1); limbs long and fragile in appearance. Fur fine; forehead, back and sides vary from grizzled buffy grey with pinkish wash in southern regions to grizzled buffy grey in northeast; grizzling due to juxtapositioning of terminal tips of guard hairs which are buffy subterminally ending in black tips; underfur greyish; underparts variable. In south and central Cape Province chin, chest and abdomen rich pinkish buff as are limbs; anal region and bottom of tail pure white; gular patch russet, fur of coarser texture than rest of underparts; laterally the pinkish buff contrasts with the grizzled appearance of the sides. In northeasterly regions chin, chest, abdomen, anal region, bottom of tail and inner surfaces of limbs pure white; gular patch ochraceous buff of similar texture to rest of underparts; lateral borders of abdomen and outside of limbs ochraceous buff separating white underparts from rest of body. Soles of feet well furred; ears sparsely haired with black tips; particularly striking in southern regions; eyes encircled in buffy or off-white; imme-

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diately above and slightly behind each eye is an elongate buffy or pinkish buff spot; white blaze or spot sometimes evident on forehead particularly in southwest Cape; nape patch varies from rufous in south to greyish buff in northeast; tail black above, white below and of silky texture.

Geographic range:

Relatively widely distributed throughout South Africa (Fig. 48) showing preference for short open grassland and sometimes open lightly wooded areas; sympatric with *L. saxatilis* throughout much of geographic range; occurs widely in Cape Province and much of Orange Free State, probably absent from eastern Cape Province, Ciskei, Transkei and northeastern Orange Free State; extends narrowly into western Lesotho but probably absent elsewhere; present in western Transvaal and in far northern Transvaal, absent from south and central Transvaal, the whole of Natal and Swaziland.

L. saxatilis F. Cuvier, *Dict. des Sci Nat.* 26 : 309, 1823.

Type locality: Cape of Good Hope (Cape Province).

Description:

Large species; most external and cranial measurements vary clinally along a SW–NE axis, but nevertheless larger on average in most measurements than *L. capensis* (Appendix II, Table 2). Fur coarse; forehead, back and sides grizzled buffy greyish; grizzling as in *L. capensis;* underfur grey; chin, chest, anal region, bottom of tail and inside of limbs white; gular patch grey to buffy grey, fur of coarser texture than rest of underparts; soles of feet well furred; ears sparsely haired, tips narrowly black; eyes encircled in off-white; sides of face and muzzle greyer than rest of upper parts, white blaze or star usually present on forehead and of varying conspicuousness; nape patch pink rufous; tail black above, white below.

Geographic range:

Widely distributed throughout South Africa and probably Lesotho and Swaziland; absent in heavily forested areas and the extreme northwestern Cape Province; less abundant than *L. capensis* in arid regions but nevertheless generally present (Fig. 49); catholic in habitat requirements, occurring in grassland with scrub associations, woodlands, rocky or stony ground, lower slopes of hillsides and on flats.

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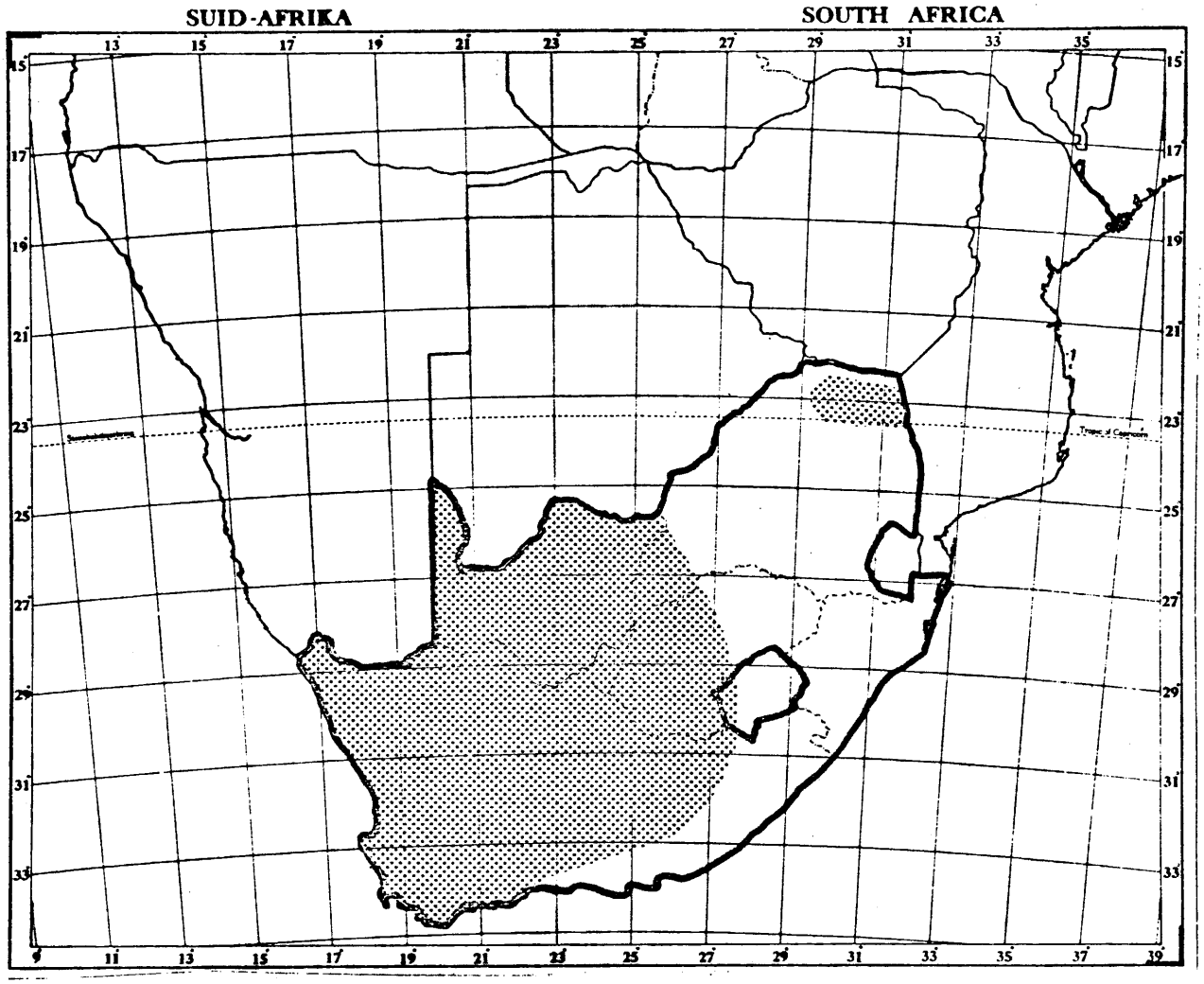

Fig. 48: *Distribution of* L. capensis *in South Africa based on specimens utilised in the present investigation and provincial mammal surveys.*

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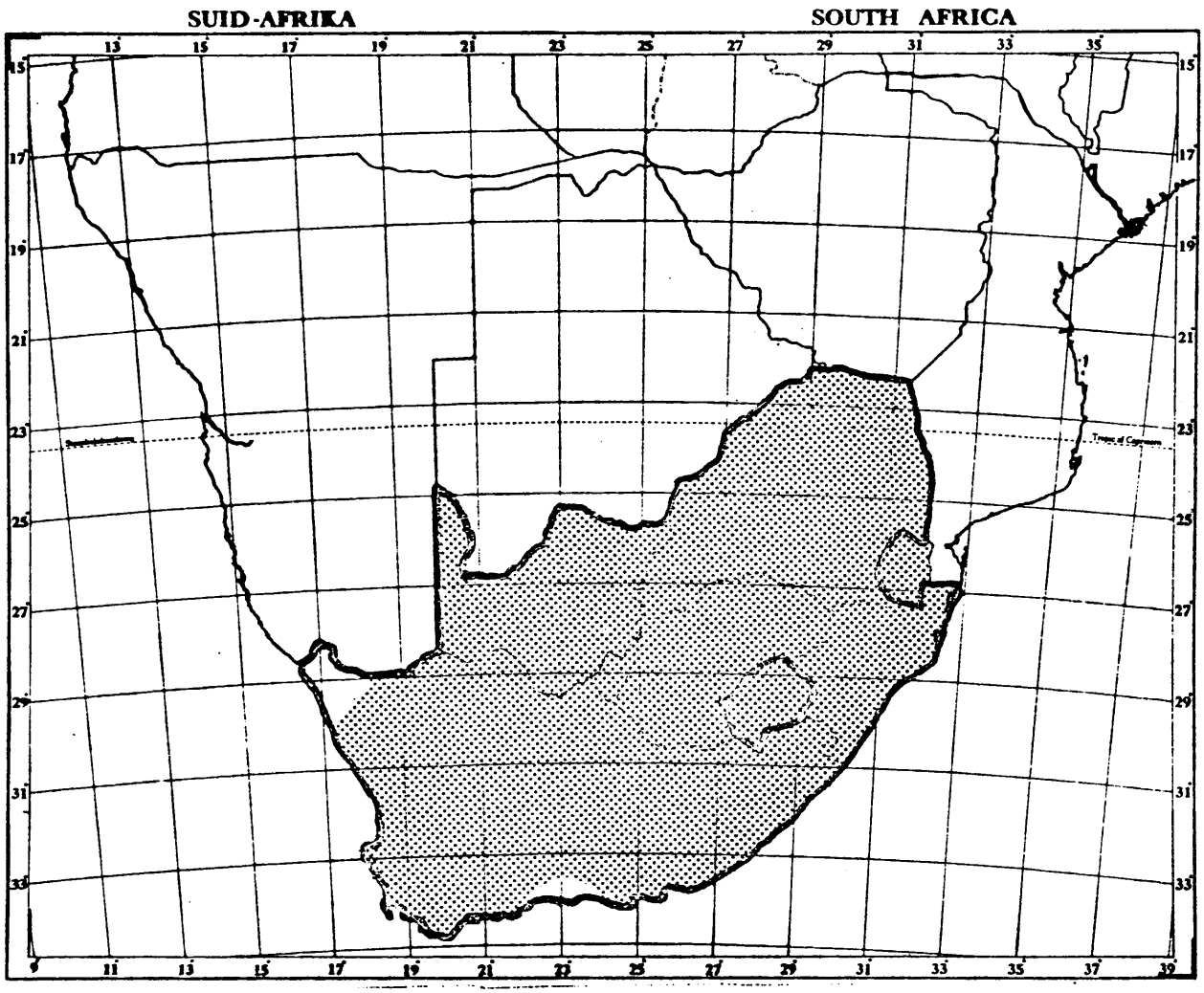

Fig. 49: *Distribution of* L. saxatilis *in South Africa based on specimens utilised in the present investigation and provincial mammal surveys.*

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Species comparisons and distinguishing characters:

Pelage: The two species can be readily distinguished on pelage characteristics. In *L. capensis* the chin, chest and abdomen are totally pinkish buff or else white, but with a diffuse ochraceous buff band laterally along the flanks separating the underparts from the grizzled greyish of the sides. No comparable markings separate the white underparts from the grizzled grey of the sides in *L. saxatilis.*

External and cranial measurements: *L. saxatilis* was generally larger in most external and cranial characters than *L. capensis* (Appendix II, Tables 1 and 2). Although the disparity in size between species is particularly evident in the southern part of their geographic ranges, the clinal variation in *L. saxatilis* (Chapter 2) renders this distinction in the more north-eastern areas less noticeable.

Discriminant function analysis: Seventy five *L. saxatilis* and 21 *L. capensis* specimens, each characterised by full data sets, were compared in a two group-discriminant function analysis. The 11 cranial parameters selected by this procedure to maximise the separation between groups and the corresponding standardised and unstandardised coefficients are presented in Table 24. The derived discriminant scores, plotted as frequency histograms (Fig. 50), clearly illustrate the separation between taxa. The scores of *L. saxatilis* ranged from −3,344 to 1,148 (mean score = −1,225) and those of *L. capensis* from 2,118 to 6,100 (mean score = 4,374). All cases were correctly classified *a posteriori.*

Karyotype: Karyotype analyses failed to reveal interspecific differences (Chapter 3) and therefore do not have diagnostic value. The diploid number was established as 2n=48. The male chromosome complement is comprised of eight pairs of metacentric to submetacentric autosomes and 15 pairs of subtelocentric and acrocentric autosomes. The X is a medium sized submetacentric and the Y a small telocentric chromosome. No detectable G- or C- band differences were noted in the species comparisons.

Incisor tooth patterns: The width of, and cross sections through the principal upper incisors are of diagnostic value (Chapter 4). *L. capensis* is characterised by relatively narrow principal upper incisor (2,3−2,8 mm; minimum and maximum values are $\bar{X} \pm 2SD$), which are squarish in cross section, and possess a simple lance shaped groove on the anterior surface containing cement. Conversely, those of *L. saxatilis* are more robust, being markedly broader on average than *L. capensis* (2,7−3,5 mm; minimum and maximum values are $\bar{X} \pm 2SD$) and more oblong in cross section. The enamel fold which contains cement is vari-

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Table 24:

Results of a two-group discriminant function analysis of *L. saxatilis* and *L. capensis*.

Measurement	*L. saxatilis* species means	*L. capensis* species means	Unstandardised coefficients	Standardised coefficients
Parietal length	20,7	19,0	– 0,184	– 0,286
Posterior muzzle breadth	21,3	18,7	0,187	0,244
Anterior frontal breadth	20,3	16,2	– 0,433	– 0,684
Basal cranial length	77,9	69,9	0,079	0,304
Maxillary premolar length	8,9	7,7	– 0,432	– 0,199
Principal I^1 breadth	3,1	2,5	– 1,903	– 0,342
Bizygomatic breadth	44,1	39,7	– 0,312	– 0,442
Mesopterygoid space	9,2	8,6	0,403	0,344
Posterior cranial height	19,0	17,4	0,157	0,205
Maximum mandibular height	45,5	38,8	– 0,236	– 0,469
Mandibular body breadth	6,3	5,4	– 0,691	– 0,229
		CONSTANT	33,061	

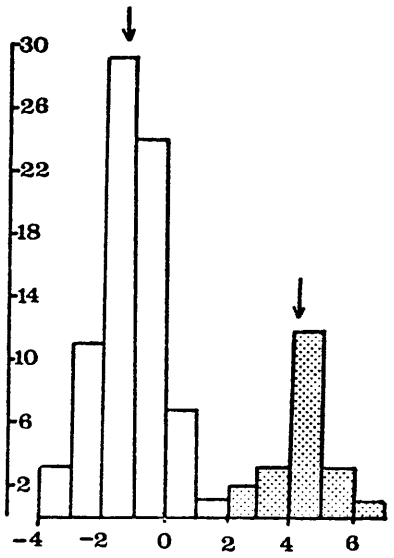

Fig. 50: *Histograms of discriminant scores (X axis = discriminant scores; Y axis = frequencies) from a two-group discriminant function analysis of* L. saxatilis *and* L. capensis. *Arrows indicate the positions of the mean discriminant scores.*

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able, being lance shaped in specimens from the southern and southwestern distribution areas tending towards increasing complexity northeastwards.

Spermatozoan morphology and silver staining patterns: The two species can be distinguished on the basis of both morphology and the silver staining patterns of the spermatozoa (Chapter 5). In *L. capensis* the head is relatively short but characteristically broad,with convex symetrical sides, while in *L. saxatilis* the head tends to be more elongate in appearance. Furthermore, the basal region of the post acrosomal sheath is flattened horizontally in *L. capensis*, as opposed to the more convex form of this structure in *L. saxatilis*. More pertinently, the silver stained spermatozoa provide unequivocable separation between the two species. *L. capensis* can be recognised by the presence of two or more strongly argentophilic bead-like structures on the equatorial segment which were never observed in the silver stained spermatozoa of *L. saxatilis*.

Electrophoresis: The use of erythrocytic carbonic anhydrase as a genetic marker is of diagnostic importance (Chapter 6). *L. saxatilis* is characterised by a monomorphic anodally migrating band which is absent in *L. capensis*.

Key to the species (minimum and maximum values are $\bar{X} \pm 2SD$)

1. Smaller species, length of adult skull 81,5 – 91,1 mm; upper principal incisor narrow across face 2,3 – 2,8 mm, squarish in cross section with simple enamel groove filled with cement; chest and abdomen either totally pinkish buff or white medially with diffuse ochraceous buff bands laterally demarcating the ventral and dorsal surfaces; silver stained spermatozoa with two or more argentophilic granules visible in equatorial segment; no detectable anodal erythrocytic carbonic anhydrase system using starch gel electrophoresis.

   ——— *L. CAPENSIS*

2. Larger species, length of adult skull 85,6 – 106,0 mm*[1]; upper principal incisor broad across the face,robust in appearance 2,7 – 3,5*[2] mm, appearing elongated in cross section with enamel fold variable and filled with cement; under parts

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Observed ranges based on individual specimens (Appendix II, Table 2):

*[1] Greatest length of skull 87,0 – 107,7 mm

*[2] Principal I^1 breadth 2,6 – 3,6 mm

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white without ochraceous buffy band separating this region from the greyish of upperparts; silver stained spermatozoa without argentophilic granules visible in equatorial segment; a single monomorphic,anodal erythrocytic carbonic anhydrase system visible using starch gel electrophoresis.

——— *L. SAXATILIS*

GENUS *PRONOLAGUS* LYON

Pronolagus Lyon, *Smithsonian Misc. Coll.* 45 : 416, 1904. Genotype *Lepus crassicaudatus.*

P. rupestris A. Smith, *S. Afr. Quart. J.* 2 : 174, 1834.
Type locality: "South Africa, rocky situations" Probably Van Rhynsdorp district (See Roberts, 1951).

Description:

Small species, limbs short, not robust, ears short, cranially smallest in most measurements (Appendix II, Table 3). Pelage variable and the species can only be described in very broad terms. Fur on back and sides grizzled rufous brown, rump and back of hind limbs brighter rufous but not pronounced; grizzling due to juxtapositioning of terminal tips of guard hairs which are buffy subterminally ending in black tips; underfur variable from pinkish buff to grey; chin buffy grey; gular patch similar in colour and texture to that of sides and back; cheeks greyish buff not in striking contrast with general colouring of sides and back; chest, abdomen, anal region and inner surfaces of limbs pinkish buff, abdomen lighter in midline; soles of feet well furred, ears sparsely furred; forehead and fur above nose with slight brownish wash; tail variable, sometimes large and bushy, of rufous colouring with distal two thirds dark brown, varying to small rusty brown with hairs at end of tail sometimes tipped in black; nape patch rufous.

Geographic range:

Relatively widely spread throughout South Africa and probably Lesotho (Fig. 51); as with all *Pronolagus* species narrowly confined to rocky situations on hill slopes and summits with adequate scrub or grass cover; occurs throughout much of Cape Province (except in heavily forested areas) and also Ciskei, Transkei, southern and central Natal; present in southeastern fringes of Transvaal and central and southern Orange Free State.

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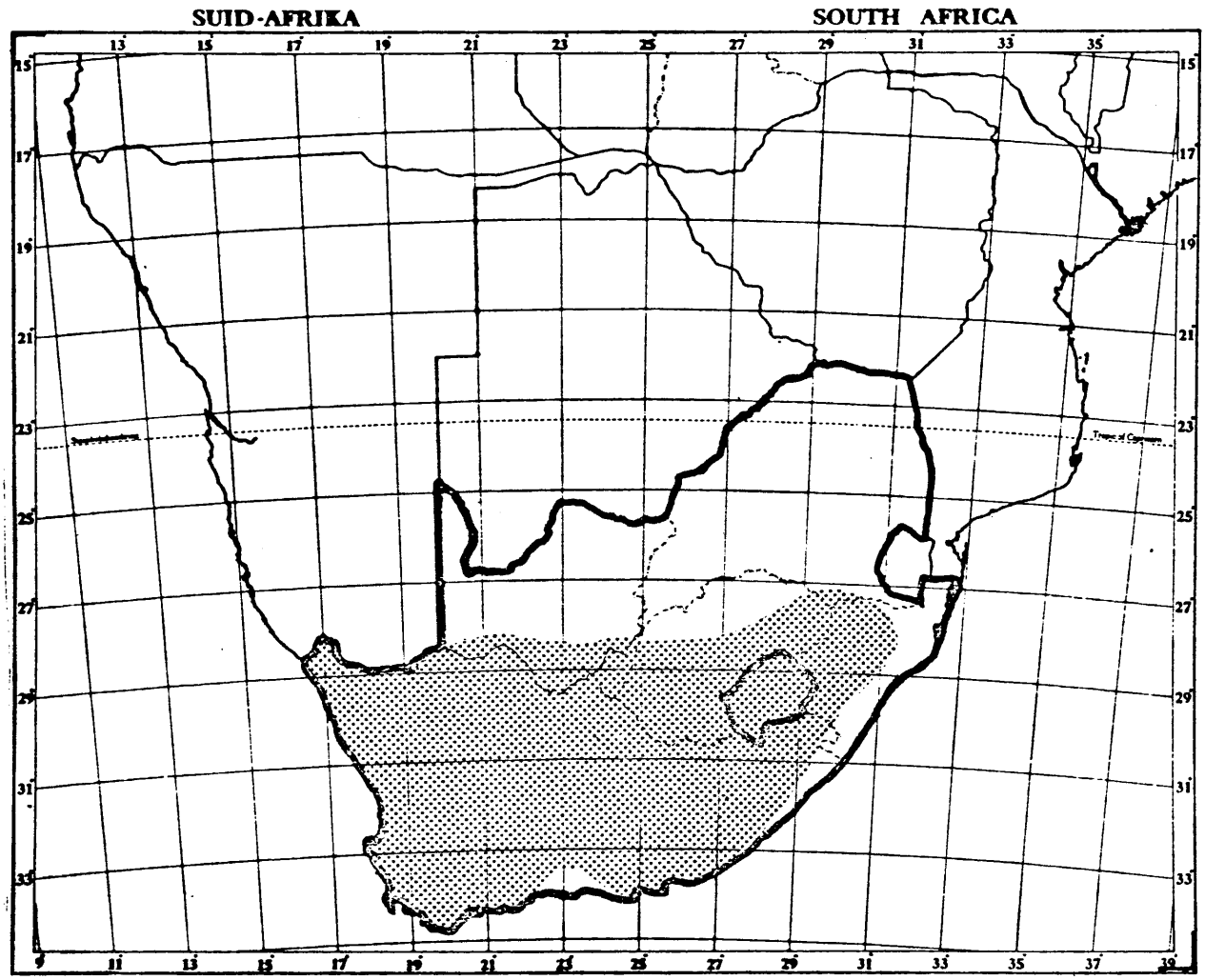

Fig. 51: *Distribution of* P. rupestris *in South Africa based on specimens utilised in the present investigation and provincial mammal surveys.*

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P. crassicaudatus (I. Geoffroy)

L. crassicaudatus I. Geoffroy, *Guérin's Mag. de Zool.*, 2 Cl 1, plate 9 and text, 1832.
Type locality: Port Natal (Durban, Natal).

Description:

Large species, limbs short and robust, ears short, cranially largest in most measurements (Appendix II, Table 3). Fur dense and coarse; back and sides grizzled rufous brown with blackish wash, rump and back of hind limbs brighter rufous attributable to brightening of underfur and less black tipped hairs; grizzling as in *P. rupestris;* underfur brown rufous above, grey beneath; chin grey white, this colour extending laterally in broad band running along borders of lower jaw to approach inferior margins of nape patch; gular patch of similar colouring and texture as back; chest, abdomen, anal region and insides of limbs rufous buff; outside of limbs and lateral borders of underparts darker rufous than rest of underparts; soles of feet well furred; ears sparsely furred; forehead and sides of face grey with brownish wash, more tawny above nose, tail short but not bushy, uniformly ochraceous brown; nape patch varies geographically from brown to grey, of taxonomic value at subspecies level (See Pringle, 1974).

Geographic range:

Restricted (Fig. 52), limited largely to Natal extending southwards into Transkei and fringes of northeastern Cape Province (?). Northwards distributed marginally into south-eastern Transvaal, southern Swaziland and southern Mozambique. Probably present in eastern Lesotho.

P. randensis Jameson

P. ruddi Jameson, *Ann. Mag. Nat. Hist.* (7) 20 : 404; 1907.
Type locality: Observatory, Johannesburg (Transvaal).

Description:

Size moderately large, ears short, cranially approaching *P. crassicaudatus* in most measurements (Appendix II, Table 3). Fur fine of silky texture; back and sides grizzled rufous brown, rump and back of limbs lighter in colour than back, with less black tipped hairs; grizzling as in *P. rupestris;* underfur brown rufous becoming lighter at base; chin whitish; cheeks, sides of neck and jaw characteristically light grey with some brown flecking in striking contrast to colouring of back and sides; gular patch comprises dense fur of similar texture and colouring to back and sides; chest, abdomen and anal region pinkish

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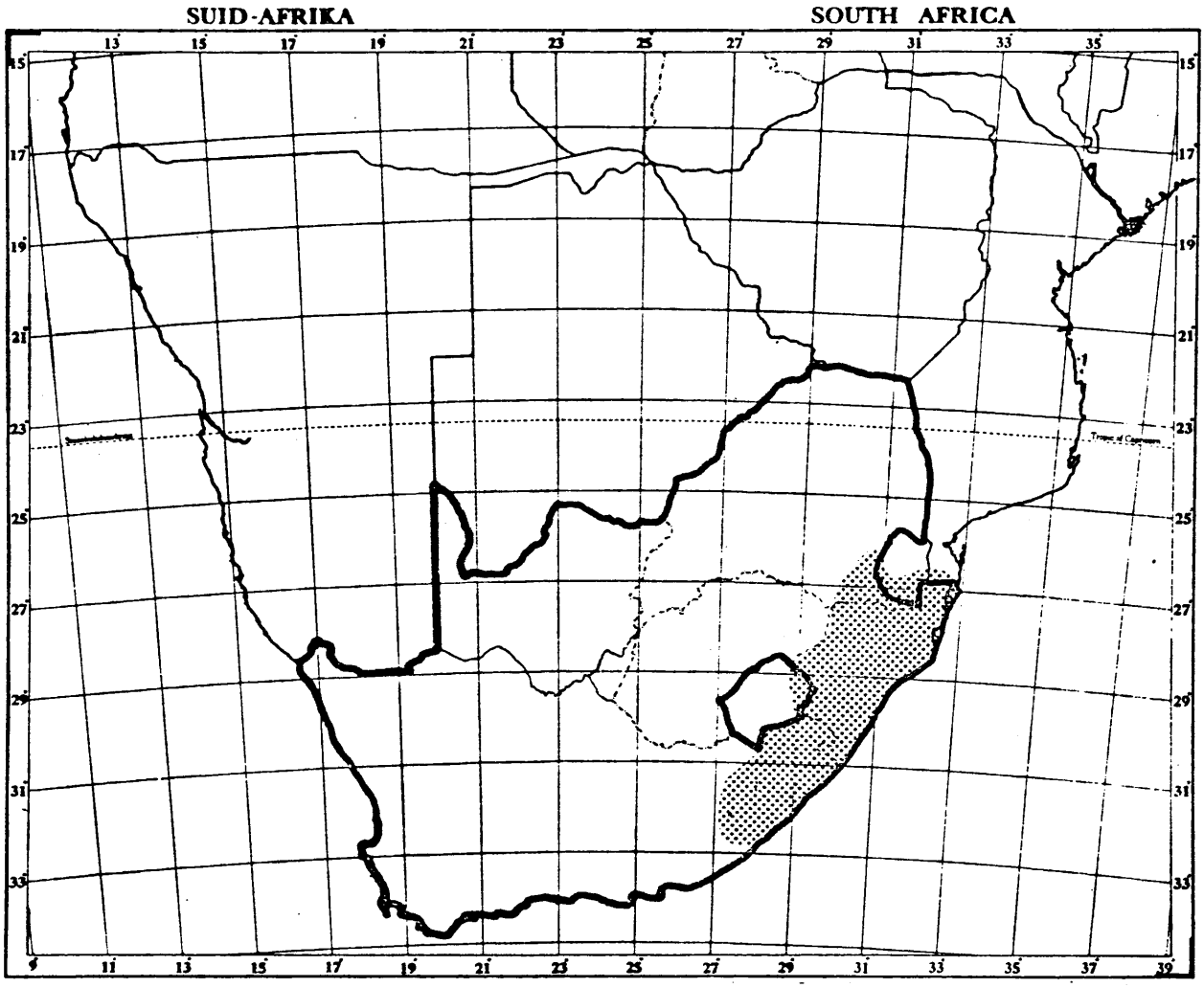

Fig. 52: *Distribution of* P. crassicaudatus *in South Africa based on specimens utilised in the present investigation and provincial mammal surveys.*

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buff, lighter near base; soles of feet well furred; ears sparsely haired, tips sometimes ribbed in black; fur on forehead with slight rufous wash; tail large and bushy, uniformly ochraceous brown, tip tinged with black; nape patch rufous.

Geographic range:

Limited distribution in South Africa (Fig. 53); occurs in central, southern and northern Transvaal, marginally in western Transvaal, but absent from extreme southwestern Transvaal.

Species comparisons and distinguishing characters:

Pelage: Although the back and sides of all three species are relatively similar in general colouring, interspecific pelage characters of important diagnostic value are evident in other regions of the body. *P. crassicaudatus* is characterised by a prominent broad white to grey stripe along the lower jaw. This stripe extends upwards towards the inferior margins of the nape patch, a pelage characteristic totally absent in either of the other species. Differences between *P. randensis* and *P. rupestris* are not as distinct being largely one of degree. Nevertheless, *P. randensis* can be readily distinguished by the striking light grey hue of the head region which contrasts strongly with the rest of the body; the same region in *P. rupestris* is much darker and consequently less noticeable.

External and cranial measurements: *P. rupestris* appears to be much smaller, on average, in most external and cranial characters than either *P. crassicaudatus* or *P. randensis* (Appendix II, Table 3). Differences between the latter species are for the most part slight. The overall size differences between the three taxa can be clearly illustrated by their greatest skull length measurements (all minimum and maximum values used below are $\bar{X} \pm 2SD$): *P. rupestris* 77,4 – 87,5 mm; *P. crassicaudatus* 86,3 – 96,3 mm; *P. randensis* 85,5 – 94,4 mm. In addition to differences in absolute cranial measurements between species, proportionate differences of diagnostic value were noted. *P. rupestris* appears to be characterised by robust bulla, both proportionately (bulla breadth x 100/greatest length of skull = 7,4 – 11,4%, bulla breadth x 100 / mandibular height = 16,5 – 25,1%), and in absolute measurements (bulla breadth 6,4 – 9,2 mm), while the two larger species, *P. crassidaudatus and P. randensis,* differ in the relative lengths of the maxillary premolar toothrow and the width of the mesopterygoid space. *P. crassicaudatus* appears to be larger in both measurements, when expressed as a percentage of frontal length, than *P. randensis* (19,9 – 32,5%; 11,7 – 19,9% *cf.* 16,9 – 22,9%; 9,4 – 14,7%).

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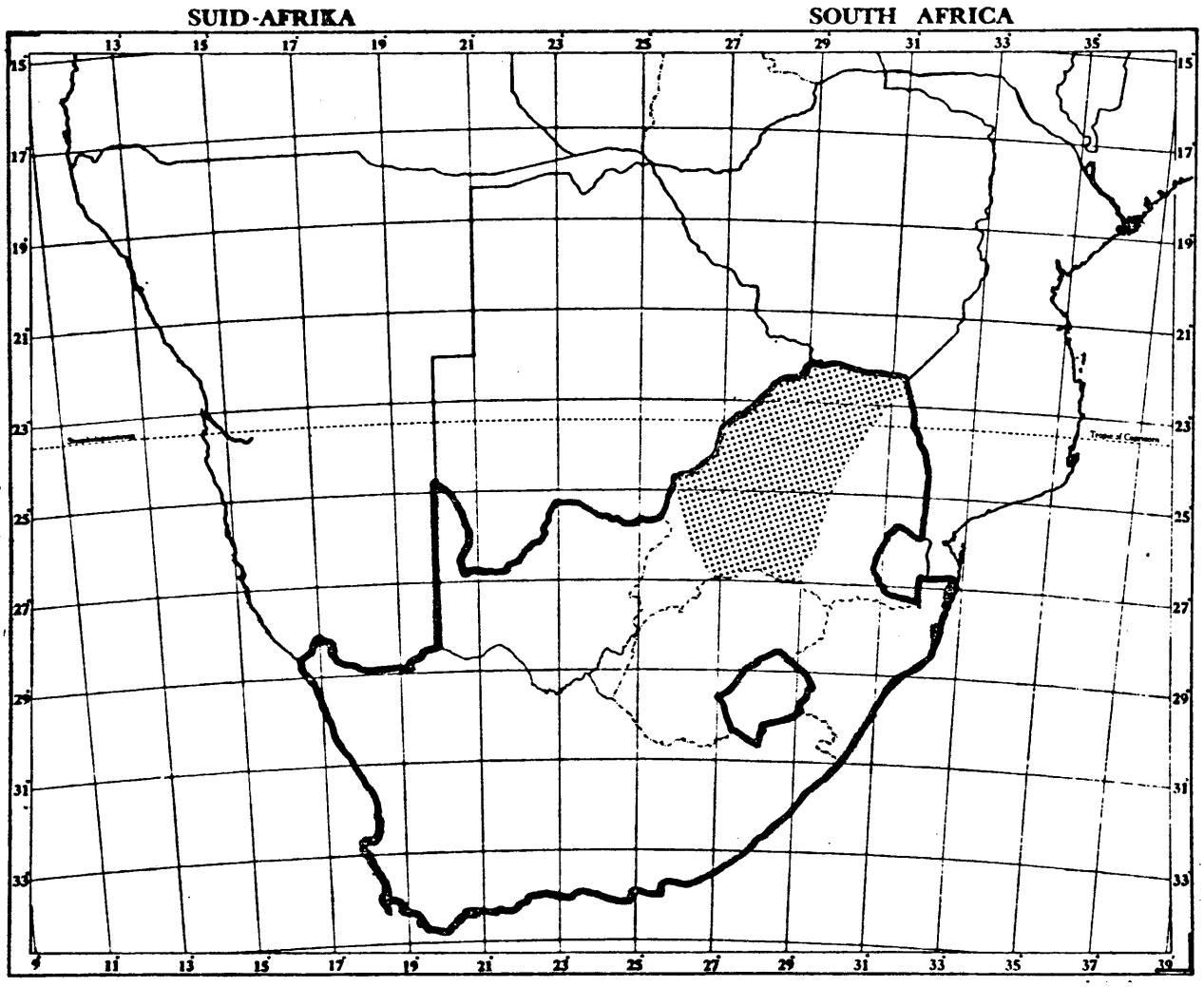

Fig. 53: *Distribution of* P. randensis *in South Africa based on specimens utilized in the present investigation and provincial mammal surveys.*

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Discriminant function analysis: The analyses were based on 14 *P. crassicaudatus,* 12 *P. randensis* and nine *P. rupestris* specimens each characterised by full data sets.

P. crassicaudatus : P. rupestris : The eight cranial characters selected by this procedure to maximise the separation between the species and the corresponding standardised and unstandardised coefficients are presented in Table 25. The standardised coefficients indicate that the breadth of a principal upper incisor, mandibular height and lower incisor breadth are primarily responsible for the discrimination between groups. The derived discriminant scores plotted as frequency histograms (Fig. 54) clearly illustrate the separation between the species. Discriminant scores for *P. crassicaudatus* range from −7,254 to −3,510 (mean score=−5,726) and those of *P. rupestris* from 6,388 to 9,579 (mean score =8,208). All specimens were correctly classified *a posteriori.*

P. randensis : P. rupestris : Fifteen characters were selected by the discriminant function procedure to maximise the separation between species. These measurements and the corresponding standardised and unstandardised coefficients are presented in Table 26. It is evident from the standardised coefficients that basal cranial length and palate incisor length were primarily responsible for the discrimination between these species. The derived discriminant scores plotted as frequency histograms (Fig. 55), clearly illustrate the separation. Discriminant scores for *P. randensis* ranged from −40,557 to −37,795 (mean score = −39,405) and those of *P. rupestris* from 28,202 to 31,655 (mean score = 29,554). All specimens were correctly classified *a posteriori.*

P. randensis : P. crassicaudatus : The nine cranial parameters selected by this procedure to maximise the separation between groups and the standardised and unstandardised coefficients are presented in Table 27. The standardised coefficients indicate that frontal length, mesopterygoid space and the mandibular toothrow length were primarily responsible for the discrimination between these species. The derived discriminant scores plotted as frequency histograms (Fig. 56) clearly show the separation between taxa. The scores for *P. randensis* ranged from −5,575 to −2,333 (mean score=−3,787) and those of *P. crassicaudatus* from 2,518 to 5,891 (mean score = 4,419). All specimens were correctly classified *a posteriori.*

Karyotype: Karyotype analyses conducted on specimens of all three species failed to reveal interspecific differences (Chapter 3) and therefore have no diagnostic value. The diploid number was established as 2n = 42. The male karyotype comprises 11 pairs of metacentric to submetacentric autosomes and nine pairs of subtelocentric and acrocentric

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Table 25:

Results of a two-group discriminant function analysis of *P. crassicaudatus* and *P. rupestris*

Measurement	*P. crassicaudatus* species mean	*P. rupestris* species mean	Unstandardised coefficient	Standardised coefficient
Posterior muzzle breadth	20,8	15,1	− 0,841	− 1,298
Palate incisor length	41,6	36,6	0,793	0,959
Palate vacuity length	26,4	24,3	− 1,552	− 1,315
Principal I^1 breadth	3,3	2,5	8,617	1,653
Posterior cranial height	15,8	15,7	1,037	1,078
Maximum mandibular height	44,4	37,7	− 1,038	− 1,599
I_1 breadth	3,3	2,6	− 8,800	− 1,651
Bulla breadth	6,0	7,5	2,369	1,152
			CONSTANT 36,149	

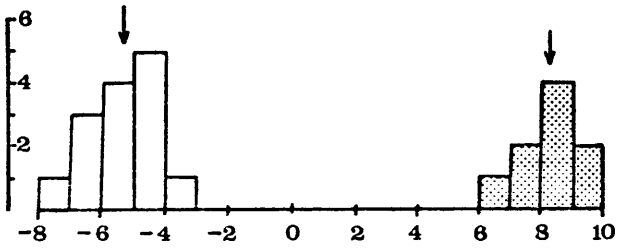

Fig. 54: *Histograms of discriminant scores (X axis = discriminant scores, Y axis = frequencies) from a two-group discriminant function analysis of* P. crassicaudatus *and* P. rupestris. Arrows indicate the positions of the mean discriminant scores.

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autosomes. The X chromosome is a medium sized submetacentric and the Y a small telocentric. No species specific G- or C-band differences were noted.

Table 26:

Results of a two-group discriminant function analysis of *P. randensis* and *P. rupestris*.

Measurement	*P. randensis* species mean	*P. rupestris* species mean	Unstandardised coefficient	Standardised coefficient
Muzzle length	39,9	35,9	–2,529	–6,289
Frontal length	40,1	34,1	2,222	4,869
Parietal length	17,9	18,1	–1,278	–1,961
Interauditory breadth	26,8	25,4	–4,845	–2,982
Basal cranial length	72,2	66,8	–11,888	–29,950
Palate incisor length	42,3	36,6	15,056	21,968
Palate vacuity length	26,6	24,3	–9,580	–9,145
Maxillary premolar length	7,9	7,6	–11,318	–6,177
Bizygomatic breadth	40,6	37,3	12,508	13,045
Cranial breadth	29,7	28,5	–17,201	–12,419
Hard palate length	9,3	7,4	–2,965	–2,195
Zygomatic arch length	35,7	32,8	–3,233	–4,383
Maximum mandibular length	66,5	60,7	7,655	16,831
Maximum mandibular height	42,8	37,7	1,305	1,973
I_1 breadth	3,0	2,6	59,121	10,547
		CONSTANT	164,541	

Incisor tooth patterns: The width of the upper incisor is of diagnostic value in *Pronolagus* particularly in the separation of *P. rupestris* from *P. crassicaudatus* and *P. randensis* (minimum and maximum values presented below are $\bar{X} \pm 2SD$). *P. rupestris* is characterised by a narrow principal upper incisor (2,2–2,8 mm), while those of *P. crassicaudatus* (2,8–3,7 mm) and *P. randensis* (2,6–3,4 mm) are wider. The increase in incisor width in *P. crassicaudatus* and *P. randensis* is attributable to an elongation of that portion of the tooth lateral to the invagination (Chapter 4) which is clearly evident in sectioned teeth and provides a further means of distinguishing both *P. crassicaudatus* and *P. randensis* from *P. rupestris*.

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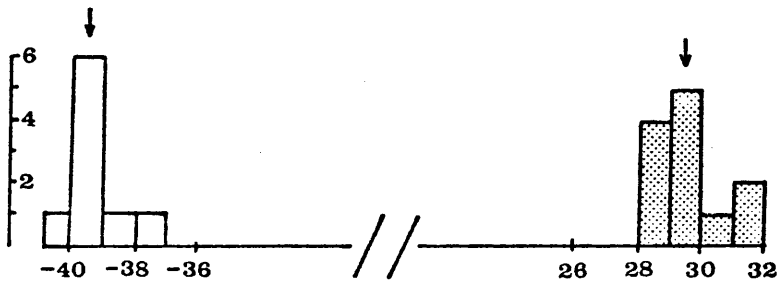

Fig. 55: *Histograms of discriminant scores (X axis = frequencies) from a two-group discriminant function analysis of* P. randensis *and* P. rupestris. *Arrows indicate the positions of the mean discriminant scores.*

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Table 27:

Results of a two group discriminant function analysis of *P. randensis* and *P. crassicaudatus*

Measurement	*P. randensis* species mean	*P. crassicaudatus* species mean	Unstandardised coefficient	Standardised coefficient
Muzzle length	39,9	41,0	0,373	0,909
Frontal length	40,1	36,8	0,429	1,125
Posterior muzzle breadth	17,5	20,8	−0,671	−0,942
Palate vacuity length	26,6	26,8	−0,660	−0,599
Palatal breadth	24,2	26,0	−0,784	−0,596
Mesopterygoid space	4,8	5,6	−2,039	−1,021
Mandibular toothrow length	41,6	41,3	0,831	1,121
Maximum mandibular height	42,8	44,4	−0,511	−0,694
Mandibular body breadth	5,3	5,8	−2,199	−0,611
			CONSTANT 29,372	

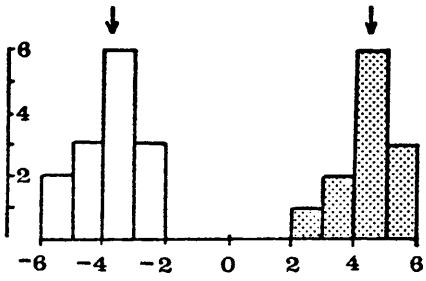

Fig. 56: *Histograms of discriminant scores (X axis = discriminant scores; Y axis = frequencies) from a two group discriminant function analysis of* P. randensis *and* P. crassicaudatus. *Arrows indicate the positions of the mean discriminant scores.*

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Although the width of the incisors of *P. crassicaudatus* and *P. randensis* are similar, subtle differences appear to exist in the shape of the sectioned incisors of the respective species. In cross section the *P. crassicaudatus* upper incisor tends to be characteristically flatter anterio-posteriorly than *P. randensis*, which is closer to *P. rupestris* in this regard. However, this difference is not striking and may only be of use when a series of incisors from *P. crassicaudatus* and *P. randensis* are available.

Spermatozoan morphology and silver staining patterns: The limited data for two of the species, *P. crassicaudatus* and *P. randensis*, confounded any taxonomic conclusions regarding differences in sperm morphology within this genus (Chapter 5). Nevertheless it would appear from the existing data that although the silver staining properties of the spermatozoa are similar, significant interspecific differences are evident in the sperm dimensions. However, in all cases extensive overlap exists between the respective species.

Electrophoresis: No diagnostic genetic marker was observed.

Key to the species (minimum and maximum values are $\bar{X} \pm 2SD$).

1. Small species (total length of adult skull = 77,4 – 87,5 mm); upper principal incisor narrow across face 2,2 – 2,8 mm; robust bulla both proportionately (bulla breadth x 100/total of skull = 7,4 – 11,4% ; bulla breadth x 100/mandibular height = 16,5 – 25,1%); and in absolute measurements (bulla breadth = 6,4 – 9,2 mm).

   ——— *P. RUPESTRIS*

1[1] Larger species (total length of adult skull = 85,5 – 96,3 mm), with upper principal incisor broad across face 2,6 – 3,7 mm; narrow bullae, both proportionately (bulla breadth x 100/total length of skull = 5,6 – 8,0% ; bulla breadth x 100/ mandibular height = 11,6 – 16,7%) and in absolute measurements (bulla breadth = 5,1 – 7,2 mm).

   ——— 2

2. Chin white to grey, this colour extending in broad band running along lower jaw to approach inferior margins of nape patch; pelage coarse; cheeks dark grey, not contrasting with sides and upper parts; ratio maxillary premolar length x 100/frontal length = 19,9 – 32,5% ; ratio mesopterygoid space x 100/frontal length = 11,7 – 19,9% .

   ——— *P. CRASSICAUDATUS*

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Chin white to grey but localised, never extending in a broad band along lower jaw towards inferior margins of nape patch; cheeks strikingly light grey, contrasting with sides and upper parts; ratio maxillary premolar length x 100/frontal length = 16,9 – 22,9% ; ratio mesopterygoid space x 100/frontal length = 9,4 – 14,7% .

P. RANDENSIS

GENUS *BUNOLAGUS* THOMAS

Bunolagus Thomas, *Proc. zool. Soc. Lond.*, p. 109, 1929. Genotype: *Lepus monticularis.*

Bunolagus monticularus (Thomas)

Lepus monticularis Thomas, *Ann. Mag. nat. Hist.* 11 : 78, 1903.

Type locality: Deelfontein (Cape Province)

Description:

Size small, limbs moderately short and relatively robust, ear moderately long, cranially small (Appendix II, Table 4) and adults invariably characterised by distinct interparietal bone. Fur fine of silky texture, forehead, back and sides buffy greyish brown; guard hairs buffy subterminally ending in black tips; grizzling due to juxtapositioning of terminal tips of guard hairs; underfur grey to roots; chin yellowish white, sharply demarcated from grey brown of cheeks by a thin black band running along border of lower jaw towards base of ears; gular patch of similar colour and texture as back and sides; chest, abdomen and anal region pale yellow with lateral margins and inner surfaces of limbs pinkish buff; soles of feet particularly hind feet extremely well furred; ears sparsely haired; eye encircled in prominent white ring; tail thick and cylindrical, uniformly vernaceous brown with some black ticking towards tip.

Geographic range:

Extremely limited distribution(Fig. 57); based on existing records, this extends approximately between 31°S – 33°S and 20° – 24°E in central and southern Karoo, Cape Province; known localities based on museum specimens are east of Calvinia (31°32' S, 20° 32' E and 31° 27' S, 190° 50' E), Deelfontein (30° 59' S, 23° 48' E), Middelpos (31° 55' S, 20° 13' E),

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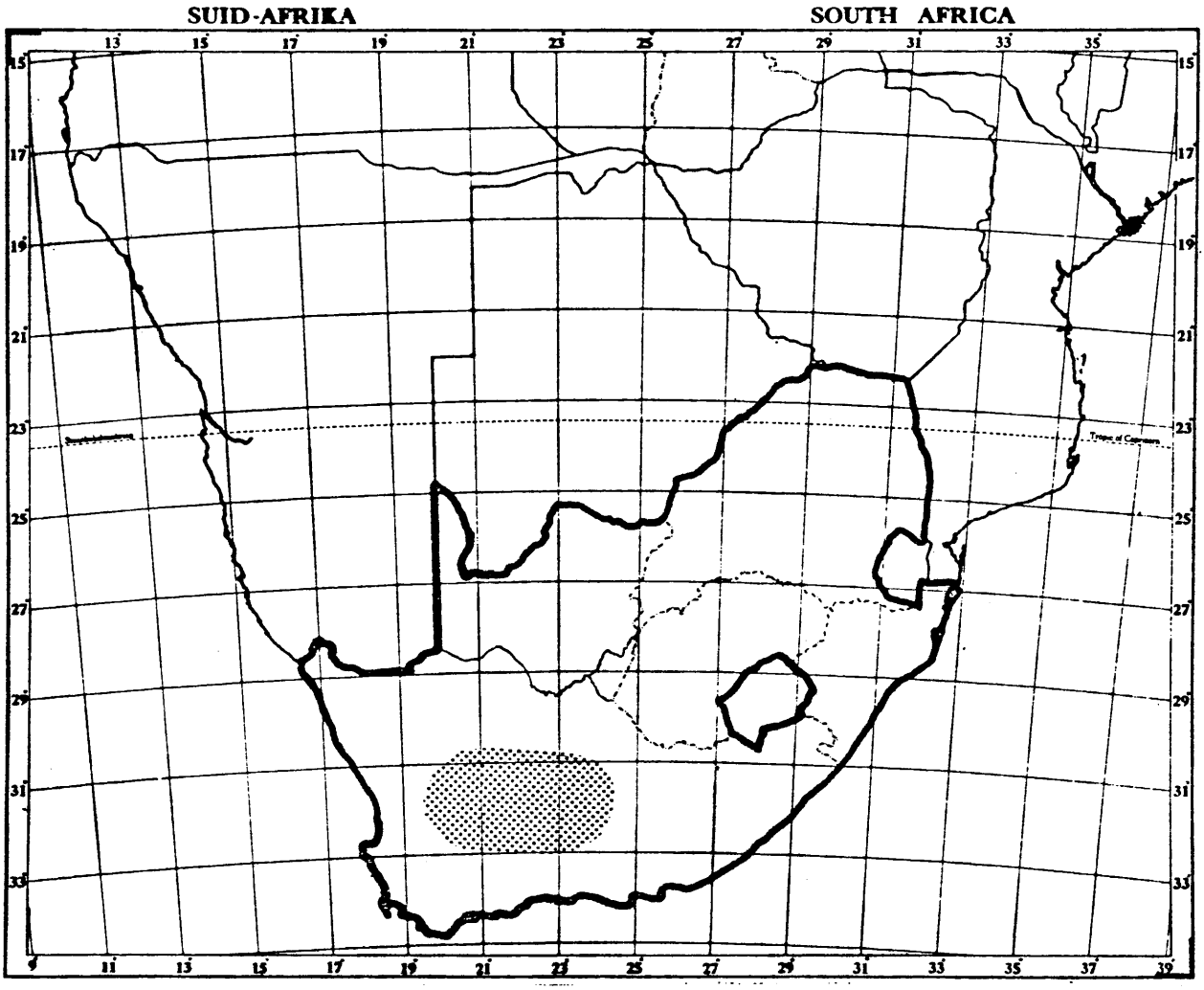

Fig. 51: *Distribution of* B. monticularis *based on all known localities from which specimen have been sampled.*

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Nelspoort (32° 07' S, 23° 00' E), north of Sutherland (32° 12' S, 20° 42' E) and Victoria West 31° 26' S, 23° 07' E); species appears habitat specific being restricted to dense riverine scrub along seasonal rivers in these areas (Robinson, 1981; Robinson and Skinner, in press).

Species comparisons and distinguishing characters:

Bunolagus is a monotypic genus. However, it does share certain external phenotypic characters with *Lepus* and *Pronolagus* species. Furthermore, if the pattern of the principal upper incisors in cross section and width are excluded, *Bunolagus* could cranially be confused with *Lepus*, particularly *L. capensis*. Consequently, while attention will be drawn to the similarities between *Bunolagus* and the other South African Leporidae, emphasis will be placed on diagnostic characters which facilitate the identification of *Bunolagus*.

Pelage: *Bunolagus* differs from all other South African leporids in the colouring of the chest and abdomen which is pale yellow medially, contrasting with the pinkish buff or white of the other taxa. Another diagnostic pelage character is the thin black band which demarcates the cheeks from the chin and which runs along the lower margins of the jaws towards the base of the ears, a characteristic never observed in *Pronolagus* and only once, albeit very faintly, in *Lepus* (*L. capensis*). Conversely, the uniformly coloured tail in *Bunolagus* is in marked contrast to the clearly bicoloured black and white tail of *Lepus*, but is similar in this feature to *Pronolagus*, particularly *P. randensis*.

External and cranial measurements: *Bunolagus* appears to be smaller on average in most external characters than either *L. capensis* or *P. rupestris*, the smallest representatives of their respective genera. A noticeable exception and a character of diagnostic value in regard to the delimitation of *Bunolagus* from any *Pronolagus* species, is ear length. *Bunolagus* is characterised by a moderately long ear (109 – 124 mm; minimum and maximum values are $\bar{X} \pm 2SD$) which differs markedly from *Pronolagus* (63 – 106 mm), including *P. rupestris*, which has the greatest ear length measurement in the genus (See Appendix II, Table 3).

Cranially, the width of the mesopterygoid space relative to the hard palate length provides unequivocable separation between *Bunolagus* and the *Pronolagus* species. In *Bunolagus* the mesopterygoid space is always wider than the minimal length of the hard palate (124,9 – 180,5% ; minimum and maximum values are $\bar{X} \pm 2SD$), while the converse holds for *Pronolagus* (38,6 – 87,7%). Consequently, on cranial characters, should the marked

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differences in their principal incisors be ignored, *Bunolagus* could only possibly be confused with *Lepus.*

The genus *Lepus,* represented by *L. capensis* and *L. saxatilis,* and *Bunolagus,* are very similar in cranial morphology (Chapter 2). With the exception of the incisor tooth pattern characteristics and the results of the discriminant function analyses presented below, no reliable diagnostic cranial features appear to exist for the delimitation of these genera.

Discriminant function analysis: The following analyses were based on 13 *B. monticularis,* 21 *L. capensis* and 75 *L. saxatilis* specimens, each characterised by full data sets.

L. capensis : B. monticularis : The 13 cranial characters selected by this procedure to maximise the separation between groups and the corresponding standardised and unstandardised coefficients are presented in Table 28. The standardised coefficients indicate that total length of the skull, interauditory breadth, palate vacuity length, maxillary premolar length and mandibular toothrow length were primarily responsible for the discrimination between groups. The derived discriminant scores plotted as frequency histograms (Fig. 58) clearly illustrate the separation between taxa. The scores of *L. capensis* ranged from −9,313 to −5,581 (mean score = −7,201) and those of *B. monticularis* from 9,799 to 13,487 (mean score = 11,633). All specimens were correctly classified *a posteriori.*

L. saxatilis : B. monticularis : In this case nine cranial characters were selected by the procedure to maximise the separation between groups. These measurements and their corresponding standardised and unstandardised coefficients are presented in Table 29. The derived discriminant scores, plotted as frequency histograms (Fig. 59), clearly illustrate the separation between taxa. The scores of *L. saxatilis* range from −4,277 to 0,590 (mean score = −1,545) and those of *B. monticularis* from 7,686 to 9,987 (mean score = 8,911). All specimens were correctly classified *a posteriori.*

Karyotype: Karyotype analyses were conducted on a single male specimen of *Bunolagus* (Chapter 3). The chromosomal data provides unequivocable identification of the species and extremely strong grounds for its generic status. The diploid number was established as 2n = 44 as opposed to the 2n = 42 for the *Pronolagus* species and 2n = 48 for *Lepus.* The male karyotype comprises five pairs of biarmed metacentric, submetacentric and subtelocentric autosomes, 16 pairs of telocentric autosomes which show a gradual transition in size from large to small. The X is medium sized submetacentric chromosome and the Y a small acrocentric.

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Table 28:

Results of a two-group discriminant function analysis of *L. capensis* and *B. monticularis*

Measurement	*L. capensis* species mean	*B. monticularis* species mean	Unstandardised coefficient	Standardised coefficient
Total length of skull	86,3	81,1	− 0,794	− 1,764
Muzzle length	38,4	34,5	0,531	0,891
Posterior frontal breadth	12,0	14,2	0,683	0,636
Interauditory breadth	26,7	24,7	− 1,477	− 1,283
Palate incisor length	34,2	32,3	− 0,807	− 0,924
Palate vacuity length	22,4	21,7	2,813	2,636
Maxillary premolar length	7,7	6,5	− 3,216	− 1,172
Principal I^1 breadth	2,6	2,1	− 9,716	− 0,946
Zygomatic arch length	33,9	30,5	− 0,599	− 0,786
Posterior cranial height	17,4	14,8	− 0,771	− 0,834
Mandibular toothrow length	35,7	33,0	1,222	1,504
Bulla breadth	9,1	9,0	− 1,416	− 1,049
Mandibular premolar-molar length	15,0	13,1	− 1,679	− 0,886
		CONSTANT	114,529	

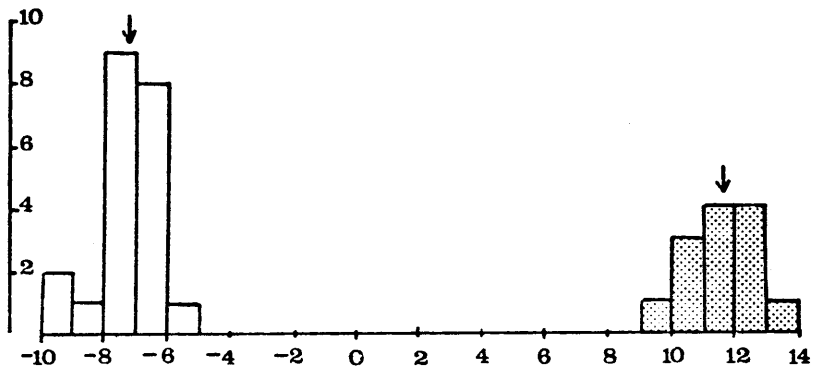

Fig. 58: *Histograms of discriminant scores (X axis = discriminant scores; Y axis = frequencies) from a two-group discriminant analysis of* L. capensis *and* B. monticularis. *Arrows indicate the positions of the mean discriminant scores.*

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Table 29:

Results of a two-group discriminant function analysis of *L. saxatilis* and *B. monticularis*.

Measurement	*L. saxatilis* species mean	*B. monticularis* species mean	Unstandardised coefficient	Standardised coefficient
Anterior frontal breadth	20,3	14,5	− 0,241	− 0,387
Interauditory breadth	28,5	24,7	0,194	0,281
Principal I^1 breadth	3,1	2,1	− 2,134	− 0,389
Palatal breadth	26,9	22,4	0,597	0,654
Bizygomatic breadth	44,1	36,5	− 0,640	− 0,907
Cranial breadth	32,0	28,6	0,262	0,377
Maximum mandibular height	45,5	33,4	− 0,236	− 0,446
Mandibular body breadth	6,3		− 1,462	− 0,471
Mandibular premolar-molar length	17,6	13,1	− 0,231	− 0,195
		CONSTANT	32,182	

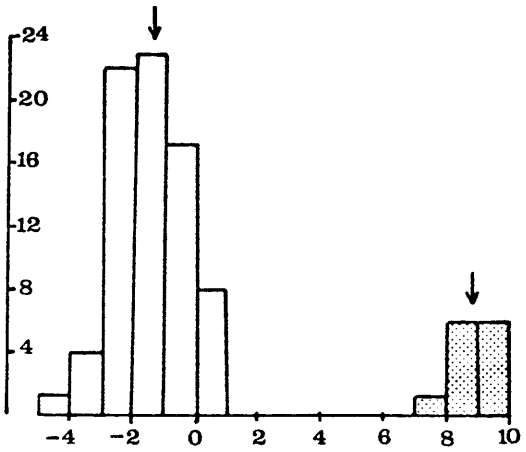

Fig. 59: *Histograms of discriminant scores (X axis = discriminant scores; Y axis = frequencies) from a two-group discriminant function analysis of* L. saxatilis *and* B. monticularis. *Arrows indicate the positions of the mean discriminant scores.*

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Incisor tooth patterns: The narrowness and the shape of the principal upper incisors in *Bunolagus* are of diagnostic importance. It is clear from absolute values that *B. monticularis* has the narrowest principal upper incisors (1,9 – 2,2 mm; minimum and maximum values are $\bar{X} \pm 2SD$) of the South African Leporidae. Comparable data for the other species are *L. capensis* (2,3 – 2,8 mm), *L. saxatilis* (2,7 – 3,5 mm), *P. rupestris* (2,2 – 2,8 mm), *P. randensis* (2,6 – 3,4 mm) and *P. crassicaudatus* (2,8 – 3,7 mm).

The absence of cement in the enamel fold of the principal upper incisor and the thinness of the incisive enamel provides unequivocable separation between *Bunolagus* and either *Lepus* species. However the absence of cement concurs with the situation for *Pronolagus.* Nevertheless, shape differences in the cross sections of the *Bunolagus* incisors are evident on comparison with those of the three *Pronolagus* species. Of these, *P. rupestris* appears closest to *B. monticularis* in both shape and width of the incisors, with the latter character perhaps the more reliable criterion in view of the larger sample sizes involved (Chapter 4).

Spermatozoan morphology and silver staining patterns: The presence of a large anteriorly pronounced acrosome in *Bunolagus* spermatozoa (Chapter 5) is of diagnostic value and can be used to separate the species from *Lepus;* it does however share this character with *Pronolagus.*

The limited data on both the quantitative aspects and silver staining patterns of the *Bunolagus* spermatozoa severely limit conclusions regarding the taxonomic significance of these criteria. In all quantitative characters except length of midpiece, the *Bunolagus* spermatozoa are grouped within the same nonsignificant subset as those of at least one species of *Pronolagus* (Table 16 p. 127). The silver staining patterns and gross overall morphology of the spermatozoa appear similar to those of the *Pronolagus* species.

Electrophoresis: No electrophoretic analyses were conducted on this species.

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CHAPTER 8

GENERAL CONCLUSIONS

The results of this investigation support the recognition of *Lepus, Pronolagus* and the monotypic *Bunolagus* as valid genera for the South African Leporidae. While the cranial distinctiveness of *Pronolagus* was clearly evident from the morphometric analyses, the recognition of the two remaining taxa using these methods of data analysis, were not clear cut. *Lepus* and *Bunolagus* tend to be of similar skull morphology and the delimitation of these taxa is largely dependent on the overall size differences of their crania, *Lepus* being larger on average than *Bunolagus.*

Karyologically however, all three genera can be unequivocally identified by both diploid number and the unique morphology of several of their chromosomes. *Lepus*, which is considered to be chromosomally very similar to the ancenstral condition, is characterised by 2n = 48, while the karyotypically derived taxa, *Pronolagus* and *Bunolagus,* have 2n = 42 and 2n = 44 respectively. None of the chromosomal rearrangements which have been responsible for the structuring of the *Pronolagus* and *Bunolagus* genomes were found to be common, implying that the rearrangements responsible for their derived chromosomal states have occurred subsequent to their separation. None of the South African genera can be considered to be chromosomally closely related. These data are particularly relevant to the controversial taxonomic status of *Bunolagus.* The presence of 44 chromosomes in this taxon, and in many cases their unique morphology, provide unusually strong support for generic status distinct from that of *Lepus,* the genus to which it has been assigned in the past.

Several other systematic criteria proved to be of diagnostic value in the delimitation of the respective genera. In various combinations, the use of cross sections through the principal upper incisors, sperm morphology with particular reference to acrosome shape, differing electrophoretic mobilities of several protein systems and quantitative pelage and other external phenotypic characters provide unequivocal identification of three discrete groups, corresponding to the genera *Lepus, Pronolagus* and *Bunolagus.*

Morphometric analyses of the South African *Lepus* gave clear indication of two Phenetically distinct species, *L. capensis* and *L. saxatilis.* Although Petter (1972) is of the opinion that the smaller more northern forms of this genus in South Africa are attributable to a third species *L. crawshayi,* this was not substantiated by the present investigation. An analysis of geographic variation clearly showed *L. saxatilis* to vary clinally, with the smaller

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forms simply representing a northeastward extension of the species cline.

The recognition of only two South African *Lepus* species was supported by almost all other test parameters. Representatives of the two species could be identified by the shape of the principal upper incisors in cross section, quantitative differences in sperm morphology as well as species specific silver staining properties of the spermatozoa, the presence or absence of erythrocytic carbonic anhydrase and certain pelage characteristics. Only one criterion, comparative karyology, failed to provide a means of distinguishing between these two well deliniated species.

Similarly, the recognition of three *Pronolagus* species, *P. rupestris, P. crassicaudatus* and *P. randensis* appears justified. While electrophoresis, chromosome analyses and sperm morphology were not useful in the delimitation of the taxa, several other parameters, principally the morphometric analyses and pelage characteristics, strongly support this treatment.

Univariate and multivariate statistical analyses gave clear indication of three cranially discrete groups corresponding to *P. rupestris, P. crassicaudatus* and *P. randensis. P. rupestris,* which is the smallest taxon on average, has the broadest bullae, both on absolute values and proportionately. The ratio of bulla breadth to mandibular height, or alternatively, to either greatest length of skull or basal cranial length, are useful diagnostic characters for the recognition of this species.

Likewise, *P. crassicaudatus* and *P. randensis,* which are of similar overall size, differ in respect to the length of the maxillary premolar toothrow and width of the mesopterygoid space, *P. crassicaudatus* being larger on average in both measurements. When contrasted to frontal length or posterior cranial height, these measurements are of diagnostic value for the delimitation of these taxa.

In addition, all three species were readily distinguished on pelage characteristics, while cross sections through, and the width of the principal upper incisors, proved reliable in the delimitation of *P. rupestris* from the remaining taxa. Although differences were apparent in the incisor patterns of *P. crassicaudatus* and *P. randensis,* these were subtle and of limited systematic value.

In a wider context, the results of the present investigation point to the need for a detailed investigation of the taxonomic status of the northern *L. crawshayi,* whose separation from *L. saxatilis* has been based primarily on size. Should the clinal variation illustrated by

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L. saxatilis extend northwards, the validity of *L. crawshayi* as a distinct species would have to be critically reassessed. Likewise, the controversial *L. capensis, L. europaeus* synonomy has been highlighted by the apparent marked differences in the incisor toothpatterns of the two species. An extensive investigation of *L. capensis* and *L. europaeus*, particularly in their presumed area of sympatry east of the Caspian Sea (Angermann, 1972), is indicated.

Above all, this study has clearly illustrated the value of using several test criteria in systematic investigations. A multiplicity of investigatory procedures can be examined for concordance, which provide a more critical evaluation of taxonomic conclusions indicated by a single criterion alone.

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SUMMARY

The taxonomy of the South African Leporidae has been characterised by considerable disagreement both with regard to the status of the monotypic *Bunolagus,* and the specific classification of representatives of the genera *Lepus* and *Pronolagus.* The history of this is lengthy and has been presented by way of introduction.

The purpose of the present investigation was to examine the taxonomic relationships of the South Africa lagomorphs using new and traditional systematic parameters. The study was easily divisable into six sections incorporating univariate and multivariate statistical techniques of data analysis, comparative karyology, interspecific variation in cross sections through the principal upper incisors, sperm morphology, electrophoretic analyses of serum proteins and red blood cell enzymes, and qualitative pelage characteristics.

Material used in the course of this investigation was derived from field specimens sampled from numerous localities throughout South Africa and supplemented in certain instances by museum specimens comprising standard skins and skulls. Material from the former category formed the data base for those investigatory procedures dependent on fresh tissue (karyology, sperm morphology, electrophoresis). The important separation of adult from subadult was based on the closure of the proximal epiphysis of the humerus in field specimens, while museum material, lacking postcranial skeletal data, was aged on visible evidence of fusion along the entire length of the suture between the exoccipital and supraoccipital bones.

Twenty eight cranial and four external body measurements were analysed statistically for sexual dimorphism in two subsamples of *L. saxatilis* and one of *P. crassicaudatus.* The results clearly indicated that females were larger than males in most parameters but, with few exceptions, not significantly so. Consequently detailed univariate and multivariate analyses were performed on samples irrespective of sex. Multivariate procedures using selected subroutines of the Numerical Taxonomy System of Multivariate Statistical Programmes (NT–SYS), illustrated the cranial distinctiveness of the genus *Pronolagus.* However, the delimitation of the controversial *Bunolagus* from *Lepus*, the genus with which it has the greatest affinity, is not clear and is dependent largely on overall size differences of their crania, *Lepus* being larger on average than *Bunolagus.*

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Univariate and multivariate analyses of specimens within the polytypic genera *Lepus* and *Pronolagus* gave clear indications of two phenetically distinct species, *L. capensis* and *L. saxatilis* in the former, and three *P. rupestris, P. crassicaudatus* and *P. randensis* in the latter. The delimitation of the *Lepus* species was heavily dependent on overall size, *L. saxatilis* being on average larger than *L. capensis.* Although Petter (1972) is of the opinion that the smaller northern forms of this genus in South Africa represent a southern extension of the northern extralimital *L. crawshayi,* this was not substantiated in the present investigation. An analysis of geographic variation using the univariate SS-STP analysis clearly showed *L. saxatilis* to vary clinally, with a marked trend for decreasing size along a SW-NE axis. The smaller forms of *L. saxatilis* simply represent the northward extension of the species cline. Of the *Pronolagus* species, *P. crassicaudatus* and *P. randensis* are, on average, of similar overall size. However marked, albeit complex, differences in the shape of their crania were evident. Interspecific differences in the length of the maxillary premolar toothrow and width of the mesopterygoid space are relatively pronounced, *P. crassicaudatus* being larger on average than *P. randensis* in both measurements. When contrasted to frontal length or posterior cranial height, these measurements are useful diagnostic characters for the delimitation of these species. Similarly the smaller *P. rupestris* is characterised by inflated bullae, the measurement of which, when contrasted to mandibular height, or alternatively to either the greatest length of the skull or basal cranial length, appears to be of diagnostic value.

Supplementary investigations using a two-group discriminant function analysis of the SPSS computer programme, resulted in all specimens of the South African leporid taxa being correctly classified *a posteriori.* The results of this analysis provide a reliable means of identification which can be used independently of other diagnostic features, and are particularly pertinent to museum taxonomy where material for several of the other tested systematic parameters may not be available.

Karyotype analyses incorporating G- and C-banding techniques were performed on fibroblast cells derived from skin biopsies grown in culture. The results revealed that all three South African leporid genera can be unequivocably identified by both diploid number and the unique morphology of several of their chromosomes. The genus *Lepus* is highly conservative karyotypically with all species analysed, including extralimital taxa, characterised by $2n = 48$. This remarkable uniformity in diploid number is mirrored by G-banding patterns of the species, which appear similar if not identical in most cases. While of no value in the delimitation of *L. saxatilis* and *L. capensis*, chromosome comparisons between these taxa and the extralimital *L. timidus, R. diazi* and the published data for *S. bachmani,* allowed speculation on the leporid ancenstral karyotype. It is proposed that the presence of the *Lepus*-like

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2n = 48 karyotype in three genera of two distinct lineages is unlikely to be due to chance alone, but indicates a common evolutionary origin and refers back to the ancestral condition from which all the species may have been derived.

The karyotype data is particularly relevant to the controversial taxonomic status of *Bunolagus*. The presence of 44 chromosomes, many of which are of unique morphology, as opposed to the remarkably uniform *Lepus* karyotype (2n = 48), the genus to which it has been assigned in the past, is considered to provide exceptionally strong support for the validity of *Bunolagus* as a genus distinct from *Lepus*. The karyotypic divergence of *Bunolagus* from the common ancestor has been characterised by three fusions, one fission and numerous non-Robertson structural changes possibly involving, amongst others, repeated pericentric inversions.

Likewise, *Pronolagus* (2n = 42) is chromosomally unique. Although of no systematic value in the species delimitation, karyotype comparisons with the ancestral condition clearly revealed that the reduction in chromosome number, which accompanied the evolution of this genus, is due to three unique fusion events. However, none of the chromosomal rearrangements responsible for the structuring of either the *Bunolagus* or *Pronolagus* genomes were found to be common, implying that the rearrangements responsible for their derived chromosomal conditions occurred subsequent to their isolation.

A single principal upper incisor was extracted from almost all specimens utilised in the present study and sectioned using a diamond bladed saw. Differences in respect of incisor width and the pattern of the tooth in cross section provide a reliable means of distinguishing representatives of *L. capensis* and *L. saxatilis*. The incisors of *L. capensis* are characterised by their narrowness ($\bar{X}$ = 2,5 mm observed range 2,3–2,8) and in cross section, their squarer appearance and more abrupt infolding of the incisive groove. Conversely, those of *L. saxatilis* are more robust ($\bar{X}$ = 3,1 mm observed range 2,6 – 3,6) and tend to be oblong in section. Within *Pronolagus*, distinct interspecific differences exist between the incisor tooth patterns of *P. rupestris* and the remaining taxa, *P. crassicaudatus* and *P. randensis*, which are similar with respect to this character. This difference, which is due largely to an elongation of the lateral section of the incisors in *P. crassicaudatus* and *P. randensis*, is reflected in the incisor breadth measurements of the three taxa : *P. rupestris* ($\bar{X}$ = 2,5 mm observed range 2,3 – 2,8), *P. crassicaudatus* ($\bar{X}$ = 3,2 mm observed range 2,9 – 3,6) *P. randensis* ($\bar{X}$ = 3,0 observed range 2,6 – 3,4). Similarly, striking differences were evident between the incisor patterns of *Bunolagus* and the South African *Lepus* species. The former taxon can clearly be distinguished by the complete absence of cement in the incisive fold, the extreme narrowness of the teeth ($\bar{X}$ = 2,1 mm observed range 2,0 – 2,2) and thinner anterior covering of enamel.

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Comparative qualitative (shape and silver staining properties) and quantitative (mensural) studies of the spermatozoa of the South African lagomorphs were conducted. Based on the shape of the acrosome all taxa could be assigned to one of two types; one type comprising the two *Lepus* species is characterised by a relatively small acrosome which does not extend much beyond the leading edge of the head, while the other containing the three *Pronolagus* species and *Bunolagus,* has an acrosome which is pronounced anteriorly. The spermatozoa of *L. capensis* and *L. saxatilis* differ significantly in the three parameters tested, with *L. saxatilis* being larger than *L. capensis* with regard to both the length of the head and midpiece, while the latter taxon was characterised by a greater head width measurement. In addition to these quantitative differences the spermatozoa of both species were recognised by their respective silver staining properties. Small sample sizes in *Pronolagus,* particularly *P. crassicaudatus* and *P. randensis,* and in *Bunolagus,* hampered evaluation of the mensural differences between species. In addition, no marked silver staining differences were apparent within the examined specimens of these taxa.

Horizontal starch gel electrophoresis of serum proteins and red blood cell enzymes were performed on material from specimens of *Pronolagus* and *Lepus;* no suitable material was available for similar investigations on *Bunolagus.* Five of the nine proteins which could be consistently scored in each specimen of this study proved monomorphic within both *Lepus* and *Pronolagus* (albumin, haemoglobin, indophenol oxidase, malate dehydrogenase, 6-phosphogluconate dehydrogenase), while three (transferrin, esterase-1, esterase-2) were polymorphic. Small sample size and in particular the low number of polymorphic systems resulting from this investigation, curtailed detailed investigations of genetic similarities between taxa. Nevertheless, one enzyme system, erythrocytic carbonic anhydrase, proved useful in the delimitation of the South African *Lepus* species. Based on this enzyme all *Lepus* specimens analysed could be assigned to one of two phenotypic classes; those specimens classified as *L. saxatilis* were characterised by a single dark staining anodal band, while those of *L. capensis* were identified by the complete absence of a band. The latter phenotype is presumed to be the expression of a null or silent allele.

All species of the South African Leporidae could be identified using pelage and other external phenotypic characters. Data from these classical taxonomic parameters, together with diagnostic data resulting from the other systematic criteria employed in this study, were incorporated in identification keys at both generic and species level. It can be concluded that the results of this investigation support the recognition of *Lepus, Pronolagus* and monotypic *Bunolagus* as valid genera for the South African Leporidae and that the recognition of two *Lepus* species, *L. capensis* and *L. saxatilis,* and three *Pronolagus* species, *P. rupestris, P. crassicaudatus* and *P. randensis,* appears justified.

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OPSOMMING

Sovêr dit die taksonomie van die Suid-Afrikaanse Leporidae betref, was daar nog altyd uiteenlopende menings m.b.t. die status van die monotipiese genus *Bunolagus* en die spesifieke klassifikasie van die verteenwoordigers in die genera *Lepus* en *Pronolagus*. Die lang geskiedenis hieromtrent word aangebied as inleiding tot die werkstuk.

Die doel van hierdie studie was derhalwe om ondersoek in te stel na die taksonomiese verwantskappe van die Suid-Afrikaanse Lagomorpha deur gebruik te maak van beide tradisionele en nuwe taksonomiese parameters. Die ondersoek het ses verskillende fasette behels naamlik enkel- en veelvoudigveranderlike statistiese tegnieke van data-ontleding, vergelykende kariologie, interspesifiese variasie aanwesig in dwarssnitte van die boonste snytande, spermmorfologie, elektroforetiese analises van serumproteïene en rooibloedselensieme en kwalitatiewe pelseienskappe.

Die materiaal waarop gewerk is, is ingesamel by talle lokaliteite dwarsoor Suid-Afrika en in sekere gevalle is dit aangevul met standaard museumeksemplare bestaande uit die vel en skedel. Eersgenoemde tipe materiaal het die basis gevorm vir daardie ondersoeke waarvoor vars weefsels benodig word (kariologie, spermmorfologie, elektroforese). In die geval van vars materiaal is onderskeid tussen volwasse en onvolwasse diere getref op grond van die mate van ossifisering van die proksimale epifise van die humerus. By museummateriaal, waar die postkraniale skelet natuurlik ontbreek, is hierdie skeiding gedoen na aanleiding van die mate van versmelting aanwesig oor die lengte van die naat tussen die exoccipitale en supraoccipitale bene.

By twee submonsters van *L. saxatilis* en een van *P. crassicaudatus* is 28 skedel- en vier uitwendige mates statisties ontleed vir aanduiding van geslagsdimorfisme. Daar was wel duidelike aanduiding dat wyfies groter is as mannetjies sovêr dit die meeste van die parameters betref maar op 'n paar uitsonderings na, egter nie genoegsaam betekenisvol nie. Gedetailleerde enkel- en veelvoudigveranderlike ontledings is gevolglik uitgevoer op monsters sonder om geslag in ag te neem. Deur gebruik te maak van geselekteerde subroetes van die Numerical Taxonomy System of Multivariate Statistical Programmes (NT-SYS) is met behulp van veelvoudigveranderlike ontledings die kenmerkendheid van die skedel van die genus *Pronolagus* aangedui. Die delimitasie van *Bunolagus* van *Lepus*, die genus waarmee dit die grootste verwantskap toon, is egter nie duidelik nie en berus hoofsaaklik op die grootteverskil van hul skedels, met dié van *Lepus* gemiddeld groter as dié van *Bunolagus*.

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Enkel- en veelvoudigveranderlike ontledings van eksemplare binne die genera *Lepus* en *Pronolagus* het die bestaan van twee duidelik onderskeibare spesies, *L. capensis* en *L. saxatilis* in eersgenoemde en drie spesies, *P. rupestris, P. crassicaudatus* en *P. randensis,* in laasgenoemde genus aangedui. Die verskil in liggaamsgrootte is die belangrikste onderskeidende kenmerk tussen *L. capensis* en *L. saxatilis* met laasgenoemde gemiddeld die grootste. Alhoewel Petter (1972) die mening toegedaan is dat die kleiner noordelike vorme van hierdie genus in Suid-Afrika 'n suidelike uitbreiding is van die verder noordelike *L. crawshayi,* het die huidige studie niks opgelewer wat sy standpunt steun nie. Deur gebruik te maak van die enkelveranderlike SS-STP-analise het 'n ontleding van geografiese variasie aangedui dat *L. saxatilis* in grootte varieer in verskillende populasies met 'n duidelike neiging tot afname in grootte in 'n SW-NO rigting oor sy verspreidingsgebied. Die kleiner, noordelike vorme van *L. saxatilis* is dus niks anders as die resultaat van hierdie kenmerkgradiënt nie. In die genus *Pronolagus* is *P. crassicaudatus* en *P. randensis* gemiddeld ewe groot maar daar is, nieteenstaande kompleks, duidelike verskille in die vorm van hulle skedels. Interspesifiese verskille in the lengte van die maksillêre premolaartandry en wydte van die mesopterygoïedspasie is uitgesproke, met dié van *P. crassicaudatus* ten opsigte van beide afmetings gemiddelde die grootste, en gestel teenoor frontale lengte of postkraniale hoogte vorm hierdie parameters betroubare kriteria om hierdie twee spesies te onderskei. *P. rupestris* word gekenmerk deur die besit van groot bullae en die afmetings hiervan gestel teenoor mandibelhoogte, of andersins gestel teenoor totale skedellengte of basale skedellengte, is diagnosties vir hierdie spesie.

Aanvullende ondersoek is uitgevoer deur gebruik te maak van 'n twee-groep-diskriminante ontleding deur die SPSS-rekenaarprogram wat gelei het tot korrekte klassifikasie van die Suid-Afrikaanse Leporidae. Die resultate van hierdie ontleding verskaf 'n betroubare manier vir identifikasie van die spesies en kan gebruik word sonder om ander diagnostiese kenmerke in ag te neem. Dit is besonder belangrik in museumtaksonomie waar materiaal vir die ander parameters wat getoets is moontlik nie beskikbaar is nie.

Kariotipe-ontledings wat G- en C-bandtegnieke behels, is uitgevoer op fibroblaste, verkry deur velbiopsies wat in kultuur gekweek is. Die resultate het aangetoon dat elk van die drie genera onder die Leporidae wat in Suid-Afrika verteenwoordig is, uniek is ten opsigte van beide die diploïede getal en die morfologie van verskeie chromosome. Die genus *Lepus* is kariotipies baie homogeen en dit is gevind dat al die spesies wat ondersoek is, insluitend taksa van ander geografiese gebiede, gekenmerk word deur 2n = 48. Hierdie merkwaardige eenvormigheid bestaan nie slegs sovêr dit die chromosoomgetal betref nie maar word verder beklemtoon deur die G-bandpatrone van die verskillende spesies deurdat dit identies, of bykans identies is in die meeste gevalle. Alhoewel hierdie verskynsels geen waarde het as onderskeidende

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kenmerke tussen *L. saxatilis* en *L. capensis* nie, bied 'n vergelyking van die chromosome van hierdie twee taksa met die van die uitheemse *L. timidus, Romerolagus diazi* en *Sylvilagus bachmani* genoegsame stof om te kan teoretiseer oor die voorouerlike kariotipe van die Leporidae. Dat die aanwesigheid van die *Lepus*-tipe 2n = 48 kariotipe in drie genera, wat twee afsonderlike afstammelingslyne verteenwoordig, as gevolg van toeval alleen verskyn het, word as onwaarskynlik beskou en moet eerder gesien word as 'n aanduiding van 'n gemeenskaplike ewolusionêre oorsprong.

Dit is veral ten opsigte van die taksonomiese status van *Bunolagus*, wat nog altyd 'n twispunt was, waaromtrent gegewens oor die kariotipe van besondere belang is. Die aanwesigheid van 44 chromosome, waarvan die morfologie van sommiges uniek is, in teenstelling met die 48 van die genus *Lepus*, waarin *B. monticularis* voorheen geplaas is, word gesien as 'n besondere sterk onderskrywing van die geldigheid vir die daarstelling van die monotipiese *Bunolagus*. Die kariotipiese afwyking van *Bunolagus* van die voorouerlike vorm was gekenmerk deur drie versmeltings, een splitsing en talle nie-Robertsoniese strukturele veranderings, wat moontlik onder andere herhaalde perisentriese inversies behels het.

Pronolagus (2n = 42) is eweneens kariotipies uniek. Alhoewel dit sistematies gesproke geen waarde het vir die delimitasie van spesies nie het 'n kariotipiese vergelyking met die voorouerlike toestand aangetoon dat 'n reduksie van die chromosoomgetal, wat gepaard gegaan het met die ewolusie van hierdie genus, die gevolg was van die unieke versmeltingsprosesse. Daar is bevind dat van die chromosoomherrangskikkings wat verantwoordelik was vir die totstandkoming van die genome van *Bunolagus* en *Pronolagus*, heeltemal van mekaar verskil het. Dit impliseer dat hierdie herrangskikking moes plaasgevind het nadat hulle geïsoleerd geraak het.

Van bykans al die eksemplare wat in die studie gebruik is, is dwarssnitte van een van die boonste snytande gemaak. Verskille in die wydte en die vorm van die tand in dwarssnit gesien, vorm 'n betroubare kriterium om tussen eksemplare van *L. capensis* en *L. saxatilis* te onderskei. Die snytande van *L. capensis* word gekenmerk deurdat hulle smal is ($\bar{X}$ = 2,5 mm waargenome variasie 2,3 – 2,8) en in dwarssnit meer vierkantig vertoon met 'n skerp invouing van die snygroef. Hierteenoor is dié van *L. saxatilis* breër ($\bar{X}$ = 3,1 mm waargenome variasie 2,6 – 3,6) en neig om meer langwerpig te wees in dwarssnit. In die genus *Pronolagus* bestaan daar duidelike interspesifiese verskille tussen die snytandpatroon van *P. rupestris* aan die een kant,en dié van *P. crassicaudatus* en *P. randensis* aan die ander kant wat ooreenstem met mekaar ten opsigte van hierdie kenmerk. Hierdie verskil, wat hoofsaaklik toe te skryf is aan die verlenging van die laterale gebied van die snytande in

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P. crassicaudatus en *P. randensis,* word weerspieël in die afmetings in breedte by die drie taksa: *P. rupestris* ($\bar{X}$ = 2,5 mm waargenome variasie 2,3 – 2,8), *P. crassicaudatus* ($\bar{X}$ = 3,2 mm, waargenome variasie 2,9 – 3,6) en *P. randensis* ($\bar{X}$ = 3,0 mm waargenome variasie 2,6 – 3,4). Soortgelyke duidelike verskille bestaan tussen die snytandpatrone van *Bunolagus* en die Suid-Afrikaanse *Lepus*-spesies. Eersgenoemde word gekenmerk deur die afwesigheid van tandsement in die snygroef, besondere smal tande ($\bar{X}$ = 2,1 mm waargenome variasie 2,0 – 2,3) en dunner glasuurbedekking op die voorkant van die tande.

Vergelykende kwalitatiewe (vorm en silwerkleurende eienskappe) en kwantitatiewe (mates) studies van die spermatosoa van die Suid-Afrikaanse Lagomorpha is ook uitgevoer. Op grond van die vorm van die akrosoom kan al die betrokke taksa geplaas word onder een van twee tipes: die een tipe waar die spermatosoon gekenmerk word deur die besit van 'n relatief klein akrosoom wat slegs effens verby die voorkant van die spermkop strek en waaronder die twee *Lepus*-spesies resorteer, en die tweede tipe waaronder die drie *Pronolagus*-spesies en *B. monticularis* val, waar die akrosoom uitgesproke vooruitstekend is. Die spermatosoa van *L. capensis* en *L. saxatilis* verskil opvallend van mekaar ten opsigte van die drie parameters wat getoets is. Dié van *L. saxatilis* besit 'n langer kop en middelstuk as dié van *L. capensis* waarvan die spermatosoon gekenmerk word deur 'n breër kop. Verder is die spermatosoa van beide spesies identifiseerbaar op grond van hulle respektiewelike silwerkleurende eienskappe. Weens die beperkte monstergroottes beskikbaar in *Pronolagus*, veral van *P. crassidaudatus* en *P. randensis,* en *Bunolagus,* kon geen betroubare evaluasie van die verskille in afmetings tussen die spesies gemaak word nie. Verder is ook geen betekenisvolle silwerkleurende verskille waargeneem in geval van die eksemplare van hierdie taksa wat ondersoek is nie.

Horisontale styseljelelektroforese van serumproteïene en rooibloedselensieme is uitgevoer op materiaal vanaf eksemplare van *Pronolagus* en *Lepus,* maar geen geskikte materiaal kon verkry word vir 'n soortgelyke ondersoek op *Bunolagus* nie. Vyf (albumien, hemoglobien, indofenoloksidase, malaatdehidrogenase, 6-fosfoglukonaatdehidrogenase) van die nege proteïene wat konstant aanwesig was in al die eksemplare wat ondersoek was, is gevind monomorfies te wees in beide *Lepus* en *Pronolagus*, terwyl drie (transferien, esterase-1, esterase-2), polimorfies is. Die klein monsters, en veral die klein aantal polimorfiese sisteme, het gedetailleerde ondersoeke na genetiese ooreenkomste tussen taksa gekortwiek. Een ensiemsisteem, eritrositiese koolstofanhidrase, het egter geblyk handig te wees by die delimitasie van die Suid-Afrikaanse *Lepus*-spesies. Op grond van hierdie ensiem kan al die eksemplare wat geanaliseer is, toegesê word aan een van twee fenotipiese klasse; die eksemplare van *L. saxatilis* was gekenmerk deur 'n enkel, donkergekleurde positiewe band, terwyl eksemplare van *L. capensis* geen tekens van so 'n band getoon het nie. Laasgenoemde fenotiep word beskou as die gevolg te wees van 'n alleel sonder sigbare effek.

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Al die Suid-Afrikaanse Leporidae-spesies kan op grond van pels- en ander uitwendige fenotipiese kenmerke geïdentifiseer word. Hierdie klassieke taksonomiese parameters sowel as diagnostiese data verkry uit die ander sistematiese kriteria wat toegepas was gedurende die studie, is gebruik in die opstelling van identifikasiesleutels op beide genus- en spesievlak. Ten slotte kan gesê word dat die resultate van hierdie ondersoek die geldigheid van die genera *Lepus, Pronolagus* en die monotipiese *Bunolagus* vir die Suid-Afrikaanse Leporidae onderskryf, en verder ook die bestaan van twee *Lepus*-spesies, *L. capensis* en *L. saxatilis*, en drie *Pronolagus*-spesies, *P. rupestris, P. crassicaudatus* en *P. randensis*, bevestig.

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APPENDIX I – GAZETTEER

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Map co-ordinates of localities, taken in most cases with reference to the nearest town, and their spelling, were derived primarily from the gazetteers of the United States Board of Geographic Names (South Africa Vol I and II Division of Geography, Department of the Interior, 1954) and Skead's Zoo Historical Gazetteer (Skead, 1973). Where place names were not listed in either of the above sources, use was made of the 1:250,000 topocadastrial map series.

Cape Province:

† Beaufort West	32°20'S	22°35'E
† Bredasdorp	34°32'S	20°03'E
† *Calvinia	31°28'S	19°50'E
† Cape Agulhas	34°50'S	20°00'E
† Clanwilliam	32°11'S	18°53'S
† Cradock	32°10'S	25°38'E
† *Deelfontein	30°59'S	23°48'E
† Grahamstown	33°19'S	26°32'E
*Hutchinson	31°30'S	23°11'E
†Kammanassie	33°39'S	22°46'E
† Lemoenshoek	33°53'S	22°25'E
† Middelpos	31°55'S	20°13'E
† Murraysburg	31°57'S	23°46'E
*Nieuwoudtville	31°23'S	19°06'E
† Prieska	29°40'S	22°45'E
*Springbok	29°40'S	17°52'E
† *Sutherland	32°23'S	20°40'E
† Uitenhage	33°46'S	25°24'E
*Victoria West	31°26'S	23°07'E
† Wolseley	33°25'S	19°12'E
† Zoetendalsvlei	34°43'S	19°58'E

Orange Free State:

*Boshof	28°33'S	25°14'E
*Rouxville	30°25'S	26°50'E
*Winburg	28°32'S	27°01'E

† Museum specimen localities
* Field specimen localities

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Natal:

*Babanango	28°22 'S	31°05 'E
† Donnybrook	29°56' S	29°52' E
† Dundee	28°10' S	30°14' E
† Estcourt	29°00' S	29°53' E
† Harding	30°35' S	29°53' E
† *Hilton	29°29' S	30°18' E
† Howick	29°28' S	30°14' E
† Louwsburg	27°35' S	31°17' E
† *Magudu	27°32' S	31°39' E
*Matatiele	30°20' S	28°48' E
† Melmoth	28°35' S	31°24' E
† Oribi Gorge	30°40' S	30°15' E
† Otto's Bluff	29°29' S	30°23' E
† Paulpietersburg	27°25' S	30°49' E
† Umzinto	30°19' S	30°40' E
*Winterton	28°48' S	29°32' E

Transvaal:

*Bandelierkop	23°19' S	29°48' E
*Bloemhof	27°39' S	25°36' E
† Bon Accord	25°38' S	28°11' E
*Derdepoort	25°43' S	28°18' E
† Hartebeespoort Dam	25°45' S	27°51' E
† Hennop's River	25°51' S	27°56' E
*Jack Scott Nature Reserve	25°52' S	27°43' E
† Johannesburg	26°12' S	28°05' E
*Kaalplaats	25°39'S	28°11'E
† Koster	25°52'S	26°54'E
*Lydenburg	25°06'S	30°27'E
† Messina	22°21'S	30°03'E
† Pafuri	22°27'S	31°18'E
† Silverton	25°44'S	28°18'E
† Waterberg	24°53'S	27°48'E
† Zeerust	25°33'S	26°05'E

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APPENDIX II

EXTERNAL AND CRANIAL MEASUREMENTS OF THE SOUTH AFRICAN LEPORIDAE

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TABLE 1: External and cranial measurements of *L. capensis*. Localities and number of specimens (in parenthesis) pooled for computing the statistics are : Bandelierkop (2), Bloemhof (4), Boshof (5), Calvinia (1), Deelfontein (2), Hutchinson (7), Nieuwoudtville (5), Rouxville (1), Springbok (5).
$\bar{X}$ = Arithmetic mean; n = Sample size; t SE = 95% Confidence limit; CV = Coefficient of variation

$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
		Head and body length		
468	32	5,84	3,5	445–500
		Tail length		
112	32	5,06	12,5	65–150
		Hind foot length		
118	32	1,42	3,3	110–125
		Ear length		
118	32	3,77	8,9	95–135
		Total length of skull		
86,3	32	0,86	2,8	81,0–90,6
		Muzzle length		
38,7	32	0,78	5,6	35,1–42,9
		Frontal length		
33,1	32	0,68	5,7	29,1–36,5
		Parietal length		
19,2	32	0,49	7,2	16,8–22,0
		Posterior muzzle length		
18,7	32	0,50	7,4	16,5–21,5
		Anterior frontal breadth		
16,2	32	0,40	6,9	14,3–18,6
		Posterior frontal breadth		
12,1	32	0,32	7,4	10,5–14,0
		Interauditory breadth		
26,7	31	0,34	3,5	24,6–28,8

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TABLE 1 : (continued)

$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
		Basal cranial length		
70,1	32	0,74	2,9	65,4–73,5
		Palate incisor length		
34,2	32	0,42	3,4	32,1–36,7
		Palate vacuity length		
22,3	32	0,36	4,5	20,6–24,8
		Hard palate length		
5,6	32	0,22	11,1	4,5–6,8
		Mesopterygoid space		
8,6	30	0,25	7,7	6,9–10,0
		Maxillary premolar length		
7,7	32	0,12	4,4	7,1–8,7
		Maxillary premolar-molar length		
14,6	32	0,22	4,2	13,4–15,8
		Principal I^1 breadth		
2,5	32	0,04	4,4	2,3–2,8
		Palatal breadth		
23,7	30	0,36	4,1	21,4–25,5
		Bizygomatic breadth		
39,7	31	0,39	2,7	37,9–42,2
		Maximum cranial breadth		
29,8	32	0,32	2,9	28,0–31,9
		Zygomatic arch length		
33,9	32	0,52	4,3	31,5–37,5
		Posterior cranial height		
17,5	30	0,44	6,7	15,4–20,8
		Bulla breadth		
9,0	32	0,31	9,4	7,3–10,6

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TABLE 1 : (continued)

$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
		Maximum mandibular length		
60,8	29	0,91	3,9	55,5–65,0
		Mandibular toothrow length		
35,7	29	0,51	3,7	32,0–38,5
		Maximum mandibular height		
38,9	29	0,72	4,9	35,3–42,9
		Mandibular body breadth		
5,5	32	0,18	9,0	4,5–7,5
		I_1 breadth		
2,6	32	0,05	5,6	2,4–3,1
		Mandibular premolar-molar length		
15,0	31	0,26	4,7	13,2–16,1

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TABLE 2 : External and cranial measurements of *L. saxatilis*. Clinal variation in most characters precluded the calculation of species mean values. Localities have been ranked in accordance with the variation exhibited by the measurement: greatest length of skull.
$\bar{X}$ = Arithmetic mean; n = Sample size; t SE = 95% Confidence limit; CV = Coefficient of variation.

Locality	$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
		Head and body length			
Beaufort West	608	1			
Murraysburg	590	1			
Hutchinson	615	1			
Calvinia	570	2	317,65	6,2	545–595
Deelfontein	588	2	31,77	0,6	585–590
Zoetendalsvlei	510	1			
Sutherland	563	2	95,30	1,9	555–570
Cape Agulhas	565	1			
Nieuwoudtville	536	5	54,54	8,2	460–570
Springbok	545	1			
Lemoenshoek	600	1			
Clanwilliam	566	2	578,12	11,4	520–611
Prieska	558	2	44,47	0,9	554–561
Rouxville	549	7	18,63	3,7	530–590
Wolseley	490	1			
Grahamstown	512	3	68,37	5,4	480–530
Boshof	510	1			
Bredasdorp	600	1			
Winburg	543	6	20,66	3,6	510–570
Uitenhage	532	1			
Matatiele	529	7	15,73	3,2	500–555
Cradock	533	1			
Bloemhof	524	4	35,20	4,2	500–545
Kammanassie	593	2	216,00	4,1	516–610
Hilton	520	9	15,49	3,9	490–555
Lydenburg	513	3	51,72	4,1	490–530
Winterton	519	12	13,05	4,0	485–550
Magudu	506	8	21,64	5,1	475–540
Kaalplaats	488	5	36,62	6,0	440–520
Babanango	475	2	65,53	1,5	470–480
Derdepoort	481	9	12,07	3,3	460–500
		Tail length			
Beaufort West	127	1			
Murraysburg	140	1			
Hutchinson	150	1			
Calvinia	138	2	158,83	12,9	125–150
Deelfontein	128	2	31,77	2,8	125–130

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TABLE 2 (continued)

Locality	$\bar{X}$ mm	n	t SE	CV(%)	Range(mm)
Zoetendalsvlei	110	1			
Sutherland	145	2	381,18	29,3	115–175
Cape Agulhas	135	1			
Nieuwoudtville	144	5	26,84	15,0	110–165
Springbok	130	1			
Lemoenshoek	100	1			
Clanwilliam	106	2	184,24	19,4	91–120
Prieska	120	2	31,77	3,0	117–122
Rouxville	131	7	8,32	6,9	125–150
Wolseley	110	1			
Grahamstown	118	3	18,98	6,5	110–125
Boshoff	120	1			
Bredasdorp	120	1			
Winburg	101	6	29,39	27,8	70–135
Uitenhage	90	1			
Matatiele	125	7	9,25	8,0	110–135
Cradock	110	1			
Bloemhof	133	4	18,94	9,0	125–150
Kammanassie	123	2	38,12	3,5	120–126
Hilton	114	9	9,76	11,2	90–125
Lydenburg	128	3	47,03	14,8	115–150
Winterton	119	12	8,88	11,7	100–140
Magudu	100	8	13,96	16,7	80–125
Kaalplaats	124	5	14,82	9,6	105–135
Babanango	113	2	31,77	3,1	110–115
Derdepoort	115	10	8,83	10,8	90–130
		Hind foot length			
Beaufort West	139	1			
Murraysburg	135	1			
Hutchinson	155	1			
Calvinia	147	2	108,00	8,2	138–155
Deelfontein	142	2	44,47	3,5	138–145
Zoetendalsvlei	125	1			
Sutherland	150	2			150–150
Cape Agulhas	125	1			
Nieuwoudtville	146	5	10,70	5,9	138–155
Springbok	140	1			
Lemoenshoek	135	1			
Clanwilliam	137	2	114,35	9,3	128–146
Prieska	130	2	31,77	2,7	127–132
Rouxville	135	7	4,83	3,9	127–140

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV(%)	Range(mm)
Wolseley	135	1			
Grahamstown	122	3	7,19	2,4	120–125
Boshoff	135	1			
Bredasdorp	115	1			
Winburg	133	6	4,39	3,2	125–135
Uitenhage	122	1			
Matatiele	132	7	4,51	3,7	125–140
Cradock	136	1			
Bloemhof	137	4	9,09	4,2	130–143
Kammanassie	146	2	12,71	1,0	145–147
Hilton	128	9	3,52	3,6	120–135
Lydenburg	130	3	12,42	3,9	125–135
Winterton	131	12	2,65	3,2	125–140
Magudu	119	8	4,14	4,2	110–125
Kaalplaats	123	5	5,55	3,6	120–130
Babanango	120	2			120–120
Derdepoort	125	10	3,56	4,0	120–130
			Ear length		
Beaufort West	140	1			
Murraysburg	150	1			
Hutchinson	130	1			
Calvinia	135	2	63,53	5,2	130–140
Deelfontein	135	2	63,53	5,2	130–140
Zoetendalsvlei	140	1			
Sutherland	140	2			140–140
Cape Agulhas	145	1			
Nieuwoudtville	148	5	5,55	3,0	140–150
Springbok	130	1			
Lemoenshoek	130	1			
Clanwilliam	147	2	38,12	2,9	144–150
Prieska	133	2	19,06	1,6	131–134
Rouxville	126	7	6,48	5,6	120–135
Wolseley	153	1			
Grahamstown	124	3	4,30	1,4	122–125
Boshoff	125	1			
Bredasdorp	152	1			
Winburg	118	6	4,29	3,5	115–125
Uitenhage	116	1			
Matatiele	121	7	6,38	5,7	110–130
Cradock	135	1			
Bloemhof	121	4	3,98	2,1	120–125
Kammanassie	136	2	57,2	4,7	131–140
Hilton	107	9	4,34	5,3	100–115

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
Lydenburg	107	3	11,48	4,3	102–110
Winterton	108	12	12,87	4,2	100–115
Magudu	102	8	4,63	5,4	95–110
Kaalplaats	103	5	3,40	2,7	100–105
Babanango	99	2	12,71	1,4	98–100
Derdepoort	103	9	2,67	3,4	100–110
		Total length of skull			
Beaufort West	107,7	1			
Murraysburg	106,0	1			
Hutchinson	105,5	1			
Calvinia	105,1	2	12,07	1,3	104,1–106,0
Deelfontein	104,5	2	28,59	3,1	102,2–106,7
Zoetendalsvlei	104,0	1			
Sutherland	103,5	2	2,54	0,3	103,3–103,7
Cape Agulhas	102,6	1			
Nieuwoudtville	101,9	5	2,23	1,8	100,5–104,9
Springbok	100,8	1			
Lemoenshoek	100,2	1			
Clanwilliam	99,3	2	37,48	4,2	96,3–102,2
Prieska	99,1	2	6,99	0,8	98,5– 99,6
Rouxville	98,9	7	2,98	3,3	95,4–103,9
Wolseley	98,5	1			
Grahamstown	97,7	3	7,67	3,2	95,5–101,2
Boshof	97,2	1			
Bredasdorp	96,9	1			
Winburg	96,5	6	2,64	2,6	92,4– 99,3
Uitenhage	96,1	1			
Matatiele	95,7	7	1,30	1,5	93,9–98,1
Cradock	95,4	1			
Bloemhof	95,3	4	1,24	0,8	94,3–96,2
Kammanassie	95,1	2	1,27	0,2	95,0–95,2
Hilton	94,2	9	2,20	3,1	90,3–99,8
Lydenburg	93,3	3	4,04	1,8	91,4–94,3
Winterton	92,8	12	1,43	2,3	88,4–95,2
Magudu	90,9	8	2,49	3,3	87,8–97,0
Kaalplaats	90,0	4	2,12	1,7	88,5–91,9
Babanango	89,6	2	5,72	0,7	89,1–90,0
Derdepoort	88,5	8	1,10	1,5	87,0–90,1

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
		Muzzle length			
Beaufort West	47,8	1			
Murraysburg	46,5	1			
Hutchinson	48,8	1			
Calvinia	48,2	2	13,34	3,1	47,1–49,2
Deelfontein	45,6	2	28,59	7,0	43,3–47,8
Zoetendalsvlei	45,7	1			
Sutherland	47,0	2	9,53	2,3	46,2–47,7
Cape Agulhas	42,5	1			
Nieuwoudtville	43,2	5	1,77	3,3	41,7–45,4
Springbok	44,4	1			
Lemoenshoek	40,2	1			
Clanwilliam	40,6	2	30,49	8,4	38,2–43,0
Prieska	43,2	2	22,24	5,7	41,4–44,9
Rouxville	44,0	7	1,51	3,7	42,1–46,9
Wolseley	40,0	1			
Grahamstown	43,1	3	1,51	1,4	42,4–43,5
Boshof	42,5	1			
Bredasdorp	43,0	1			
Winburg	42,1	6	1,79	4,1	39,9–44,5
Uitenhage	41,6	1			
Matatiele	41,7	7	1,42	3,7	39,5–43,8
Cradock	39,0	1			
Bloemhof	43,0	4	2,89	3,4	41,1–44,6
Kammanassie	41,8	2	9,53	2,5	41,0–42,5
Hilton	41,7	9	1,08	3,4	38,6–43,2
Lydenburg	40,8	3	4,60	4,5	39,0–42,7
Winterton	40,7	11	0,79	2,9	38,6–42,0
Magudu	41,1	8	1,70	4,9	38,6–44,8
Kaalplaats	39,2	5	1,53	3,1	37,6–40,6
Babanango	38,0	2	4,45	1,3	37,6–38,3
Derdepoort	39,4	10	1,37	4,9	36,3–43,1
		Frontal length			
Beaufort West	38,6	1			
Murraysburg	42,2	1			
Hutchinson	40,4	1			
Calvinia	40,7	2	16,52	4,5	39,4–42,0
Deelfontein	40,3	2	12,07	3,3	39,3–41,2
Zoetendalsvlei	39,0	1			
Sutherland	38,5	2	3,81	1,1	38,2–38,8
Cape Agulhas	39,6	1			
Nieuwoudtville	40,6	5	3,58	7,1	38,0–44,2
Springbok	34,9	1			
Lemoenshoek	38,5	1			

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Clanwilliam	40,5	2			40,5–40,5
Prieska	38,0	2	10,80	3,2	37,1–38,8
Rouxville	38,8	7	2,83	7,9	34,2–42,4
Wolseley	37,7	1			
Grahamstown	38,7	3	2,83	2,9	37,8–40,0
Boshof	42,0	1			
Bredasdorp	34,3	1			
Winburg	39,7	6	2,68	6,4	36,9–43,0
Uitenhage	36,3	1			
Matatiele	39,9	7	1,41	3,8	38,0–42,5
Cradock	36,3	1			
Bloemhof	36,9	4	3,89	6,6	33,2–38,4
Kammanassie	36,2	2	10,16	3,1	35,4–37,0
Hilton	37,7	9	2,37	8,2	32,6–41,9
Lydenburg	37,5	3	2,52	2,7	36,6–38,6
Winterton	38,3	12	1,10	4,5	34,6–40,5
Magudu	37,4	7	0,64	1,8	36,5–38,2
Kaalplaats	35,8	5	1,95	4,4	33,8–38,0
Babanango	38,4	2	1,91	0,6	38,2–38,5
Derdepoort	36,2	9	1,41	5,1	33,2–38,5
			Parietal length		
Beaufort West	23,9	1			
Murraysburg	20,8	1			
Hutchinson	20,2	1			
Calvinia	22,9	2	15,88	7,7	21,6–24,1
Deelfontein	21,6	2	15,25	7,9	20,4–22,8
Zoetendalsvlei	20,7	1			
Sutherland	20,9	2	8,26	4,4	20,2–21,5
Cape Agulhas	20,0	1			
Nieuwoudtville	22,1	5	1,96	7,1	20,3–24,2
Springbok	–				
Lemoenshoek	22,1	1			
Clanwilliam	21,7	2	3,18	1,6	21,4–21,9
Prieska	20,5	2	12,07	6,6	19,5–21,4
Rouxville	21,9	7	1,33	6,6	20,0–23,6
Wolseley	22,9	1			
Grahamstown	21,6	3	4,84	9,0	20,1–23,8
Boshof	19,7	1			
Bredasdorp	23,7	1			
Winburg	19,6	6	1,28	6,2	18,1–21,2
Uitenhage	21,9	1			
Matatiele	20,1	7	0,57	3,1	19,4–20,9
Cradock	22,2	1			
Bloemhof	19,3	4	1,16	3,8	18,5–20,2

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TABLE 2 (continued)

Locality	$\bar{X}$(mm)	n	t SE	CV(%)	Range (mm)
Kammanassie	20,0	2	1,91	1,1	19,8–20,1
Hilton	20,5	9	1,23	7,4	18,3–22,0
Lydenburg	20,2	3	3,43	6,8	18,6–21,2
Winterton	20,3	12	0,81	6,3	18,1–22,8
Magudu	19,0	7	1,07	6,1	17,9–21,0
Kaalplaats	19,3	5	0,92	3,8	18,5–20,4
Babanango	19,8	2	3,18	1,8	19,5–20,0
Derdepoort	19,1	8	0,40	2,5	18,5–20,0
		Posterior muzzle breadth			
Beaufort West	23,2	1			
Murraysburg	22,7	1			
Hutchinson	22,6	1			
Calvinia	23,3	2	20,33	9,7	21,7–24,9
Deelfontein	21,0	2	1,91	1,0	20,8–21,1
Zoetendalsvlei	21,1	1			
Sutherland	22,6	2	6,99	3,5	22,0–23,1
Cape Agulhas	22,6	1			
Nieuwoudtville	22,2	5	0,93	3,4	21,0–22,9
Springbok	21,7	1			
Lemoenshoek	22,0	1			
Clanwilliam	21,3	2	13,34	7,0	20,2–22,3
Prieska	22,2	2	0,64	0,3	22,1–22,2
Rouxville	21,3	7	1,18	6,0	18,6–22,2
Wolseley	20,1	1			
Grahamstown	21,3	3	4,60	8,7	20,1–23,4
Boshof	21,2	1			
Bredasdorp	21,4	1			
Winburg	21,4	6	1,17	5,2	20,3–23,0
Uitenhage	19,6	1			
Matatiele	20,9	7	1,25	6,5	18,4–22,5
Cradock	19,0	1			
Bloemhof	22,9	4	2,07	5,7	21,5–24,3
Kammanassie	21,0	2	4,45	2,4	20,6–21,3
Hilton	21,7	9	0,74	4,4	19,8–22,9
Lydenburg	20,7	3	4,28	8,3	19,3–22,6
Winterton	20,7	11	0,77	5,7	19,0–22,5
Magudu	20,3	8	0,97	5,7	18,9–22,2
Kaalplaats	19,8	5	1,05	4,3	18,9–21,0
Babanango	19,5	2	13,98	8,0	18,4–20,0
Derdepoort	20,7	10	0,69	4,7	18,8–22,5

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TABLE 2 (continued)

Locality	$\bar{X}$(mm)	n	t SE	CV(%)	Range (mm)
		Anterior frontal breadth			
Beaufort West	24,4	1			
Murraysburg	21,8	1			
Hutchinson	23,2	1			
Calvinia	23,1	2	26,68	12,9	21,0–25,2
Deelfontein	19,2	2	25,41	14,7	17,2–21,2
Zoetendalsvlei	20,4	1			
Sutherland	21,0	2	12,07	6,4	20,0–21,9
Cape Agulhas	21,3	1			
Nieuwoudtville	21,0	5	2,50	9,6	18,3–23,5
Springbok	–				
Lemoenshoek	21,2	1			
Clanwilliam	20,6	2	22,24	12,0	18,8–22,3
Prieska	21,1	2	6,99	3,7	20,5–21,6
Rouxville	20,9	7	1,02	5,3	19,8–23,0
Wolseley	19,0	1			
Grahamstown	21,5	3	4,62	8,7	19,5–23,2
Boshof	19,4	1			
Bredasdorp	18,4	1			
Winburg	20,2	6	1,22	5,7	19,4–22,5
Uitenhage	18,8	1			
Matatiele	19,0	7	0,83	4,8	17,5–20,0
Cradock	18,0	1			
Bloemhof	22,6	4	1,51	4,2	21,6–23,6
Kammanassie	23,2	2	10,80	5,2	22,3–24,0
Hilton	20,4	9	1,47	9,4	17,0–22,4
Lydenburg	21,6	3	6,96	13,0	19,9–24,8
Winterton	19,5	12	1,10	8,9	16,0–22,5
Magudu	19,3	8	0,83	5,2	18,2–21,5
Kaalplaats	18,6	5	1,65	7,2	17,0–20,5
Babanango	18,5	2	15,88	9,6	17,2–19,7
Derdepoort	19,3	10	0,53	3,9	18,4–20,4
		Posterior frontal breadth			
Beaufort West	14,7	1			
Murraysburg	15,0	1			
Hutchinson	11,6	1			
Calvinia	14,4	2	14,61	11,3	13,2–15,5
Deelfontein	11,3	2	1,91	1,9	11,1–11,4
Zoetendalsvlei	12,2	1			
Sutherland	13,3	2	10,16	8,5	12,5–14,1
Cape Agulhas	13,8	1			
Nieuwoudtville	14,6	5	0,93	5,3	13,9–15,5
Springbok	–				
Lemoenshoek	13,9	1			

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TABLE 2 (continued)

Locality	$\bar{X}$(mm)	n	t SE	CV(%)	Range (mm)
Clanwilliam	14,8	2	0,64	0,5	14,7–14,8
Prieska	13,3	2	0,64	5,4	13,2–13,3
Rouxville	13,4	7	1,25	10,1	10,8–14,9
Wolseley	12,5	1			
Grahamstown	13,0	3	3,02	9,4	12,2–14,4
Boshof	11,8	1			
Bredasdorp	13,4	1			
Winburg	12,2	6	0,51	4,0	11,4–12,7
Uitenhage	14,3	1			
Matatiele	12,3	7	0,76	6,7	11,2–13,4
Cradock	14,4	1			
Bloemhof	13,1	4	1,50	7,2	11,8–14,0
Kammanassie	13,7	2	8,89	7,2	13,0–14,4
Hilton	13,0	9	1,48	14,8	11,1–17,7
Lydenburg	12,8	3	3,39	10,7	11,2–13,7
Winterton	12,4	12	0,77	9,9	10,7–14,5
Magudu	12,6	7	1,00	8,6	10,9–13,8
Kaalplaat	12,5	5	1,13	7,3	11,4–13,9
Babanango	12,7	2	5,08	4,5	12,3–13,1
Derdepoort	12,1	10	0,52	6,0	10,5–13,0
		Interauditory breadth			
Beaufort West	33,0	1			
Murraysburg	31,1	1			
Hutchinson	30,4	1			
Calvinia	31,1	2	11,44	4,1	30,2–32,0
Deelfontein	30,1	2	1,91	0,7	29,9–30,2
Zoetendalsvlei	30,0	1			
Sutherland	30,3	2	3,18	1,2	30,0–30,5
Cape Agulhas	29,5	1			
Nieuwoudtville	29,4	5	0,75	2,1	28,8–30,0
Springbok	–				
Lemoenshoek	29,2	1			
Clanwilliam	28,6	2	13,34	5,2	27,5–29,6
Prieska	29,4	2	9,53	3,6	28,6–30,1
Rouxville	28,8	7	0,75	2,8	27,8–29,8
Wolseley	29,3	1			
Grahamstown	27,6	3	0,80	1,2	27,4–28,0
Boshof	28,0	1			
Bredasdorp	29,6	1			
Winburg	29,5	6	1,71	5,5	27,9–31,8
Uitenhage	28,1	1			
Matatiele	27,6	5	0,63	2,1	27,0–28,5
Cradock	29,2	1			
Bloemhof	28,6	4	1,05	2,3	27,9–29,4

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TABLE 2 (continued)

Locality	$\bar{X}$(mm)	n	t SE	CV(%)	Range (mm)
Kammanassie	29,1	2	1,91	0,7	28,9–29,2
Hilton	27,6	9	0,78	3,7	25,0–28,5
Lydenburg	27,5	3	3,90	5,7	26,3–29,3
Winterton	28,1	11	0,73	3,9	26,0–29,8
Magudu	27,1	6	1,35	4,8	25,6–28,8
Kaalplaats	26,9	3	0,74	1,7	26,4–27,2
Babanango	26,8	1			
Derdepoort	26,4	7	0,71	2,9	25,5–27,8
		Basal cranial length			
Beaufort West	88,8	1			
Murraysburg	85,2	1			
Hutchinson	84,5	1			
Calvinia	83,7	2	6,35	0,8	83,2–84,2
Deelfontein	83,9	2	32,40	4,3	81,3–86,4
Zoetendalsvlei	83,3	1			
Sutherland	83,6	2	7,62	1,0	83,0–84,2
Cape Agulhas	83,6	1			
Nieuwoudtville	82,3	5	2,39	2,3	80,6–85,2
Springbok	82,3	1			
Lemoenshoek	81,1	1			
Clanwilliam	79,9	2	34,94	4,9	77,1–82,6
Prieska	79,6	2	10,16	1,4	78,8–80,4
Rouxville	78,6	7	2,56	3,5	75,2–82,5
Wolseley	80,5	1			
Grahamstown	78,4	3	6,92	3,6	76,5–81,6
Boshof	78,8	1			
Bredasdorp	78,3	1			
Winburg	78,0	6	2,76	3,4	74,2–81,3
Uitenhage	76,5	1			
Matatiele	77,5	7	1,19	1,7	75,9–79,2
Cradock	76,8	1			
Bloemhof	77,0	4	1,87	1,5	75,6–78,4
Kammanassie	75,8	2	2,54	0,4	75,6–76,0
Hilton	76,4	9	2,00	3,4	72,6–81,1
Lydenburg	74,6	3	4,85	2,6	72,4–76,0
Winterton	74,6	11	1,12	2,3	71,7–77,0
Magudu	73,5	6	2,83	3,7	70,9–78,4
Kaalplaats	72,2	4	0,92	0,9	71,4–73,0
Babanango	72,1	2	2,54	0,4	71,9–72,3
Derdepoort	71,4	7	1,00	1,5	70,1–73,4

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TABLE 2 (continued)

Locality	$\bar{X}$(mm)	n	t SE	CV(%)	Range (mm)
		Palate incisor length			
Beaufort West	44,6	1			
Murraysburg	43,5	1			
Hutchinson	42,8	1			
Calvinia	42,6	2	22,87	6,0	40,8–44,0
Deelfontein	43,0	2	18,42	4,8	41,5–44,4
Zoetendalsvlei	43,0	1			
Sutherland	41,3	2	7,62	2,1	40,7–41,9
Cape Agulhas	41,9	1			
Nieuwoudtville	40,2	5	1,32	2,6	38,8–41,7
Springbok	42,5	1			
Lemoenshoek	40,4	1			
Clanwilliam	39,7	2	26,05	7,3	37,6–41,7
Prieska	38,9	2	10,80	3,1	38,0–39,7
Rouxville	40,0	7	1,27	3,4	38,0–42,2
Wolseley	38,8	1			
Grahamstown	38,3	3	3,25	3,4	36,8–39,2
Boshof	40,0	1			
Bredasdorp	39,6	1			
Winburg	39,1	6	1,15	2,8	37,6–40,3
Uitenhage	38,1	1			
Matatiele	38,5	7	0,75	2,1	37,7–39,9
Cradock	38,7	1			
Bloemhof	38,8	4	0,88	1,4	38,1–39,4
Kammanassie	37,9	2	14,67	4,3	36,7–39,0
Hilton	38,1	9	1,09	3,7	36,1–40,2
Lydenburg	37,5	3	3,31	3,6	36,4–39,0
Winterton	38,0	11	0,72	2,8	35,6–39,7
Magudu	37,5	8	1,14	3,6	35,5–40,2
Kaalplaats	36,4	5	0,46	1,0	35,9–36,8
Babanango	36,5	2	7,62	2,3	35,9–37,1
Derdepoort	35,8	10	0,72	2,9	34,0–37,7
		Palate vacuity length			
Beaufort West	29,0	1			
Murraysburg	24,7	1			
Hutchinson	26,5	1			
Calvinia	27,2	2	0,64	0,3	27,1–27,2
Deelfontein	27,5	2	10,80	4,4	26,6–28,3
Zoetendalsvlei	28,1	1			
Sutherland	27,2	2	12,07	5,0	26,2–28,1
Cape Agulhas	26,4	1			
Nieuwoudtville	26,9	5	1,07	3,2	26,2–28,0
Springbok	27,8	1			
Lemoenshoek	26,2	1			

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t.SE	CV(%)	Range (mm)
Clanwilliam	26,1	2	15,67	9,2	24,4–27,8
Prieska	24,6	2	3,18	1,4	24,3–24,8
Rouxville	25,3	7	0,61	2,6	24,6–26,5
Wolseley	25,7	1			
Grahamstown	24,0	3	1,58	2,7	23,6–24,7
Boshof	25,7	1			
Bredasdorp	26,0	1			
Winburg	24,6	6	0,55	2,1	24,0–25,2
Uitenhage	24,4	1			
Matatiele	24,3	7	0,66	2,9	23,5–25,2
Cradock	22,7	1			
Bloemhof	24,9	3	1,88	4,1	23,7–25,6
Kammanassie	24,0	2	10,81	5,0	23,1–24,8
Hilton	24,4	9	0,61	3,2	23,1–25,3
Lydenburg	23,9	3	1,49	2,5	23,3–24,5
Winterton	23,9	11	0,58	3,6	22,4–25,4
Magudu	23,3	8	0,56	2,9	22,2–24,0
Kaalplaats	23,0	5	0,85	3,0	22,4–24,1
Babanango	23,6	2	3,18	1,5	23,3–23,8
Derdepoort	22,2	10	0,57	3,6	20,9–23,0
		Hard palate length			
Beaufort West	7,2	1			
Murraysburg	7,4	1			
Hutchinson	8,1	1			
Calvinia	7,5	2	1,27	18,9	6,5–8,5
Deelfontein	8,0	2	1,91	2,7	7,8–8,1
Zoetendalsvlei	6,5	1			
Sutherland	6,4	2	1,91	3,3	6,2–6,5
Cape Agulhas	7,9	1			
Nieuwoudtville	6,3	5	0,34	4,3	6,0–6,7
Springbok	6,8	1			
Lemoenshoek	6,4	1			
Clanwilliam	6,8	2	1,91	3,1	6,6–6,9
Prieska	7,0	2	12,71	20,2	6,0–8,0
Rouxville	7,1	7	0,69	10,5	6,0–8,2
Wolseley	6,0	1			
Grahamstown	6,9	3	2,16	12,6	5,9–7,5
Boshof	7,3	1			
Bredasdorp	6,3	1			
Winburg	7,0	6	0,15	2,1	6,8–7,2
Uitenhage	5,8	1			
Matatiele	7,0	7	0,54	8,4	6,4–8,2
Cradock	8,3	1			
Bloemhof	6,7	4	0,50	4,7	6,3–7,0
Kammanassie	6,7	2	6,35	10,6	6,2–7,2

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Hilton	6,8	9	0,50	9,6	6,0–7,8
Lydenburg	6,9	3	1,14	6,6	6,5–7,4
Winterton	7,0	12	0,40	8,9	5,8–8,0
Magudu	6,7	8	0,67	12,1	5,4–8,0
Kaalplaats	7,2	5	0,74	8,3	6,2–7,7
Babanango	6,3	2	2,54	4,5	6,1–6,5
Derdepoort	6,5	10	0,36	7,8	5,9–7,2
		Mesopterygoid space			
Beaufort West	10,0	1			
Murraysburg	10,5	1			
Hutchinson	9,8	1			
Calvinia	10,4	2	2,54	2,7	10,2–10,6
Deelfontein	9,8	2	3,18	3,7	9,5–10,0
Zoetendalsvlei	9,9	1			
Sutherland	10,9	2	6,35	6,5	10,4–11,4
Cape Agulhas	10,0	1			
Nieuwoudtville	10,1	5	0,84	6,7	9,2–11,0
Springbok	9,6	1			
Lemoenshoek	10,0	1			
Clanwilliam	9,5	2	4,45	5,2	9,1–9,8
Prieska	9,6	2	2,54	3,0	9,4–9,8
Rouxville	9,8	7	0,51	5,6	9,2–10,7
Wolseley	10,5	1			
Grahamstown	10,1	3	2,29	9,2	9,0–10,6
Boshof	9,3	1			
Bredasdorp	9,4	1			
Winburg	8,8	6	0,38	4,2	8,2–9,2
Uitenhage	10,7	1			
Matatiele	9,2	7	0,55	6,4	8,5–10,0
Cradock	7,5	1			
Bloemhof	9,5	4	1,75	11,6	8,1–10,7
Kammanassie	8,7	2	5,08	6,5	8,3–9,1
Hilton	8,6	9	0,42	6,3	7,8–9,4
Lydenburg	8,2	3	0,62	3,1	8,0–8,5
Winterton	8,4	12	0,33	6,2	7,7–9,2
Magudu	8,1	8	0,48	7,2	7,3–8,8
Kaalplaats	8,4	5	0,51	4,8	7,9–8,8
Babanango	8,8	2	3,18	4,1	8,5–9,0
Derdepoort	8,3	10	0,30	5,1	7,7–9,0
		Maxillary premolar length			
Beaufort West	9,1	1			
Murraysburg	9,1	1			
Hutchinson	9,3	1			
Calvinia	8,5	2	1,27	1,7	8,4–8,6

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Deelfontein	9,6	2	6,35	7,4	9,1–10,1
Zoetendalsvlei	9,2	1			
Sutherland	9,2	2	1,27	1,5	9,1–9,3
Cape Agulhas	9,6	1			
Nieuwoudtville	9,2	5	0,45	4,0	8,9–9,8
Springbok	8,9	1			
Lemoenshoek	9,0	1			
Clanwilliam	8,7	2	3,18	4,1	8,4–8,9
Prieska	8,9	2	1,91	2,4	8,7–9,0
Rouxville	9,3	7	0,55	6,5	8,3–9,9
Wolseley	8,8	1			
Grahamstown	8,8	3	1,43	6,5	8,5–9,5
Boshof	9,2	1			
Bredasdorp	8,6	1			
Winburg	8,6	6	0,46	5,1	7,8–9,0
Uitenhage	8,4	1			
Matatiele	9,0	7	0,23	2,8	8,8–9,4
Cradock	8,2	1			
Bloemhof	9,6	4	0,59	3,9	9,1–9,9
Kammanassie	9,3	2	9,53	11,5	8,5–10,0
Hilton	8,8	9	0,45	6,7	8,0–9,9
Lydenburg	8,2	3	0,53	3,8	7,9–8,5
Winterton	8,7	12	0,22	3,9	7,9–9,0
Magudu	8,6	8	0,38	5,3	8,1–9,4
Kaalplaats	9,0	5	0,57	5,2	8,6–9,5
Babanango	9,0	2	3,18	4,0	8,7–9,2
Derdepoort	9,2	10	0,43	6,6	8,2–10,0
		Maxillary toothrow length			
Beaufort West	16,6	1			
Murraysburg	17,5	1			
Hutchinson	17,0	1			
Calvinia	16,2	2			16,2–16,2
Deelfontein	17,8	2	6,99	4,4	17,2–18,3
Zoetendalsvlei	16,9	1			
Sutherland	17,4	2	7,62	4,9	16,8–18,0
Cape Agulhas	16,4	1			
Nieuwoudtville	17,5	5	1,08	5,0	16,1–18,3
Springbok	16,7	1			
Lemoenshoek	16,5	1			
Clanwilliam	16,5	2	8,26	5,6	15,8–17,1
Prieska	15,7	2	6,35	4,5	15,2–16,2
Rouxville	17,0	7	0,86	5,5	16,0–18,8
Wolseley	16,2	1			

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Grahamstown	16,2	3	1,79	4,5	15,4–16,8
Boshof	17,2	1			
Bredasdorp	15,9	1			
Winburg	16,5	6	0,48	2,8	16,0–17,0
Uitenhage	15,7	1			
Matatiele	16,5	7	0,20	1,3	16,2–16,9
Cradock	16,5	1			
Bloemhof	17,2	4	0,53	1,9	16,8–17,5
Kammanassie	16,7	2	12,71	8,5	15,7–17,7
Hilton	16,3	9	0,56	4,5	15,4–17,6
Lydenburg	15,5	3	1,37	3,6	14,9–16,0
Winterton	15,7	12	0,46	4,7	13,8–16,6
Magudu	15,4	8	0,36	2,8	14,8–16,2
Kaalplaats	16,3	5	0,54	2,7	16,0–17,0
Babanango	16,1	2	0,64	0,4	16,0–16,1
Derdepoort	16,6	10	0,59	5,0	15,5–17,8
		Principal I[1] breadth			
Beaufort West	3,4	1			
Murraysburg	3,4	1			
Hutchinson	3,2	1			
Calvinia	3,2	2			3,2–3,2
Deelfontein	3,2	2	1,27	4,4	3,1–3,3
Zoetendalsvlei	3,1	1			
Sutherland	3,3	2	0,64	2,2	3,2–3,3
Cape Agulhas	3,1	1			
Nieuwoudtville	3,2	5	0,20	4,9	3,0–3,4
Springbok	3,2	1			
Lemoenshoek	3,0	1			
Clanwilliam	3,0	2	5,08	18,9	2,6–3,4
Prieska	3,1	2	1,91	7,0	2,9–3,2
Rouxville	3,0	7	0,15	5,4	2,8–3,3
Wolseley	3,0	1			
Grahamstown	2,9	3	0,74	10,3	2,6–3,2
Boshof	3,0	1			
Bredasdorp	3,0	1			
Winburg	3,1	6	0,15	4,7	3,0–3,3
Uitenhage	2,9	1			
Matatiele	3,4	7	0,11	3,6	3,2–3,6
Cradock	2,8	1			
Bloemhof	3,0	4			3,0–3,0
Kammanassie	3,0	2			3,0–3,0
Hilton	3,2	9	0,11	4,4	3,1–3,4
Lydenburg	2,9	3	0,29	3,9	2,8–3,0

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Winterton	3,1	11	5,6	5,6	2,8–3,4
Magudu	3,1	8	0,11	4,2	3,0–3,3
Kaalplaats	3,0	4			3,0–3,0
Babanango	3,0	2			3,0–3,0
Derdepoort	3,0	9	0,09	3,7	2,8–3,2
		Palatal breadth			
Beaufort West	28,4	1			
Murraysburg	28,0	1			
Hutchinson	28,5	1			
Calvinia	27,5	2	6,35	2,6	27,0–28,0
Deelfontein	28,0	2	0,64	0,3	27,9–28,0
Zoetendalsvlei	27,4	1			
Sutherland	29,0	2	1,91	0,7	28,8–29,1
Cape Agulhas	28,9	2			28,9–28,9
Nieuwoudtville	28,0	5	0,88	2,5	27,2–29,0
Springbok	25,8	1			
Lemoenshoek	27,6	1			
Clanwilliam	28,0	2	17,79	7,1	26,6–29,4
Prieska	26,5	2	1,27	0,5	26,4–26,6
Rouxville	26,5	7	0,89	3,7	25,5–28,1
Wolseley	25,5	1			
Grahamstown	26,3	3	4,79	7,3	24,8–28,5
Boshof	27,2	1			
Bredasdorp	26,6	1			
Winburg	26,7	6	0,75	2,7	26,1–28,0
Uitenhage	27,4	1			
Matatiele	27,1	7	0,75	3,1	26,0–28,0
Cradock	26,6	1			
Bloemhof	27,0	4	2,08	4,8	26,0–28,9
Kammanassie	26,7	2	3,81	1,6	26,4–27,0
Hilton	26,8	9	0,84	4,1	25,3–28,4
Lydenburg	25,4	3	3,69	5,8	23,8–26,7
Winterton	26,5	12	0,53	3,2	25,5–28,4
Magudu	25,8	7	0,25	1,1	25,6–26,3
Kaalplaats	25,7	4	0,55	1,5	25,1–26,0
Babanango	25,9	2	6,99	3,0	25,3–26,4
Derdepoort	25,7	10	0,57	3,1	24,1–26,7
		Bizygomatic breadth			
Beaufort West	45,1	1			
Murraysburg	46,0	1			
Hutchinson	46,0	1			
Calvinia	45,8	1			

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Deelfontein	46,1	2	0,64	0,2	46,0–46,1
Zoetendalsvlei	44,0	1			
Sutherland	45,0	2	12,71	3,1	44,0–46,0
Cape Agulhas	45,4	1			
Nieuwoudtville	45,7	5	1,38	2,4	43,9–46,8
Springbok	–				
Lemoenshoek	44,5	1			
Clanwilliam	44,9	2	14,61	3,6	43,7–46,0
Prieska	43,9	2	6,35	1,6	43,4–44,4
Rouxville	45,2	7	1,38	3,3	43,4–47,9
Wolseley	43,7	1			
Grahamstown	43,8	3	2,83	2,6	42,9–45,1
Boshof	43,9	1			
Bredasdorp	44,5	1			
Winburg	44,1	6	1,53	3,3	43,0–46,5
Uitenhage	44,0	1			
Matatiele	43,8	7	1,11	2,7	42,0–45,4
Cradock	45,0	1			
Bloemhof	43,9	4	1,01	1,5	43,0–44,5
Kammanassie	45,3	2	1,91	0,5	45,1–45,4
Hilton	44,4	9	0,98	2,9	42,4–46,3
Lydenburg	42,4	2	4,30	3,3	41,4–43,4
Winterton	43,0	11	0,77	2,7	41,5–45,6
Magudu	43,1	6	1,03	2,3	41,6–44,6
Kaalplaats	42,5	4	1,38	2,1	41,4–43,4
Babanango	42,9	2	5,72	1,5	42,4–43,3
Derdepoort	41,9	10	0,60	2,0	40,2–42,8
		Maximum cranial breadth			
Beaufort West	32,5	1			
Murraysburg	33,8	1			
Hutchinson	32,0	1			
Calvinia	33,5	2	12,71	4,2	32,5–34,5
Deelfontein	34,0	2	4,45	1,5	33,6–34,3
Zoetendalsvlei	34,2	1			
Sutherland	33,8	2	17,15	5,7	32,4–35,1
Cape Agulhas	33,2	1			
Nieuwoudtville	33,7	5	2,17	5,2	31,0–35,5
Springbok	–				
Lemoenshoek	33,1	1			
Clanwilliam	32,4	2	21,60	7,4	30,7–34,1
Prieska	32,0	2	1,97	0,7	31,8–32,1
Rouxville	33,3	7	0,77	2,5	32,2–34,7
Wolseley	32,6	1			

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Grahamstown	32,1	3	0,94	1,2	31,8–32,5
Boshof	31,3	1			
Bredasdorp	33,0	1			
Winburg	31,4	6	0,99	3,0	30,0–32,5
Uitenhage	32,1	1			
Matatiele	32,0	6	0,93	2,9	30,6–33,1
Cradock	31,0	1			
Bloemhof	31,5	3	2,75	3,5	30,5–32,7
Kammanassie	33,0	2	8,89	3,0	32,3–33,7
Hilton	31,5	8	0,97	3,8	29,6–33,2
Lydenburg	30,8	3	0,87	1,1	30,5–31,2
Winterton	31,2	10	0,59	2,7	29,6–32,5
Magudu	30,6	6	0,89	2,8	29,6–32,1
Kaalplaats	30,1	3	3,57	4,8	29,0–31,7
Babanango	30,8	2	0,64	0,2	30,7–30,8
Derdepoort	29,4	8	0,57	2,4	28,1–30,0
		Zygomatic arch length			
Beaufort West	39,6	1			
Murraysburg	40,5	1			
Hutchinson	43,5	1			
Calvinia	40,7	1			
Deelfontein	41,8	2	3,81	1,0	41,5–42,1
Zoetendalsvlei	40,0	1			
Sutherland	40,2	2	1,91	0,5	40,0–40,3
Cape Agulhas	38,9	1			
Nieuwoudtville	39,7	5	0,58	1,1	39,0–40,3
Springbok	37,3	1			
Lemoenshoek	39,8	1			
Clanwilliam	39,2	2	13,34	3,8	38,1–40,2
Prieska	39,2	2	2,54	0,7	39,0–39,4
Rouxville	39,9	7	1,04	2,8	38,3–41,8
Wolseley	38,6	1			
Grahamstown	39,0	3	5,59	5,8	37,4–41,6
Boshof	39,1	1			
Bredasdorp	38,4	1			
Winburg	39,9	6	1,53	3,7	37,6–41,4
Uitenhage	39,0	1			
Matatiele	39,1	7	0,79	2,2	38,1–40,7
Cradock	37,8	1			
Bloemhof	39,3	4	2,21	3,5	37,5–40,7
Kammanassie	38,3	2	8,89	2,6	37,6–39,0
Hilton	39,3	8	1,37	4,3	37,8–42,7
Lydenburg	38,2	3	5,45	5,7	36,5–40,7

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Winterton	38,5	11	1,07	4,1	34,6–40,6
Magudu	38,0	8	1,38	4,4	36,2–41,1
Kaalplaats	37,6	5	1,32	2,8	36,2–39,1
Babanango	37,9	2	4,45	1,3	37,5–38,2
Derdepoort	36,1	10	0,85	3,3	33,9–37,8
		Posterior cranial height			
Beaufort West	20,2	1			
Murraysburg	21,2	1			
Hutchinson	23,3	1			
Calvinia	21,2	2	1,91	1,0	21,0–21,3
Deelfontein	19,2	2	4,45	2,6	18,8–19,5
Zoetendalsvlei	19,0	1			
Sutherland	19,8	2	8,89	5,0	19,1–20,5
Cape Agulhas	21,0	1			
Nieuwoudtville	19,7	5	1,01	4,1	18,8–20,8
Springbok	20,0	1			
Lemoenshoek	19,9	1			
Clanwilliam	20,1	2	6,35	3,5	19,6–20,6
Prieska	19,4	2	7,62	4,4	18,8–20,0
Rouxville	20,3	6	1,07	5,0	19,0–22,0
Wolseley	19,8	1			
Grahamstown	18,4	3	1,62	3,5	17,8–19,1
Boshof	18,3	1			
Bredasdorp	19,1	1			
Winburg	19,5	6	0,90	4,4	18,2–20,5
Uitenhage	19,1	1			
Matatiele	18,5	7	1,46	8,5	16,2–21,0
Cradock	18,2	1			
Bloemhof	18,9	4	2,02	6,7	17,4–20,5
Kammanassie	19,8	2	3,18	1,8	19,5–20,00
Hilton	18,1	9	0,50	3,6	17,0–19,3
Lydenburg	19,1	3	1,97	4,2	18,5–20,0
Winterton	18,1	12	0,56	4,8	16,6–19,4
Magudu	17,5	6	0,55	3,3	16,6–18,3
Kaalplaats	18,3	4	2,08	7,2	16,6–19,8
Babanango	17,6	2	5,72	3,6	17,1–18,0
Derdepoort	17,3	8	0,40	2,9	16,2–17,8
		Bulla breadth			
Beaufort West	10,0	1			
Murraysburg	9,2	1			
Hutchinson	9,6	1			
Calvinia	9,0	2	3,18	4,0	8,7–9,2

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Deelfontein	9,9	2	0,64	0,7	9,8–9,9
Zoetendalsvlei	9,2	1			
Sutherland	9,4	2	5,08	6,0	9,0–9,8
Cape Agulhas	10,0	1			
Nieuwoudtville	9,0	5	0,28	2,5	8,7–9,2
Springbok	8,6	1			
Lemoenshoek	9,8	1			
Clanwilliam	9,7	2	1,91	2,2	9,5–9,8
Prieska	9,9	2	4,4	5,0	9,5–10,2
Rouxville	8,8	7	0,49	6,0	8,4–9,9
Wolseley	8,7	1			
Grahamstown	8,9	3	12,9	1,9	8,7–9,0
Boshof	8,5	1			
Bredasdorp	9,0	1			
Winburg	9,0	6	0,62	6,6	8,2–9,8
Uitenhage	8,6	1			
Matatiele	8,6	7	0,46	5,8	7,9–9,3
Cradock	9,5	1			
Bloemhof	8,8	4	1,08	7,8	8,1–9,5
Kammanassie	9,3	2	5,08	6,1	8,9–9,7
Hilton	8,2	9	0,48	7,6	7,4–9,0
Lydenburg	8,2	3	0,94	4,6	7,8–8,5
Winterton	8,3	11	0,31	5,7	7,4–8,8
Magudu	8,7	6	0,49	5,3	8,2–9,4
Kaalplaats	8,4	5	0,75	7,2	7,5–9,2
Babanango	8,6	1			
Derdepoort	8,4	9	0,42	6,5	7,6–9,2
		Maximum mandibular length			
Beaufort West	75,0	1			
Murraysburg	73,1	1			
Hutchinson	76,1	1			
Calvinia	72,8	2	29,22	4,5	70,5–75,1
Deelfontein	74,5	2	10,16	1,5	73,7–75,3
Zoetendalsvlei	72,1	1			
Sutherland	73,0	2	11,44	1,7	72,1–73,9
Cape Agulhas	72,9	1			
Nieuwoudtville	71,1	5	2,52	2,7	68,4–73,7
Springbok	70,1	1			
Lemoenshoek	72,2	1			
Clanwilliam	70,3	2	37,48	5,9	67,3–73,2
Prieska	68,2	2	8,26	1,4	67,5–68,8
Rouxville	69,4	7	2,30	3,6	65,3–72,4
Wolseley	67,2	1			

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Grahamstown	68,9	3	6,03	3,5	67,4–71,7
Boshof	68,9	1			
Bredasdorp	67,4	1			
Winburg	69,2	6	2,18	3,0	66,2–71,5
Uitenhage	68,1	1			
Matatiele	68,5	7	1,71	2,7	66,8–72,1
Cradock	66,5	1			
Bloemhof	67,8	4	4,61	4,3	63,6–70,2
Kammanassie	66,8	1			
Hilton	66,9	9	1,74	3,4	63,1–71,2
Lydenburg	66,8	2	3,01	1,5	66,1–67,5
Winterton	66,5	12	0,99	2,3	63,6–68,8
Magudu	65,4	6	3,29	4,8	62,2–71,0
Kaalplaats	64,5	4	1,35	1,5	63,5–65,8
Babanango	64,0	2			64,0–64,0
Derdepoort	62,8	10	0,96	2,1	61,0–65,0
		Mandibular toothrow length			
Beaufort West	44,9	1			
Murraysburg	43,5	1			
Hutchinson	43,5	1			
Calvinia	44,2	2	14,61	3,7	43,1–45,4
Deelfontein	44,4	2	4,45	1,1	44,0–44,7
Zoetendalsvlei	44,0	1			
Sutherland	43,4	2	2,54	0,6	43,2–43,6
Cape Agulhas	43,7	1			
Nieuwoudtville	42,8	5	1,92	3,6	40,9–45,2
Springbok	43,6	1			
Lemoenshoek	41,9	1			
Clanwilliam	41,0	2	32,40	8,8	38,4–43,5
Prieska	40,1	2			40,1–40,1
Rouxville	41,7	7	1,23	3,2	40,0–43,3
Wolseley	41,7	1			
Grahamstown	40,3	3	4,53	4,5	38,7–42,3
Boshof	40,4	1			
Bredasdorp	40,9	1			
Winburg	40,7	6	1,47	3,4	38,6–42,9
Uitenhage	40,1	1			
Matatiele	40,5	7	2,41	3,2	38,6–43,0
Cradock	38,3	1			
Bloemhof	40,9	4	1,24	1,9	40,3–42,0
Kammanassie	39,5	1			
Hilton	39,9	9	1,90	3,8	36,5–41,5
Lydenburg	38,5	3	2,49	2,6	37,5–39,5

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Winterton	39,4	12	0,68	2,7	37,8–41,6
Magudu	38,7	8	1,06	3,3	37,4–40,9
Kaalplaats	38,3	4	1,54	2,9	36,6–39,0
Babanango	37,6	2	5,72	1,7	37,1–38,0
Derdepoort	37,4	10	0,68	2,5	36,0–38,7
		Maximum mandibular height			
Beaufort West	47,0	1			
Murraysburg	48,3	1			
Hutchinson	49,3	1			
Calvinia	46,5	2	14,61	3,5	45,3–47,6
Deelfontein	49,1	2	9,53	2,2	48,3–49,8
Zoetendalsvlei	46,8	1			
Sutherland	47,9	2	26,68	6,2	45,8–50,0
Cape Agulhas	44,8	1			
Nieuwoudtville	45,5	5	0,97	1,7	44,1–46,1
Springbok	44,9	1			
Lemoenshoek	44,2	1			
Clanwilliam	45,2	2	10,71	5,8	43,3–47,0
Prieska	45,0	2	20,96	5,2	43,3–46,6
Rouxville	46,4	7	1,39	3,3	44,0–48,3
Wolseley	43,2	1			
Grahamstown	43,6	3	3,38	3,1	42,5–45,1
Boshof	45,4	1			
Bredasdorp	42,9	1			
Winburg	46,8	6	1,57	3,2	44,9–49,2
Uitenhage	43,2	1			
Matatiele	46,5	7	1,39	3,2	44,3–48,5
Cradock	44,4	1			
Bloemhof	46,4	4	1,56	2,1	45,5–47,5
Kammanassie	45,3	2	1,91	0,5	45,1–45,4
Hilton	46,3	9	1,59	4,2	42,9–49,4
Lydenburg	45,1	3	3,28	2,9	43,7–46,3
Winterton	45,1	12	1,59	5,5	41,1–49,6
Magudu	44,9	7	1,48	3,7	42,8–48,1
Kaalplaats	43,5	4	1,66	2,4	42,3–44,7
Babanango	44,9	2	9,53	2,4	44,1–45,6
Derdepoort	43,2	10	0,90	2,9	41,8–45,1
		Mandibular body breadth			
Beaufort West	6,3	1			
Murraysburg	6,5	1			
Hutchinson	6,8	1			
Calvinia	6,1	2	0,64	1,2	6,0–6,1

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
Deelfontein	6,1	2	1,91	3,5	5,9–6,2
Zoetendalsvlei	6,3	1			
Sutherland	6,5	2	0,64	1,1	6,4–6,5
Cape Agulhas	6,4	1			
Nieuwoudtville	6,3	5	0,24	3,1	6,0–6,5
Springbok	6,1	1			
Lemoenshoek	5,7	1			
Clanwilliam	6,2	2	2,54	4,6	6,0–6,4
Prieska	5,9	2	6,35	12,0	5,4–6,4
Rouxville	6,1	7	0,24	4,3	5,8–6,6
Wolseley	5,8	1			
Grahamstown	6,2	3	0,76	5,0	5,9–6,5
Boshof	6,5	1			
Bredasdorp	6,2	1			
Winburg	6,2	6	0,20	3,1	5,9–6,4
Uitenhage	6,1	1			
Matatiele	6,7	7	0,38	6,1	6,0–7,3
Cradock	6,4	1			
Bloemhof	6,4	4	3,75	3,7	6,2–6,7
Kammanassie	5,9	2	2,54	4,8	5,7–6,1
Hilton	6,4	9	0,40	8,1	5,3–7,1
Lydenburg	6,0	3	0,80	5,4	5,6–6,2
Winterton	6,4	12	0,35	8,7	5,8–7,9
Magudu	6,1	8	0,27	5,4	5,7–6,6
Kaalplaats	6,3	4	0,52	6,0	5,8–6,7
Babanango	6,4	2	0,64	1,1	6,3–6,4
Derdepoort	6,2	10	0,27	6,1	5,6–6,7
			I_1 breadth		
Beaufort West	3,4	1			
Murraysburg	3,2	1			
Hutchinson	3,2	1			
Calvinia	3,1	2	0,64	2,3	3,0–3,1
Deelfontein	3,1	2	1,27	4,5	3,0–3,2
Zoetendalsvlei	3,2	1			
Sutherland	3,2	2			3,2–3,2
Cape Agulhas	3,0	1			
Nieuwoudtville	3,2	5	0,20	5,1	3,0–3,4
Springbok	3,2	1			
Lemoenshoek	3,0	1			
Clanwilliam	3,1	2	3,81	13,7	2,8–3,4
Prieska	3,0	2	1,27	4,7	2,9–3,1
Rouxville	3,1	7	0,15	5,4	2,8–3,3
Wolseley	2,9	1			
Grahamstown	3,0	3	0,66	8,8	2,7–3,2

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TABLE 2 (continued)

Locality	$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
Boshof	2,9	1			
Bredasdorp	3,0	1			
Winburg	3,1	6	0,08	2,4	3,0–3,2
Uitenhage	3,0	1			
Matatiele	3,4	7	0,12	4,0	3,2–3,6
Cradock	2,8	1			
Bloemhof	3,2	4	0,28	5,5	2,9–3,3
Kammanassie	3,0	2	0,64	2,4	2,9–3,0
Hilton	3,1	9	0,08	3,2	3,0–3,3
Lydenburg	3,0	3	0,43	5,8	2,8–3,1
Winterton	3,1	12	0,14	7,1	2,8–3,4
Magudu	3,0	8	0,15	6,0	2,8–3,3
Kaalplaats	3,0	4	0,08	1,7	2,9–3,0
Babanango	3,1	2	0,64	2,3	3,0–3,1
Derdepoort	3,0	10	0,10	4,6	2,7–3,2
		Mandibular premolar-molar length			
Beaufort West	18,1	1			
Murraysburg	17,8	1			
Hutchinson	18,8	1			
Calvinia	17,6	2	6,99	4,4	17,0–18,1
Deelfontein	18,2	2	0,64	0,4	18,1–18,2
Zoetendalsvlei	17,9	1			
Sutherland	18,9	2	4,45	2,6	18,5–19,2
Cape Agulhas	19,2	1			
Nieuwoudtville	18,4	5	1,30	5,6	17,4–20,0
Springbok	17,8	1			
Lemoenshoek	18,1	1			
Clanwilliam	17,8	2	15,25	9,5	16,6–19,0
Prieska	16,8	2	0,64	0,4	16,7–16,8
Rouxville	18,0	7	0,43	2,6	17,4–18,8
Wolseley	17,3	1			
Grahamstown	16,9	3	2,49	5,9	16,1–18,0
Boshof	17,4	1			
Bredasdorp	16,2	1			
Winburg	17,8	6	0,75	4,0	17,0–18,9
Uitenhage	17,0	1			
Matatiele	17,7	7	0,53	3,2	17,1–18,5
Cradock	15,7	1			
Bloemhof	18,0	4	1,20	4,2	17,3–18,9
Kammanassie	17,3	2	16,52	10,6	16,0–18,6
Hilton	17,8	9	0,62	4,6	16,5–18,8
Lydenburg	17,1	3	0,90	2,1	16,8–17,5
Winterton	17,3	12	0,44	4,0	15,8–18,2
Magudu	16,7	8	0,41	3,0	16,2–17,8
Kaalplaats	17,2	4	1,38	5,1	16,2–18,3
Babanango	16,6	2	0,64	0,4	16,5–16,6
Derdepoort	17,4	10	0,54	4,4	16,2–18,5

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TABLE 3 : External and cranial measurements of *P. rupestris*, *P. crassicaudatus* and *P. randensis*. Localities and number of specimens (in parenthesis) pooled for computing the statistics are *P. rupestris* : Boshof (1), Matatiele (3), Nieuwoudtville (1), Springbok (9), Winterton (1). *P. crassicaudatus:* Donnybrook (2), Dundee (1), Estcourt (2), Harding (1), Hilton (12), Howick (1), Louwsburg (1), Magudu (3), Melmoth (1), Oribi Gorge (1), Otto's Bluff (1), Paulpietersburg (1), Umzinto (1). *P. randensis:* Bon Accord (1), Hartebeespoort Dam (1), Hennop's River (2), Jack Scott Nature Reserve (3), Johannesburg (1), Koster (1), Messina (1), Pafuri (1), Silverton (1), Waterberg (1), Zeerust (1).
$\bar{X}$ = Arithmetic mean; n = Sample size; tSE = 95% Confidence limit;
CV = Coefficient of variation.

Pronolagus species	$\bar{X}$ (mm)	n	t SE	CV (%)	Range (mm)
		Head and body length			
P. rupestris	447	15	20,14	8,1	380–535
P. crassicaudatus	508	28	10,29	5,2	460–560
P. randensis	463	12	19,21	6,5	420–500
		Tail length			
P. rupestris	86	15	8,65	18,2	50–115
P. crassicaudatus	65	26	6,62	25,2	35–110
P. randensis	98	13	12,46	21,1	60–135
		Hind foot length			
P. rupestris	92	15	2,22	4,4	85–100
P. crassicaudatus	112	12	4,78	6,7	100–125
P. randensis	100	13	4,74	7,9	87–110
		Ear length			
P. rupestris	94	15	5,06	9,7	80–105
P. crassicaudatus	74	12	3,27	7,0	60–80
P. randensis	84	13	3,48	6,9	80–100
		Total length of skull			
P. rupestris	82,4	13	1,53	3,1	78,4–87,8
P. crassicaudatus	91,3	27	0,99	2,7	85,3–94,8
P. randensis	90,0	14	1,29	2,5	86,1–92,9
		Muzzle length			
P. rupestris	35,6	14	1,43	7,0	32,5–39,2
P. crassicaudatus	41,3	27	0,95	5,8	37,4–45,0
P. randensis	40,1	14	1,41	6,1	36,4–43,7
		Frontal length			
P. rupestris	34,1	15	0,94	5,0	31,4–37,6
P. crassicaudatus	36,6	28	1,19	8,4	30,4–44,2
P. randensis	39,8	14	1,26	5,5	36,5–44,0

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TABLE 3 (continued)

Pronolagus species	$\bar{X}$(mm)	n	t SE	CV (%)	Range(mm)
		Parietal length			
P. rupestris	17,6	14	0,79	7,8	15,5–19,9
P. crassicaudatus	20,7	27	0,60	7,3	17,8–26,4
P. randensis	17,9	14	0,95	9,2	14,5–19,6
		Posterior muzzle length			
P. rupestris	15,2	15	0,60	7,1	12,5–17,0
P. crassicaudatus	20,6	28	0,65	8,2	17,5–23,6
P. randensis	17,5	14	1,95	5,2	16,5–19,0
		Anterior frontal breadth			
P. rupestris	14,1	15	1,00	13,1	11,3–19,0
P. crassicaudatus	16,8	28	0,48	7,3	14,7–19,5
P. randensis	16,9	14	0,43	4,4	15,5–17,7
		Posterior frontal breadth			
P. rupestris	10,9	15	0,56	9,3	9,5–13,3
P. crassicaudatus	13,5	28	0,30	5,7	12,0–15,1
P. randensis	13,7	14	0,67	8,5	12,4–16,5
		Interauditory breadth			
P. rupestris	25,4	11	0,38	2,2	24,7–26,4
P. crassicaudatus	27,4	23	0,53	4,5	25,4–30,6
P. randensis	26,8	13	0,35	2,2	25,7–27,7
		Basal cranial length			
P. rupestris	66,7	12	1,21	2,9	63,2–71,0
P. crassicaudatus	73,9	26	0,93	3,1	68,3–76,6
P. randensis	72,4	14	1,53	2,2	67,0–76,3
		Palate incisor length			
P. rapestris	36,6	14	0,72	3,4	35,3–39,1
P. crassicaudatus	41,9	27	0,78	4,7	37,2–48,4
P. randensis	42,4	14	0,97	4,0	39,7–44,9
		Palate vacuity length			
P. rupestris	24,4	14	0,51	3,7	23,0–26,1
P. crassicaudatus	26,3	27	0,49	4,7	22,1–28,5
P. randensis	26,8	14	0,64	4,2	25,1–28,9

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TABLE 3 (continued)

Pronolagus species	$\bar{X}$ (mm)	n	± SE	CV(%)	Range(mm)
		Hard palate length			
P. rupestris	7,2	15	0,37	9,3	6,5– 8,7
P. crassicaudatus	9,5	28	0,24	6,5	8,4–11,2
P. randensis	9,3	14	0,40	7,6	8,1–10,1
		Mesopterygoid space			
P. rupestris	5,6	15	0,20	6,6	4,8– 6,4
P. crassicaudatus	5,8	27	0,23	10,1	4,6– 6,8
P. randensis	4,8	14	0,24	8,6	3,9– 5,3
		Maxillary premolar length			
P. rupestris	7,4	15	0,32	7,9	6,5– 9,0
P. crassicaudatus	9,5	28	0,41	11,1	7,7–11,2
P. randensis	7,9	14	0,21	4,7	7,1– 8,6
		Maxillary premolar-molar length			
P. rupestris	14,1	15	0,37	4,7	13,2–15,6
P. crassicaudatus	16,5	28	0,26	4,1	15,2–18,2
P. randensis	15,5	14	0,28	3,1	14,7–16,3
		Principal I^1 breadth			
P. rupestris	2,5	14	0,08	5,5	2,3– 2,8
P. crassicaudatus	3,2	24	0,09	6,9	2,9– 3,6
P. randensis	3,0	14	0,11	6,5	2,6– 3,4
		Palatal breadth			
P. rupestris	22,2	15	0,40	3,3	20,9–24,0
P. crassicaudatus	25,9	28	0,32	3,2	24,1–27,8
P. randensis	24,3	14	0,29	2,1	23,4–25,3
		Bizygomatic breadth			
P. rupestris	37,3	13	0,61	2,7	35,5–38,8
P. crassicaudatus	39,6	25	0,64	3,9	36,0–41,8
P. randensis	40,6	14	0,56	2,4	38,6–42,6
		Maximum cranial breadth			
P. rupestris	28,6	14	0,35	2,1	27,4–29,5
P. crassicaudatus	29,5	26	0,38	3,2	27,3–31,2
P. randensis	29,7	14	0,40	2,4	28,6–30,8

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TABLE 3 (continued)

Pronolagus species	$\bar{X}$ (mm)	n	t SE	CV(%)	Range(mm)
		Zygomatic arch length			
P. rupestris	32,7	15	0,76	4,2	30,3–35,0
P. crassicaudatus	37,0	27	0,53	3,6	33,7–40,0
P. randensis	36,0	14	0,87	4,2	33,0–38,1
		Posterior cranial height			
P. rupestris	16,0	13	0,73	7,5	14,3–18,8
P. crassicaudatus	15,8	26	0,42	6,7	14,2–18,1
P. randensis	16,7	13	0,62	6,1	15,5–18,4
		Bulla breadth			
P. rupestris	7,8	13	0,43	9,1	6,6– 9,4
P. crassicaudatus	6,1	27	0,19	8,0	5,1– 7,0
P. randensis	6,3	14	0,27	7,4	5,5– 7,0
		Maximum mandibular length			
P. rupestris	60,5	15	1,11	3,3	56,5–64,0
P. crassicaudatus	68,0	27	0,85	3,2	62,8–71,4
P. randensis	66,7	13	1,34	3,4	61,4–69,9
		Mandibular toothrow length			
P. rupestris	37,0	15	0,66	3,2	35,4–39,1
P. crassicaudatus	41,4	25	0,62	3,6	37,4–43,8
P. randensis	41,8	13	0,96	3,8	39,3–44,2
		Maximum mandibular height			
P. rupestris	37,5	15	10,90	4,3	35,6–41,3
P. crassicaudatus	44,6	26	0,61	3,4	42,4–48,1
P. randensis	43,0	14	0,77	3,1	41,0–45,0
		Mandibular body breadth			
P. rupestris	4,9	15	0,12	4,4	4,4– 5,4
P. crassicaudatus	5,7	28	0,11	5,1	5,1– 6,2
P. randensis	5,3	14	0,12	3,9	5,0– 5,6
		I_1 breadth			
P. rupestris	2,6	15	0,08	5,2	2,4– 2,9
P. crassicaudatus	3,2	27	0,08	6,4	2,9– 3,6
P. randensis	3,0	14	0,11	6,1	2,7– 3,3

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TABLE 3 (continued)

Pronolagus species	$\bar{X}$ (mm)	n	t SE	CV(%)	Range(mm)
		Mandibular premolar-molar length			
P. rupestris	13,9	15	0,33	4,3	13,1–15,1
P. crassicaudatus	16,2	28	0,24	3,7	15,0–17,5
P. randensis	15,6	14	0,35	3,9	14,4–16,8

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TABLE 4:(continued)

$\bar{X}$ (mm)	n	t SE	CV(%)	Range (mm)
		Hard palate length		
5,1	15	0,18	6,4	4,7–5,9
		Mesopterygoid space		
7,8	15	0,24	5,5	7,2–8,6
		Maxillary premolar length		
6,5	15	0,17	4,7	5,7–7,1
		Maxillary premolar-molar length		
13,0	15	0,23	3,2	12,4–13,7
		Principal I^1 breadth		
2,1	15	0,05	4,0	2,0–2,2
		Palatal length		
22,4	15	0,37	3,0	21,4–23,4
		Bizygomatic breadth		
36,4	14	0,45	2,1	35,1–37,9
		Maximum cranial breadth		
28,6	14	0,44	2,7	27,3–30,3
		Zygomatic arch length		
30,5	14	0,62	3,5	28,9–32,2
		Posterior cranial height		
14,8	14	0,43	5,1	13,4–15,6
		Bulla breadth		
8,9	15	0,28	5,7	8,0–9,7
		Maximum mandibular length		
56,0	14	1,14	3,5	51,4–58,3
		Mandibular toothrow length		
33,0	14	0,40	2,1	31,8–34,1
		Maximum mandibular height		
33,3	14	0,66	3,4	32,0–35,9
		Mandibular body breadth		
4,7	15	0,12	4,9	4,4–5,3
		I_1 breadth		
2,4	14	0,08	6,0	2,1–2,6
		Mandibular premolar-molar length		
13,1	15	0,17	2,3	12,4–13,5

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